AGRO-MORPHOLOGICAL CHARACTERIZATION OF ARABICA COFFEE CULTIVARS IN BURUNDI

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Agro-morphological Characterization of Arabica Coffee Cultivars in Burundi

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DECLARATION

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

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DEDICATION

To my loving wife Niyonkuru Joselyne and my child Irishura Eli Bernice for giving me encouragement during my study period. Their prayers and moral support have been the driving force that has enabled me to achieve the goals in my studies.

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LIST OF ABBREVIATIONS/ACRONYMS

CWD	Coffee Wilt Disease
FAO	Food and Agriculture Organization
GA	Genetic Advance
GCV	Genotypic coefficient of variation
GLM	General Linear Model
GEI	Genotype by Environment Interaction
GPS	Global Position System
h^2_{bs}	Broad sense heritability
H'	Shannon-Weaver diversity index
HCA	Hierarchal Cluster Analysis
ICO	International Coffee Organization
IPGRI	International Plant Genetic Resource Institute
ISABU	Institut des Sciences Agronomiques du Burundi
ISTEEBU	Institut des Statistiques et d'Etudes Economiques du **Burundi**
LSD	Least Significant Difference
MINAGRIE	Ministere d'Agriculture et d'Elevage
P	Probability level
PACSC	Projet d'Appui a la Competitivite du Secteur Café
PCA	Principle Component Analysis
PCV	Phenotypic Coefficient of Variation

RCBD	Randomized Complete Block Design
REML	The Restricted Maximum Likelihood
US$	United States Dollar
$\boldsymbol{\sigma^2_G}$	Genotypic variance
$\boldsymbol{\sigma^2_{GE}}$	Variance due to G x E interaction
$\boldsymbol{\sigma^2_e}$	Error variance

ABSTRACT

Coffee is the most valued commodity among the stimulant crops. Its production is important in over 80 increasing countries including Burundi, for which it is the key foreign currency earner. The present study sought to contribute to the knowledge base required for the improvement of Arabica coffee in Burundi. The specific objectives of the study were to (i) determine diversity among Arabica coffee cultivars in Burundi using agro- morphological characters; (ii) assess the effect of locations on agro-morphological diversity among cultivated Arabica coffee cultivars in Burundi. In line with these objectives, two trials were conducted using two sets of coffee cultivars: Trial one with twenty accessions was conducted at Rukoba; while Trial two with fifteen genotypes was conducted at three locations (Kayanza , Rukoba and Nyange) in Burundi. For the first trial, data was collected for 11 qualitative and 17 quantitative morphological traits over one season and 10 quantitative traits for second trial using IPGRI coffee descriptors. Frequency distribution of the qualitative traits was assessed using visual counts. Analysis of variance (ANOVA) was conducted on the quantitative morphological traits for within and across location. The information on the relative importance of the morphological characters assessed was obtained using Principal Component Analysis (PCA) and the relationships among the accessions evaluated was determined using cluster analysis. Results from the Trial one showed variation in most of the traits assessed as evidenced by the coefficient of variation and frequencies of the qualitative and quantitative traits, respectively. The first and second principal components accounted for 44.79% and 19.61% of the total variability, respectively. PCA shown that Plant height (0.88), number of internodes per branch (0.89), internode length of primary branch (0.82), stem girth (0.69), length of primary branches (0.9), number of cherries per internode (0.75), hundred fruits weight (0.63), fruit length (0.68) and number of primary branches (0.63) were the chief characters to categorize the Arabica coffee genotypes studied. Cluster analysis clustered the Arabica coffee genotypes into four clusters. The diversity amongst the Arabica coffee accessions in both quantitative and qualitative traits revealed by this study can be used for trait enhancement through selection and germplasm conservation. Results from Trial two showed that the effects of environments were significant ($p < 0.05$) to highly significant ($p < 0.01$) for most of the traits. The accession and environment interaction (GEI) were also significant ($p < 0.05$) to highly significant ($p < 0.01$) for fruit and bean traits, demonstrating the presence of variability among the established materials for these traits. Phenotypic variations for fruit and bean quantitative traits were relatively different across environments indicating that these characters were under strong effect of environment. In contrast, phenotypic variation for leaf quantitative traits of tested coffee accession were not relatively different across environments. Cluster analysis based on the distance measures and PCA biplot graph grouped the fifteen accessions into three main clusters according to their genetic background. Evaluation of morphological traits showed that large phenotypic variation among fifteen coffee genotypes for most of the traits as expressed by the moderate to high plot-basis broad sense heritability (h^2_{bs}= 0.24 to 0.82). Highest heritability estimates were for leaf area,

leaf width, fruit length and leaf length (h2 bs= 0.502 to 0.82). The character leaf area, hundred cherries weight, hundred beans weight, leaf width had relatively highest GCV compared with the others with respective value of 23.58%, 13.78%, 6.107 % and 5.340% with corresponding PCV of 26.70%, 16.37%, 9.88% and 6.702) indicating the relative importance of these traits for improvement of coffee in Burundi.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Coffee is a key beverage crop in world trade. It belongs to Rubiaceae family and has more than 70 species out of which only two species, Arabica coffee and Robusta coffee, have commercial value (Steiger *et al.*, 2002). *Coffea arabica* originated from Ethiopia from where it was distributed all over the world by European colonialists and traders (Steiger *et al*., 2002). Arabica coffee accounts for 167.4 million bags of the world coffee trade while Robusta coffee accounts for 71.72 million bags (ICO, 2020).

Coffee is an important cash crop in Burundi. *Coffea arabica* is the more prevalent coffee type. According to (MINAGRIE ,2014), Coffee cultivation began in Burundi in the 1930s when the Belgian colonialists introduced it from Kivu (eastern Democratic Republic of Congo) and Mibirizi. Today the crop is developed in most portions of the country and accounts for about 10% of cultivated land. About 25 million trees are cultivated on an estimated 60,000 hectares by small scale farmers with each farmer growing an average of 50-250 trees on 0.5-0.8 ha farms (Bisekwa *et al.*, 2020). Coffee is the main source of income for farmers in northern, eastern and polar western parts of the country and it contributes on average, 60-70% of the country's foreign exchange incomes (Anaclet *et al*., 2013). The total number of coffee growers is estimated to be 800 000, or 40% of the country's households (ISTEEBU, 2005). Coffee production in the country (Table 1.1) has struggled over time as result of political instability (MINAGRIE, 2015).

Table 1.1: Estimated annual coffee production in Burundi (in thousands 60kg bags)

Year	Production (In thousand 60kg bags)
2012	405,960
2013	159,220
2014	245,550
2015	274,100
2016	254.310
2017	203,620
2018	221,530
2019	268,410
2020	257,290

Source : International Coffee Organisation (ICO) **(2020)**

Coffee is the main export product from Burundi and is exported mostly to Switzerland, the United Kingdom, and Germany. Over the past three decades, it has produced a regular of 40 to 50 million per year of export earnings (ICO, 2018). Coffee production in the country encounters a number of limitations, counting low yielding cultivars, low and deteriorating soil fertility, drought, pests and diseases (Anaclet *et al.,* 2013; MINAGRIE, 2015).

The Burundian government has in recent years advocated for expansion of coffee cultivation areas in a bid to boost production. But this approach has not been effective in regions with high population densities/growth rates, since it has led to competition with food crops for available land (Anaclet *et al.,* 2013; ISABU, 2018). As the farmers' acceptance of coffee as a commercial undertaking increases, the need for information to assist its improvement has become more apparent. The existing strategy for coffee improvement in the country has been introduction of cultivars from various sources and selection of adapted clones (ISABU, 1999).

Over the years, coffee research in Burundi have tried to widen the genetic base of Arabica coffee through introductions of exotic germplasm to increase locally adapted collections (ISABU, 1999). For most crop species, successful cultivars must be adapted to a wide range of climatic and soil conditions. The main coffee growing areas in Burundi are in three altitude zones namely Mumirwa, Buyenzi and Imbo. The low altitude (1125m and 1400 m), the medium altitude (between 1500 m and 1650 m) and high altitude (over 1850 m) (ISTEEBU, 2005). According to Yonas & Tarekegn (2015), evaluation of Arabica coffee genotypes across different environments is a suitable approach and it plays an essential role in documentation of appropriate germplasm. Variability is affected by environmental conditions such as soils, temperature and rainfall (Abrar *et al*., 2014b; Gimase *et al*., 2014).

The extent and magnitude of variation among the coffee cultivars and across environment in the country has not yet been quantified in these collections. For an effective crop enhancement program, the analysis of assortment is one of the suitable tools and plays a crucial role in identification of appropriate germplasm. (Gichimu & Omandi, 2010a; Mazid *et al*., 2013; Muvunyi *et al*., 2017). Moreover, better information of diversity among the cultivars could help to accomplish long-term selection gain (Chowdhury *et al*, 2002). As a traditional method, quantitative and qualitative traits have been used to assess genetic variance and classify current germplasm materials (Mehmood *et al*., 2008; Muvunyi *et al*., 2017; Siise *et al*., 2013).

The commonly used morphological traits used for diversity studies have been leaf, stem, flower, and fruit characteristics (Siise *et al*., 2013). For example, in Ethiopia Olika *et al.* (2011) evaluated 49 coffee accessions and reported that they significantly varied for most of the traits evaluated while (Tounekti *et al.*, 2017) described significant genetic differences for quantitative traits examined among germplasm cultivated in the South-western region of Saudi Arabia.

The coffee germplasm currently maintained in the fields of the Institut des sciences Agronomics du Burundi (ISABU), on which this study was conducted, were introduced

from different countries (ISABU, 2018).The Burundian government is currently embarking on a rehabilitation of the coffee sector through diverse approaches and this calls for an understanding of the diversity present among coffee accessions in the country in a bid to identify suitable parental genotypes. However, the diversity among the accessions has not been previously quantified and hence, this study was conducted.

1.2 Problem Statement

Coffee is an important cash crop in Burundi especially for small-holder farmers. However, its production is greatly constrained by many abiotic and biotic stresses. (ICO, 2018). Coffee productivity in the country is estimated to be about 10-30% lower than those of Ethiopia or Uganda. In addition, cultivars currently in circulation, burbon 139, burbon 71, mibirizi 49, mibirizi 68 and jakson2 are susceptible to pests and diseases and the phenomenon of degeneration through aging makes them poor performers (MINAGRIE, 2015). ISABU is required to conduct research on and conserve coffee plant genetic resources. To date, only limited studies have been conducted on the crop. In order to improve the production of coffee and quality, keep up with environmental changes and coffee market demands, a strong research base is required. Strategic conservation also demands comprehensive knowledge of existing genetic diversity. It is of uttermost importance therefore, to establish the phenotypic variability of Arabica coffee in Burundi.

1.3 Justification

Assessment of genetic diversity constitutes an important basis for the selection of suitable varieties for use in coffee improvement. In the search of better genotypes, the use of genetic variability in crosses of genetically divergent clusters is an important plan for achieving gains occasioning from selection. The importance of genetic assortment for improvement lies in the point that it offers factors for the identification of greater genotypes, since the selection of parents to form segregating populations is one of the crucial decisions that the breeder wants to make (Bertrand *et al.*, 2006).According to

Bertrand *et al*. (2006), much of the achievement of coffee improvement in Brazil was based on information of the available germplasm, and the agro-morphological variation between species in the genus, among populations within species, and among individuals within a population (Eskes, 1989). Genetic variability in Arabica coffee has been studied for agro-morphological traits by several investigators. Al Hakimi *et al. (*2005) and Eskes (1989) reported the existence of high variability among coffee genotypes for the various traits studied in Saudi Arabia. Moreover, variability is affected by weather conditions such as soils, temperature and rainfall. In Burundi, collections of new cultivars and cultivated Arabica coffee cultivars are maintained in the three stations of ISABU, namely Kayanza, Rukuba and Nyange (ISABU, 2010). There has been a gradual sizable loss of trees in all the three stations due to varying moisture, pests and diseases. To develop an effective coffee enhancement programme, it is important to first investigate the diversity among existing cultivars.

Given the existing opportunities in the country, Burundi has adopted a National Strategy for the Development of the Coffee Sector (2015-2021) under the Coffee Sector Competitiveness Support Project (PACSC). This project supports the development of new coffee cultivars through breeding. It is anticipated that the country will obtain high yielding cultivars which will undoubtedly increase the country's coffee sales. The outcomes of this study will be of benefit to Burundian coffee growers in identifying the best varieties to grow and for coffee breeders interested in improving coffee.

1.4 Hypotheses

1.4.1 Null hypotheses

1. There are no differences among Arabica coffee cultivars grown in Burundi with respect to agro- morphological traits.
2. Locations have no effect on agro-morphological diversity among cultivated *C. arabica* cultivars in Burundi.

1.5 Objectives

1.5.1 Main objective

The main objective of the study was to assess diversity among Arabica coffee accessions in Burundi using morphological characters.

1.5.2 Specific objectives

1. To determine diversity among Arabica coffee cultivars in Burundi based on agro-morphological characters.
2. To assess the effect of locations on agro-morphological diversity among cultivated Arabica coffee cultivars in Burundi.

1.6 Scope of the study

The scope of this study is to contribute to the understanding of the diversity among coffee cultivars in Burundi to inform the country's breeding program.1.7 Significance of the study

The data generated from the study will provide useful information on the level of morphological parameters in identification of superior genotypes for enhancement and provide critical information on coffee management in Burundi.

CHAPTER TWO

LITERATURE REVIEW.

2.1 Introduction

Arabica Coffee is an important crop which belongs to the Rubiaceae family (Davis *et al*., 2011). Rubiacea is one of the largest flowering plant families containing some 500 genera and 6000 species. The number of species recognized by different authors as belonging to genus *Coffea* ranges from 25 to 100 ((Wrigley, 1988), but (Bridson,1982) considers that there are, in Africa, probably 25 good species with an additional 11 poorly identified ones. However, the most key economic species of the genus are *Coffea arabica* L. producing about 70 per cent of the world's coffee and *Coffea canephora* contributing about 30%. Coffee is the main export product from Burundi and is exported mostly to Switzerland, the United Kingdom, and Germany. Over the past three decades, it has produced a usual of million per year of export earnings (ICO, 2018). Coffee production in Burundi faces a number of constraints, including low yielding cultivars, low and declining soil fertility, drought, pests and diseases among others (Anaclet *et al*., 2013; MINAGRIE, 2015).

Table 2.1: Top 10 coffee producing countries in the world (in thousand 60kg bags).

coffee year production	2015	2016	2017	2018
	(In thousand 60kg bags)			
Brazil	52 326	56787	52735	61700
Vietnam	28 737	25540	30540	29500
Colombia	14 009	14634	13824	14200
Indonesia	12 585	11541	10802	10200
Ethiopia	6 714	7297	7454	7500
Honduras	5 786	7457	7560	7450
India	5 830	6161	5813	5200
Uganda	3 650	4962	4797	4900
Mexico	2 903	3781	4392	4500
Peru	3 304	4223	4279	4300

Source: International Coffee Organization (ICO) (2018) Statistics (ICO , 2018).

World Coffee Production and Consomption

Coffee is produced in a large number of countries worldwide. In terms of regions, South America contributes around 43%, Asia 24%, Central America 18%, and Africa 16%. The four largest coffee producing countries in the world are Brazil, Vietnam, Colombia and Indonesia who produce more than half of the world supply (ICO, 2020). The two greatest vital species of coffee are *Coffea arabica* and *Coffea canephora*. While negligible quantities of *Coffea liberica* and *Coffea excelsa* are grown in a few areas. Arabica coffee accounts for 70% of the World coffee trade while Robusta coffee accounts for 30%. (Wrigley, 2003).

Arabica coffee is developed in many tropical and sub-tropical countries. Majority of these countries export the product to world market (Bisekwa *et al.,* 2020). Burundi is

among these countries which heavily depend on coffee to earn its foreign exchange earnings. About 40% of its export is covered from Arabica coffee (Bisekwa *et al*., 2020).

The efficiency in terms of greater quality and yield deteriorate, it results from the extensive agricultural practice of relying on old coffee tree with the absence of variation. the vagaries of unpredicted climate and the cumulative existence of pest and diseases also play a prominent role (Barampama & Flémal, 1986;Ngayempore,2007). The Coffee germplasm genotypes maintain at the Coffee experimental station of Institute des Sciences Agronomiques du Burundi (ISABU), were presented from diverse world coffee assortment centers, and characterize the key cultivars developed in Burundi from more than three decades ago (Anaclet *et al*., 2013).

2.3 Description of Arabica coffee

The coffee trees are perennial, woody and place from shrubs to trees in size that can develop to a height of 10 meters (Wintgens, 2009). It is a shrub with persistent and opposite leaves, generally growing some shade. Young shoots are tanned green, young pale green, leaves colour range from dark green to yellowish. The leaves are generally waxy, elliptical in shape and the apex is apiculated, the inflorescence is axillary, that is, the flowers are born at the armpit of buds and twigs. The fruit is often fleshy fruit of purple, red or yellow color and contains two cores, each containing a coffee bean. The latter is sometime enclosed in a transparent semi-rigid shell, with the parchment-like appearance corresponding to the wall of the core. It contains many primary branches and very few secondary branches. The angle of insertion of the primary branches on the main stem is horizontal or spread (Wintgens, 2009)..

2.4 Coffee taxonomy

The principal noted taxonomic description of coffee was done by French naturalist Antoine de Jussieu, who designated a coffee plant from the botanical garden of Amsterdam. The complete taxonomies of coffee were made by (Montagnon *et al*., 1996)

and the most recent taxonomy of coffee, lists 103 species of *Coffea* (Wintgens, 2009). The most common coffee species are *Coffea arabica* and *Coffea canephora*, better known as Robusta. There are a large number of coffee species, but their cultivation and consumption is without any measure with those of Arabica and Canephora (Montagnon *et al.*, 1996).The differences between these two main species are in the size of the fruits, the drupes, the shape of the leaves and also the caffeine and the aromas contain of these species (Clifford, 2012).

Arabica coffee beans are slightly lengthened while those of Robusta are minor and rounder (Clifford, 2012) Arabica has Kahweol' diterpene and as have much caffeine as Robusta, but a abundant higher sugar concentration, which contributes meaningfully to its aroma compared to that of Robusta. ((Clifford, 2012). *C. arabica* has a narrow genetic base. Deviations from the normal chromosome number occur in exceptional cases and chromosomes, occur in low frequencies, in seedling offspring as weak plants with narrow leaves and have been called monosperma (Montagnon *et al.*, 1996).

2.5 Coffee botany

Arabica coffee is from the family Rubiaceae, subfamily Ixoridaeae, tribe Coffeae. It is a perennial woody plant (resistant to winter seasons) dicotyledonous. It can live up to 50 years but production decreases after 50 years. It is about the size of a shrub, a shrub or even a tree. Often, it is composed of several trunks, which gives it a bushy appearance (Wintgens, 2004).

Usually the leaves are glossy and dark green, 10–15 cm (4–6 in) long , entire, and opposite, characteristic of Rubiaceae, positioned on the branch often opposite, but sometimes whorled by 3 leaves on the same node ((Clifford, 2012). The flowers are white, fragrant, gathered in glomeruli (tight clusters) in the axils of the leaves, it is the inflorescence. The fruit is called drupe (fruit fleshy or vulgarly cherry) red when ripe, containing two seeds / stones ((Clifford, 2012).

2.6 Ecological and climatic requirements of coffee

2.6.1 Altitude and latitude

Arabica coffee prefers the tropical lands at medium altitude (200 to 2000 meters of altitude) where there is high temperature and dry seasons and rainy must be well defined. Robusta coffee enjoys more warmth, and tolerates poor drought periods, and it grows best at low altitude in warm, humid tropical regions (Decazy *et al.*, 2003). *Coffea arabica* can also grows in the equatorial zone in altitudes ranging from 600 to 2000 meters (Wrigley, 2003). .The different varieties of coffee are located between the two tropics and more precisely between the latitude 15°N and the tropic of cancer 23.4°N. Altitude is also necessary for the development of the best coffees (Wrigley, 2003).

2.6.2 Temperature

Temperature is a key factor in coffee production, and the strongest influences on temperature are latitude and elevation. Coffee is grown around the world at latitudes from 24°N to 25°S (Coste, 1992). Coffee needs a normal temperature between 20°-27 °C. It is suitable for cool temperatures with annual averages of 20 to 25 °C, although, it produces in day temperature over 32 °C in the Arabian Peninsula. Development is greatest rapid during hot rainy season and during cool arid season berries ripen and became for picking. Bright sunshine and earnest weather are necessary for harvesting (Coste, 1992).The optimal mean annual temperature ranges from 18 to 22 °C. Temperatures above 23 ºC accelerate the growth and maturity of fruits

The best environmental growth of Arabica coffee is milder average temperatures and higher advancements with less extreme climate conditions. These discoveries in tropical high regions with important elevations, such as East Africa (Kenya, Tanzania, Burundi and Ethiopia) and the tropics inside the Americas, preliminary in the central and southern parts of Mexico. Under ideal conditions Arabica prefers a low atmospheric humidity comparable to that of the Ethiopian highlands (Snoeck *et al.*, 2009).

2.6.3 Rainfall

Coffee production plant requires a certain amount of rainfall. Ideally 1500 to 2500 mm per year of precipitation allow optimal development of the plant and the fruit (Coste, 1992). In some areas of low rainfall, mechanical irrigation compensates the lack of rainfall. However, the coffee tree still supports short periods of drought (about 2 to 4 months), which may even be beneficial for production (Geromel *et al.*, 2006). In addition, a period of water stress (rain after a dry period) can cause a uniform flowering and thus promotes a clearly defined harvest season (Coste, 1992). If the rainfall exceeds 2500 mm / year, coffee trees will be more susceptible to diseases, to the degradation of their branches (Cannell, 1985). The Arabica coffee shrub typically grows between 2.5-4.5 meters in height, requires an annual rainfall of about 1200-2200 mm/yr while Robusta coffee grows slightly taller at 4.5-6.5 meters, requires generally a slightly more rainfall 2200-3000 mm/yr than Arabica (Geromel *et al.*, 2006).

Frequent rainfall causes almost continuous flowering, which results in two coffee harvesting seasons. The period of highest rainfall determines the main harvesting period, while the period of least rainfall determines the second harvest season. Because rainfall is too frequent for court drying to occur, artificial drying with mechanical dryers is performed in this type of coffee growing environment (Coste, 1992).

2.7 Genetic diversity among coffee genotypes

Diversity can be defined as the amount of assortment between or within species. Crop improvement can change the genetic diversity of a plant. If all the individuals within the species would have been similar, then improving plant performance for different traits would not be easy (Bhandari *et al.*, 2017).The great heterogeneity in populations of *Coffea arabica* is due to the great morphological traits variability of this species which may be abundant to express differences in the growth of new leaves after the implementation of the bending technique (Wrigley, 1988). Genetic divergence is the procedure in which many populations of a family species accumulate independent

genetic variations over time (Yigzaw , 2005).

In some cases, the populations living in different ecological environments are able to show genetic variance from the rest of a population, specifically where the assortment of a population is very large. The genetic modifications among different populations can implicate silent mutations or provide growth to significant morphological and/or physiological changes. Genetic variance will always supplement multiplicative isolation, either due to different adapted environmental condition via selection and/or due to genetic drift, and is the major mechanism fundamental speciation (Wrigley, 1988).

As a traditional process, morphological traits are used to evaluate phenotypic variance and classify current germplasm materials (Van der Vossen, 1985). However, this method, a low level but powerful taxonomic tool, has been employed for the initial grouping of germplasm previous to their description using more precise marker tools. According to Din *et al*., 2010 scientific organization of the cultivar still relies on morphological traits. Moreover, this procedure is easier, cost active, and easy to score and needs less time and finally it does not necessity any practical knowledge. Walyaro, 1983 positively determined the assortment of eleven coffee accessions using agro-morphological traits in Ethiopia. Gichimu & Omondi, 2010b also determined the morphological assortment amongst approximately newly developed and present commercial genotypes in Kenya. Based on the organoleptic attributes, (Giomo *et al*., 2010) successfully discriminated Ethiopian coffee accessions from Indian. In coffee, a lot of germplasm diversity assessments have been based on morphological and agronomic traits as well as reaction to pests, diseases and other stresses (IPGRI. 1996). IPGRI, 1996 listed a number of morphological, agronomical and biochemical traits for characterization of coffee. However, some of these descriptors require at least five years to be expressed. (Bertrand *et al*., 2010) is reported that new coffee genotypes are considerably increasing the narrow genetic base of Arabica coffee in those regions. Genetic diversity in Arabica coffee has also been studied for quantitative and qualitative traits by numerous investigators. (Abrar *et al*., 2014b; Dharmaraj &Gopal, 1986; Gichimu *et al*., 2012; Gimase *et al*., 2014; Getachew *et al*., 2017; Mistro *et al*., 2008;

Olika *et al*., 2011; Petek *et al*., 2008; Yigzaw, 2005). All these investigators reported the presence of high variability among coffee genotypes for various traits.

The assessment of genetic diversity among plant populations is habitually completed using several traits such as morphological, biochemical characterization/evaluation (allozyme), and DNA (or molecular) marker analysis (Mohammadi *et al*, 2003).

Morphological markers are created on visually available traits such as flower color, seed shape, growth habits, and coloration, and it does not require expensive technology but large tracts of land area are often required for field experiments (Falconer, 1996).The selection efficiency for higher bean yield have shown genetic diversity in Arabica coffee by taking into account various growth parameter and yield components, such as fruit length ,bean length and number of berries per node, percentage of fruit bearing nodes, weight of 100 beans , stem girth, number of primary branches, length of primary branches , number of internodes, internode length of primary branches , leaf length and leaf width (Falconer, 1996). Marandu *et al*. (2008) and Muvunyi *et al*. (2017) showed that simple correlation and path analysis in selection for coffee trait assessment, greater emphasis should be given to number of berries per nodes and plant height as they had significant positive correlation and relatively high direct effect on yield of clean coffee.

2.8 Evaluation of coffee genotypes across locations

The successful coffee cultivars are those adapted to a wide range of climatic and soil conditions. For arabica coffee, different environmental conditions show the presence of greater genetic diversity of arabica coffee in Ethiopia (Paul &Teketay, 2000).

Arabica coffee has been studied for variation in agro-morphological traits by several investigators who reported the presence of high variability between and within coffee genotypes for various traits studied (Getachew *et al.*, 2017). For example, Yonas *et al*.(2014) reported the presence of significant variances among 30 Arabica coffee cultivars evaluated across four to eight environments in south western Ethiopia for all

15 different agronomic traits and high broad sense heritability estimates for growth traits such as canopy diameter, internode length of principal stem , stem girth ,plant height , , and number of primary branches, moderate to high bean traits such as bean width, bean length and hundred beans weight .

Most of the researchs among the multivariate methods proposed by Falconer (1996) consists of an excellent strategy for quantifying the variability, using quantitative and/or qualitative variables. In order to increase yield and coffee production, (Abrar *et al*., 2014b) carried out adaptation test on different genotypes of coffee across diverse environments. The results showed that some genotypes were better adapted at some locations while others did not perform well at other locations. Some varieties also fail to exhibit wide adaptation at the major coffee growing environments even within south-western region other than the fertile forest soils (Abrar *et al*., 2014b).

2.9 Principal Coordinates Analysis

Multivariate examination procedures have gained significance in classifying many accessions by ordering genetic changeability or examining genetic relations among breeding resources based on the characteristics they have (Mohammadi *et al*., 2003). Multivariate numerical algorithms concurrently observe multiple extents of each individual studied (whether morphological, biological or molecular indicator data). Individuals with comparable descriptions are mathematically grouped together providing groups with high internal (within cluster) similarity. Grouping procedures such as the multidimensional scaling (MDS) , principal component analysis (PCA) and principal organize analysis (PCoA), are at current most normally employed and appear mainly valuable (Perrier *et al*., 2003). These investiture systems produce a geometric design of individuals with each genotype different from the mean by its individual mean characteristics. Ordination approaches facilitate the finding of individuals or populations that display some intermediate among two groups.

The sum of squares of distinctly unit from the mean point is what the factorial examination numbers as inertia of spreading. To reveal their influences to genetic assortment in individuals (Mohammadi *et al*., 2003). With use of a Modified Location Model (MLM), all the definite variables can be joined into one multinomial variable which should be then used with the present unceasing traits (Mohammadi *et al*., 2003). To extract extreme material from the molecular marker data, measurable data ordinations such as principal organize analysis (PCoA) or PCA can be used in grouping with cluster analysis, mainly when the primary two or three PCA or PCoA explain over 25% of the original distinction (Muchugi *et al*., 2008; Mohammadi *et al*., 2003) . With relatively limited characters and no lost data, the output of PCoA and PCA are similar. PCoA is a well grouping option with absent data for the reason that it substitutes missing values with individually computed coefficient of two individuals although the PCA uses mean values. The PCA is very suitable for resolving pattern recognition problems rising from assortment studies, chromatographic and spectroscopic characters; (Muchugi *et al*., 2008; Perrier *et al*., 2003). Varimax rotation fixed in numerical plans such as XLSTAT version 2011.2.05 (Addinsoft SARL Company, Paris, France) was used to improve the principal component analysis plot dependability (Perrier *et al*., 2003). Multidimensional scaling (MDS) or perceptual recording of varieties in a limited dimension is appropriate when use of ranked algorithms gets narrow with nonhierarchical and reticular designs of assortment (Mohammadi *et al*., 2003). Qualitative data can be assessed using Numerous Correspondence Analysis (MCA) and the measureable tables by PCA. Joining data from agro-morphological measures probably biases distances assessed on the basis of numerical traits and increases the level of correlations between qualitative traits. The most highly planned tables may unfavorably render the input of the extra loosely planned tables. It is desirable to assign weights to qualitative traits for grouping purposes (Perrier *et al*., 2003).

2.10 Cluster analysis

Two major grouping systems are commonly used, the model and the distance based methods. The model created method of grouping adopts observations from each group

are random draws from some parametric model, and extrapolations/deductions from parameters conforming to each group and group members are realized together using standard numerical methods like Bayesian methods or maximum-likelihood or (Mohammadi *et al.*, 2003).

The distance based method of joining cultivars uses a pair wise distance matrix as an investigation input for an accurate grouping algorithm (Mohammadi *et al*., 2003; Perrier *et al*., 2003) giving a graphical image such as a dendrogram or tree or. Hierarchical grouping methods such as the Ward's minimum difference method and UPGMA (Unweighted Paired Cluster Method using Arithmetic averages) and (Mohammadi *et al*., 2003) are typically used in genetic assortment analysis in accessions to combine a series of smaller sets of individuals initial with the most comparable.

In UPGMA grouping, a Factorial Examination of Variation uses a distinction matrix data of genetic distances to graphically describe a heterotic tree clusters or dendrogram (Saitou *et al.,* 1987). UPGMA offers consistent heterotic and pedigree indication on biological resources and groups that relate to cultivars of various data groups. Ward's method avoids the binding effects often observed with UPGMA. Equally Ward and UPGMA are suitable for comparable and different assembly size study (Mohammadi *et al*., 2003). The nonhierarchical technique, normally discussed to as K-means collecting, are founded on sequential threshold, comparable threshold, or optimizing systems for assigning individuals to specific collections, after agreeing the most appropriate number of groups (Mohammadi *et al*., 2003; Perrier *et al*., 2003). Lack of prior information on collection number constrains the use of this process. Descriptive statistics are valuable approaches for characterizing precise assortment clusters using the mean, median, difference and inter quartile assortment in form of box plots. Though many grouping methods exist like, Single Linkage, Median, UPGMA, UPGMC (Unweighted Paired Group Method using Centroids), and Complete Linkage, a single grouping way may not reveal genetic relations magnificently and each process has relative fortes and limits. For instance, UPGMA and UPGMC appear similar with a comparatively high level of accuracy for pedigrees whereas Single Linkage and Median clustering methods are

linked with the binding effect and poor resolution of distinct collections that confuse the clarification of results (Mohammadi *et al*., 2003).

To compare the efficacy of dissimilar grouping algorithms, cophenetic correlation coefficient can be used to amount the agreement among the distinction specified by a dendrogram and the distance comparison matrix as contribution of cluster examination. A way with the highest cophenetic correlation coefficient is detected as the best (Mohammadi *et al*., 2003). Meanwhile a factorial step discriminant studies created on the principal components analyzes Mahalanobis distance (D^2) among group centroids (vectors of means) to classify the best grouping algorithm and to confirm whether the derived clusters were significantly diverse or qualify to be divergent populations.

The best assemblage method produces the largest distance D^2 between clusters or groups and is suitable for quantitative data. Fisher inter-group distances and significance also quantity genetic assortment. The weakness of grouping methods is that the algorithms do not clarify what produces an optimal tree or dendrogram and systemic errors acquired during cluster analysis reconstructions. Use of neighbor connection removes the statement that the data are ultra-metric (Perrier *et al*., 2003). Specific distances and Euclidean distances are illustrations of ultrametric distances. The neighbor assembly system has generally been used for phylogenetic studies but not for intraspecific distinction in crop plants (Mohammadi *et al*., 2003). Joined data analysis can consequently reveal information shared to all data sets or specific to a given table. Before joining diverse data collections, it is most significant to consider the comparison or linking between the results derived from individual data sets to establish whether a well estimate of genetic assortment will be obtained with joined data sets (Saitou *et al*., 1987).

2.11 Genotype by environment interactions and stability of quantitative traits in coffee

The term environment relates to sets of climatic, soils, biotic (pests and diseases) and management conditions in individual trial carried out at a given location in one year or over several years (Annicchiarico, 2002; Rashidi *et al*., 2013). The performance of coffee cultivars is likely to vary with changing environments. This differential yield response of cultivars from one environment to another is called genotype by environment (GE) interaction (Allard, 1960; Vargas *et al*., 1998). GEIs have been extensively studied (Annicchiarico, 2002; Elberhart & Russel, 1966; Kang, 2002). Knowledge of the presence and magnitude of GEI is important to coffee breeders in making decisions regarding the development and evaluation of new cultivars (Wamatu *et al*., 2003) and allows making of informed choices regarding which locations and input systems to be used in the breeding efforts. In coffee like many other perennial crops, there is a marked tendency for yield fluctuations from year to year, as a result of successive vegetative-reproductive cycles, but also due to genotype-environment interactions (Mesfin &Bayetta, 1987; Walyaro, 1983; Yonas *et al*., 2014). GEIs for yield traits such as bean yield, yield related growth traits /yield components have been studied with contrasting findings. Mesfin & Bayetta, 1987; Yonas & Trekking, 2015 observed significant genotype by environment relations for coffee yield and yield components in Ethiopia. Similarly, Wamatu *et al*. (2003) described significant genotype by environment interaction effects on coffee yield in Kenya but the relationship among clones and environments is not strictly linear. Contrastingly, Gichimu & Omondi, 2010a; Wamatu & Thomas, 2001 reported non-significant genotype by environment interactions effects on yield and yield traits of arbica coffee in Kenya. Identification of superior genotypes is generally complicated by the presence of GEIs, where by cultivar relative yields vary across different environments. A variety of statistical procedures are available to analyze results of multi-environment trials which include the combined analysis (Aremu *et al.,* 2007), regression coefficient and deviation from regression (Elberhart & Russel, 1966), AMMI (Gauch, 1992) and GGE (Yan *et al*., 2007). Ideally,

varieties that show low GE interaction and have high stable yields are desirable for crop breeders and farmers, because that indicates that the environments have less effect on the performance of genotypes and their yields are largely due to their genetic composition. According to (Eberhart & Russel, 1966) stability parameters like regression coefficient (b), deviation from regression (S^2 d) of the genotypes is estimated following linear regression model. Genotypes giving b-value close to unity are considered to be adapted to all environments, while those showing b-value greater than or less than unity would show specific adaptation to rich or poor environment, respectively, and the genotypes showing low and non-significant S^2 d are considered to possess stability of performance over the range of environments. Assessment of changeability for yield and its component characters converts absolutely crucial before development for an appropriate breeding plan for genetic enhancement (Solomon, 2009). Description of this variability in a population is pertinent since genetic assortment in population and in species controls the rates of adaptive growth and the amount of response in crop enhancement (Solomon, 2009). Genetic strictures such as genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) are suitable in detecting the quantity of variability existing in the germplasm.

2.12 Heritability estimates of quantitative traits in coffee

Heritability in broad sense according to (Bayetta , 2001) is the ratio of total genotypic variance to phenotypic variance, generally expressed in percentage. For making effective improvement in a trait under selection, heritability has been adopted by the large number of workers as a reliable indicator. Heritability shows the effectiveness with which choice of cultivars can be built on phenotypic performance. However, heritability alone offers no indication of the extent of genetic development that would result from selection of separate genotypes. Hence, heritability fixed with high genetic improvement would be more valuable tool in predicting the resultant effect in selection of the extreme genotypes for yield and its assigning characters. The heritability of various morphological traits of coffee has been valued in *C. arabica* L. Mesfin , 1980 assessed 68 coffee germplasm cultivars of national coffee collection during the year 1975-1978 at

Jimma and specified broad sense heritability values of 55% for extreme of coffee character studied. Cilas *et al*.(1998) also defined that yield, stem girth and tree height had high heritability. Bayetta, 2001 also stated high heritability estimations for all morphological traits measured in *Coffea arabica* L., suggesting that the effect of environment on phenotypic display of the characters was minor.

2.13 Correlations among quantitative traits in coffee

Correlation is a measure of the relationship between two or more variables. The measurement scales used should be at least interval scales, but other correlation coefficients are available to handle other types of data (Lemi *et al*., 2017). Correlation coefficients can range from -1.00 to +1.00. The value of -1.00 represents a perfect negative correlation while a value of +1.00 represents a perfect positive correlation. A value of 0.00 represents a lack of correlation (Lemi *et al.*, 2017). Correlation coefficient quantifies the relationship between two variables is simply measures mutual association without cause and effect relationship (Lemi *et al.*, 2017). The existing relationships between traits are, generally determined by the genotypic, phenotypic and environmental correlations. Correlation coefficients, although very useful in quantifying the size and direction of trait associations, can be ambiguous if the high correlation between two traits is a consequence of the indirect effect of other traits (Ariyo *et al*., 1987). Hence, path coefficient analysis is a very important statistical tool that indicate which variables (causes) exert influence on other variables (responses), while recognizing the impacts of multi co linearity (Ariyo *et al.*, 1987). Path coefficient analysis partitions the genetic correlation between yield and its component traits into direct and indirect effects and has effectively been used in identifying useful traits as selection criteria to improve yield (Getachew, 2019). In coffee, the outcome of yield depends on various growth characters and their combinations, such as stem girth, canopy width, number of secondary branches and number of primary branches (Getachew, 2019). In addition, a number of other agronomic characters; such as number of nodes on primary branches, plant height, number of fruits, leaf area, etc can directly or indirectly influence yield (Getachew, 2019; Olika *et al*, 2011). It is important to have a clear understanding about the

magnitudes of the relationships between yield and other agronomic traits, because yield is influenced by all factors that determine productivity (Lemi *et al*., 2017). Several correlation studies showed that the quantitative traits like number of stem nodes, plant height, length of the longest primary branch, canopy diameter, and stem diameter etc. have positive correlation with yield and such characters could be used as a selection measure for improving the yield of the crop meanwhile they characterize the lion's portion in the changeability of the coffee population in the specified area (Gessese *et al.*, 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Determination of diversity among Arabica coffee cultivars in Burundi using agro- morphological characters.

This trial aimed at quantifying the diversity in coffee accessions in Burundi as a basis for cultivar development and germplasm management in the country.

3.1.1 Experimental site

The study was conducted in the 2019-2020 rainy season in Rukoba Research Station of the Institut des Sciences Agronomiques du Burundi (ISABU). Rukoba is located in Dry Central Plateau in Mumirwa Region at latitude 3° 30'S and longitude 29°57'E. Its altitude is between 1500 and 1260 masl with mean total annual rainfall of about 1200-1300mm received in two rainy seasons, long rains (March – June) and short rains (October – December). The average annual temperature is 17-19 °C, while the major soil types are the Acrisols.

3.1.2 Plant materials

The genotypes in this trial comprised of nineteen accessions and one commercial cultivar. The accessions were Mi49RS1, Mi49RS2, Mi49RS3, J2RS1, J2RS2, J2RS3, J2RS4, J2RS5, J2RS6, S795ET55, S795ET49, Mik8914ET1, Mik8914ET2, B71ET55, B71ET19, B71RS, SL28RS, B139K9006ET49, and B139K9006ET55, they were selected for their resistance to coffee diseases, especially anthracose and rust during a preliminary evaluation carried out at Rukoba. One commercial cultivar, J2(T) was used as a check. The name and source of accessions are noted in table 3.1.

Table 3.1: Names and sources of Arabica coffee cultivars evaluated in this study.

No	Accessions name	Code	source	Status
1	Mi49RS1	M1	Rwanda	Germplasm accession
2	Mi49RS2	M2	Rwanda	Germplasm accession
3	Mi49RS3	M3	Rwanda	Germplasm accession
4	J2RS1	J1	Sudan	Germplasm accession
5	J2RS2	J2	Sudan	Germplasm accession
6	J2RS3	J3	Sudan	Germplasm accession
7	J2RS4	J4	Sudan	Germplasm accession
8	J2RS5	J5	Sudan	Germplasm accession
9	J2RS6	J6	Rwanda	Germplasm accession
10	S795ET55	S1	Cameroon	Germplasm accession
11	S795ET49	S2	Cameroon	Germplasm accession
12	MiK8914ET49	MK1	India	Germplasm accession
13	MiK8914ET55	MK2	India	Germplasm accession
14	B71ET55	B1	Cameroon	Germplasm accession
15	B71ET19	B2	Cameroon	Germplasm accession
16	B139K9006ET49	B4	Colombia	Germplasm accession
17	B139K9006ET55	B5	Colombia	Germplasm accession
18	B71RS	B3	Rwanda	Germplasm accession
19	SR28RS	SL1	Sudan	Germplasm accession
20	J2 (T)	J2(T)	Rwanda	Commercial variety

3.1.3 Experimental design and field management

The experiment consisted of twenty Arabica coffee cultivars planted out in Randomized Complete Block Design (RCBD) with three replications. The experimental plots comprised of ten coffee trees planted in one row for each accession at a spacing of 2 m by 2 m between rows and trees respectively. All field management practices, such as weeding and spraying for pest control were applied as per the recommendations of the center (ISABU, 2018). The experimental plot was surrounded by one row of commercial coffee cultivar border trees to provide competition to peripheral plots and at the same time to protect the trees from wind and wildlife.

3.1.4 Data collection

3.1.4.1 Qualitative data

Qualitative data were collected from each coffee accession at different stages of crop growth as shown in Table 3.2 Overall plant architecture data including plant habit, overall appearance, branching habit, angle of lateral insertion, and young leaf color data were scored in February 2020.

Table 3.2: Qualitative morphological traits studied and their descriptions

N^0	Qualitative trait	Categories used for data collection
1	Young leaf color	1: Greenish, 2: Green, 3: Brownish, 4: Reddish brown, 5 : Bronze.
2	Leaf shape	1 : Obovate, 2 : Ovate, 3 : Elliptic, 4 : Lanceolate
3	Leaf apex shape	1 : Round, 2 : Obtuse, 3 : Acute, 4 : Acuminate, 5 :Apiculate, 6 : Spatulate.
4	Fruit colour	1 : Yellow, 2 : Yellow-orange, 3 : Orange, 4 : Orangered, 5 : Red, 6 : Red-purple, 7 : Purple, 8 : Purpleviolet ,9 : Violet, 10 : Black.
5	Fruit shape	1 : Roundish, 2 : Obovate, 3 : Ovate, 4 : Elliptic, 5 :Oblong
6	Seed shape	1 : Round, 2 : Obovate, 3 : Ovate, 4 : Elliptic, 5 : Oblong
7	Angle of insertion of primary branches	1 : Drooping, 2 : Horizontal or spreading, 3 : Semi-erect .
8	Plant height	1 :very short 3 : Short 4 : Tall 9 : Very tall

9	Branching habit	1 : Very few branches (primary) 2 : Many branches (primary) with few secondary branches 3: Many branches (primary) with many secondary branches 4 : Many branches (primary) with many secondary and tertiary branches
10	Overall appearance	1 : Elongated conical 2 : Pyramidal 3 : Bushy
11	Disease reaction (Rust)	1: absent 2: present

Source: IPGRI, 1996

3.1.4.2 Quantitative data

Quantitative data were collected from four randomly selected coffee trees of each accession using the coffee descriptors presented in Table 3.3 (IPGRI, 1996). The traits considered comprised plant height (m), percentage of fruit bearing nodes (%) among primary branches, stem girth (cm), number of primary branches, length of primary branches (cm), number of internodes, internode length of primary branches (cm), Number of cherries per internode, leaf length(cm), leaf width(cm), weight of 100 fruits (g), fruit length (mm), width (mm) and thickness(mm), bean length (mm), width (mm), and thickness (mm).

Leaf data were scored in November 2019 whereas berry data were measured in April 2020. For uniformity and consistency, all leaf measurements were made on five leaves selected from the third or fourth node from the apex of the five randomly selected primary branches in the middle portion of coffee tree. Similarly, berry measurements were made on five randomly selected fully matured berries from the third or fourth node from the apex of the five randomly selected primary branches in the middle portion of coffee tree.

Table 3.3: List of quantitative traits analyzed and the method of evaluation in this study.

Quantitative traits	Abbreviation	Method of evaluation
Growth traits		
Plant height (m)	PH	The length from the ground level to the top of the tree canopy
Stem girth (cm)	GIRTH	Diameter of main stem at 10 cm from the ground
Number of primary branches	NPB	Total number of primary branches counted per tree
Percent of fruit bearing primary branches per tree	PFBPB	Percent fruit bearing primary branches obtained by the ratio between the number primary branches produced and the number of fruiting branches per tree
Length of primary branches(cm)	LPB	Average of five primary branches at the middle of the stem, measured from point of attachment to main stem to apex of branch
Internode length on primary branches (cm)	ILB	Average of five primary branches at the middle of the stem per tree, calculated as length divided by the number of nodes
Number of internodes per branch	NIB	Average of five primary branches nodes counted per tree
Number of fruit per internode of primary branches	NCB	Number of cherries per internode on five primary branches ,obtained by the ration between the total number of cherries bearing node of primary branch and the number of node bearing per each primary branch selected
Leaf traits		
Leaf length (cm)	LL	Average of five mature (> node 3 from the terminal bud) leaves, measured from petiole end to apex
Leaf width (cm)	LW	Average of five mature (> node 3 from the terminal bud) leaves, measured at the widest part
Fruit and bean traits		
Fruit length (mm)	FL	Average of five normal and mature green fruits of each tree measured at the longest part
Fruit width (mm)	FW	Average of five normal and mature green fruits of each tree measured at the widest part
Fruit thickness	FT	Average of five normal Fruits of each tree measured at the thickest part
100 fruits weight (gm)	100CW	Hundreds of normal cherries of each coffee tree weight using sensitive balance

Bean length (mm)	BL	Average of five normal beans of each tree measured at the longest part
Bean width (mm)	BW	Average of five normal beans of each tree measured at the widest part
Bean thickness	BT	Average of five normal beans of each tree measured at the thickest part

Source: IPGRI, 1996

3.1.5 Data analysis

Qualitative data were analyzed using the Statistical Package for Social Scientists (SPSS) software to determine the mean and frequencies of each parameter.

The frequency distribution and the number of phenotypic classes were used to compute the Shannon-Weaver Diversity Index (H') for each qualitative trait as per the formula described by (Zeven, 1991)

Where, pi is the proportion of total number of individuals (genotypes) in the i^{th} class and n is the number of phenotypic classes. The Shannon-Weaver Diversity Index (H) can range from 0 to 1. A value near 0 indicates that every individual belong to one and the same class. Conversely, a value 1 indicates maximum diversity i.e. the numbers of individuals are evenly distributed among the n class. H' was estimated for each character. Each value of H' was divided by its maximum value, log (n), in order to keep the values of H' in the range of 0-1.

The quantitative data were assessed using GenStat (2012) Discovery Edition 3.0 Statistical Software (VSNI, 2012). Analysis of variance (ANOVA) was computed using the general linear model in GenStat, assuming accession effects was random. Means for each morphological traits studied were separated by the least significant difference (LSD) at P = 0.05 as recommended by (Gomez et al , 1984) .

Phenotypic correlation coefficients were calculated to examine the degree of association between the traits. Cluster analysis was used to determine phenotypic modification among the cultivars. A dendrogram was constructed from the Eucledian distance matrix using an agglomerative, hierarchical cluster classification technique with average linkage strategy.

Principal component analysis of the designed accessions was performed according to (Chahal *et al*., 2002). The PCA was used to aggregate cultivars with similar morphological traits into high internal similarity and high external heterogeneity. The

pair wise comparison of morphological characters was done to arise a multi-dimensional scatter plot of individuals. Genetic distances for agro morphological traits was assessed using Euclidean straight line method (Mohammadi *et al*., 2003).

Also, genetic distance based on phenotypic traits for all pair-wise comparisons of 20 coffee cultivars was determined according to the average intra cluster distance as indicated by Singh *et al* .(1985) in the following formula:

$$D^2 = D^2 = \sum \frac{D2\ i}{N}$$

Where, D^2 i = Sum of distances between all possible combinations of genotypes included in a cluster, and N = All possible combinations.

3.2 Assessment of the effect of locations on agro-morphological diversity among cultivated Arabica coffee cultivars in Burundi

3.2.1 Experimental sites.

The trial was conducted in three research stations of the Institut des Sciences Agronomiques du Burundi (ISABU), namely Kayanza, Rukoba and Nyange**.**

Kayanza research station is located in the humid Central Plateau, in Mugamba region at latitude 3°4'S and longitude 29°40'E. Elevations range from 1500 - 1850 m with mean total rainfall of 1300-1400 mm, average annual temperature of 15-17 °C and the major soil type is the Ferrisol.

Rukoba is located in the dry Central Plateau, in Mumirwa region at latitude 3°30'S and longitude 29°57'E. Elevations range from 1500 and 1650 m with mean total rainfall of 1200-1300mm, average annual temperatures of 17-19 °C and the major soil type is the Acrisol

Nyange is in the Eastern depression, in Imbo region at latitude 4°10'S and longitude 29°45'E. Its altitude varies from 1125m to 1400 m with total rainfall of 1200-1300mm, while average annual temperatures are 19-23 °C and the major soil type is the ferrisols.

3.2.2 Plant materials

The genotypes in this trial comprised of 10 introductions and 5 commercial cultivars. The introductions were Mysore, ABK5691, ABK5718, Blue Mountain, Tekisic, K7, SL28, S288, S795 and Mibirizi Bouts bruns. The five commercial cultivars were Bourbon 139, Burbon 71, Mibirizi 49, Mibirizi 68, and Jackson2. The names and sources of these genotypes are noted in Table 3.4.

Table 3.4: Names and sources of Coffee Arabica accessions evaluated in this study.

No	Accession name	Abbreviated	Code	Source	Status
1	Jackson 2/1257	J2/1257	J1	ISAR Rwanda	Commercial variety
2	Bourbon Mayaguez 71	B 71	B1	ISAR Rwanda	Commercial variety
3	Bourbon Mayaguez 139	B 139	B2	ISAR Rwanda	Commercial variety
4	Mibirizi bouts bruns	Mi T V	B4	ISAR Rwanda	Germplasm accession
5	Mibirizi 49/1848	Mi 49/1848	M2	ISAR Rwanda	Commercial variety
6	Mibirizi 68/1589	Mi 68/1589	M1	ISAR Rwanda	Commercial variety
7	Mysore	Mysore	M3	ISAR Rwanda	Germplasm accession
8	Ainamba-Babaca-Kaffa-ABK 5691	ABK 5691	A1	Abyssinie via Rubona (Rwanda)	Germplasm accession
9	Ainamba-Babaca-Kaffa-ABK 5718	ABK 5718	A2	Abyssinie via Rubona (Rwanda)	Germplasm accession
10	Blue Mountain	Blue Mountain	B3	Kenya	Germplasm accession
11	Tekisic	Tekisic	T	San Salvador	Germplasm accession
12	K7	K7	K	Kenya	Germplasm accession
13	SL 28	SL 28	S3	Kenya	Germplasm accession
14	S 288	S 288	S2	India	Germplasm

					accession
15	S 795	S 795	S1	India	Germplasm accession

3.2.3 Experimental design and field management

The experimental treatments consisted of fifteen Arabica coffee genotypes planted out in Randomized Complete Block Design (RCBD) with three replications at each site. Each plot comprised of ten coffee trees planted in one row for each accession. The spacing between rows and trees within the rows were 2 m by 2 m. The experiments were surrounded by one row of border Burbon 71 coffee trees to provide competition to peripheral plots and at the same time to protect the trees from wind and wild life at each site. Fertilizer application and crop management were applied at all the sites as per the recommended practices (ISABU, 2018).

3.2.4 Data collection

The data were collected in the trial from September 2019 to Jun 2020. They were collected from 3 coffee trees selected randomly from each accession at each location. Such traits concern the tree leaves (length (cm), width (cm) and leaf area (cm2), fruits (100 of fruits weight (g), length (mm), width (mm), beans (100 beans weight (g), length (mm), width (mm), and thickness (mm) as shown in Table 3.5. Leaf data were scored in November 2019 whereas berry data were measured in April 2020. For uniformity and consistency, all leaf measurements were made on five leaves selected from the third or fourth node from the apex of the five randomly selected primary branches in the middle portion of coffee tree. Similarly, berry measurements were made on five randomly selected fully matured berries from the third or fourth node from the apex of the five randomly selected primary branches in the middle portion of coffee tree.

Table 3.5: List of quantitative traits analyzed in 15 Arabica coffee genotypes and method of evaluation.

Quantitative traits	Code	Method of evaluation
Leaf traits		
Leaf length (cm)	LL	Average of five mature (> node 3 from the terminal bud) leaves, measured from petiole end to apex
Leaf width (cm)	LW	Average of five mature (> node 3 from the terminal bud) leaves, measured at the widest part
Leaf area (cm2)	LA	Average of five leaves Calculated as =(length*Width) *0.88 (Walyaro,1983)
Fruit length (mm)	FL	Average of five normal and mature green fruits of each tree measured at the longest part
Fruit width (mm)	FW	Average of five normal and mature green fruits of each tree measured at the widest part
100 fruits weight (gm)	100CW	Hundreds of normal cherries of each coffee tree weighed using a sensitive balance
Bean length (mm)	BL	Average of five normal beans of each tree measured at the longest part
Bean width (mm)	BW	Average of five normal beans of each tree measured at the widest part
Bean thickness (mm)	BT	Average of five normal beans of each tree measured at the thickest part
100 bean weight (gm)	100BW	At 11% moisture (gm) –calculated as: ("bean weight at 0% moisture content" X 100)/ (Bean No X 0.89). Oven was used for drying of beans to obtain 0% moisture and weight recorded using sensitive balance

Source: IPGRI, 1996

3.2.5 Data analysis

Analysis of variance for all traits was conducted using Statistical Analysis System (SAS, 2008) software. The statistical model used for individual location variation was:

$Yij = \mu + Gi + Rj + \varepsilon ij$, Where Yij is the plot value of each trait, μ is the trial mean of a given trait, Gi, is the effect of genotypes, Rj is the effect of replications, and εij is the plot error.

Analysis of variance for across locations/environments was conducted using the statistical model $Yijk = \mu + Ek + R(E)k(j) + Gi + GEik + \varepsilon ijk$, Where Ek, R(E)k(j) and

GEik are the effects of locations/environments, the effect of replications nested within locations/environments and genotype-environment interactions, respectively, μ is the trial mean of a given trait, Gi, is the effect of genotypes, Rj is the effect of replications.

To reveal significant differences in the analyzed traits means among genotypes, a least significant difference, LSD ($P \leq 0.05$) was applied. The restricted maximum likelihood (REML) method of SAS PROC MIXED was used to approximate the components for each trait. The pair wise comparison of quantitative traits was done to derive a multi-dimensional scatter plot of each genotype.

A multivariate extension of the mixed model (Holland, 2006) was used to estimate phenotypic correlations for each pair of agro-morphological traits through environments and path coefficient analysis was conducted according to (Holland, 2006). Cluster analysis was conducted to determine phenotypic relationships between the genotype using the restricted maximum likelihood (REML) method of SAS PROC MIXED. Principal component analysis of the assessed genotypes was performed according to (Chahal *et al.*, 2002).

The stability analysis was done over three environments using Joint linear regression analysis (JRA) called heterogeneity of regression (Eberhart & Russel, 1966) was used to obtain the linear and non-linear component of GxE interaction variance.

The linear regression model is

$$Y\ddot{U} = \mu + B_{ij} + \delta_{ij},$$

Where:

$Y\ddot{U}$ = is the mean of the i th genotype at the j th environment

u, = the mean of the i th genotype overall environments

Bj= the regression coefficient that measures the response of i^{th} genotype to varying environment

δij = the deviation from regression of the i^{th} variety of j^{th} environment and

Ij = the environmental index obtained as the mean of all genotypes at the

J^{th} environment minus the grand mean

The regression coefficient (B_j) was estimated as:

$$Bj = \frac{\sum yi\,j}{\sum I2I_j} + \delta\, i\, j$$

and the deviation from regression (S^2 di) is obtained as:

$$S^2\, d_i = \frac{\sum \delta\, i\, j}{n-2} - \frac{s^2_e}{r} \ ,$$

where

$\frac{s^2_e}{r}$ is the pooled error

Heritability on plot-basis for each agro- morphological trait and on entry-mean basis for each quality trait with their approximate standard error across environments were estimated using SAS Proc MIXED model of SAS after (Holland *et al*., 2003). Analyses at each site/environment confound the genetic modification estimation with the GEI variance, so an assessment pooling the data from both sites/environments and including the site/environment as a fixed effect was required to produce unbiased results (Holland *et al*., 2003). The pooled-sites heritability estimate was comparable to the average heritability estimate of each site, signifying that GEI was minimal. Heritability on a plot basis estimated as: $h^2_{bs} = (\sigma^2_g)/ [\sigma^2_g + \sigma^2_{ge} + \sigma^2_e/re]$ using SAS Proc MIXED model of SAS after (Holland *et al*., 2003), where σ^2_g is the estimate of genotypic variance, σ^2_{ge} is

the estimate of environment x genotype variance, σ^2_e is the estimate of error variance, r is the number of replications per environment and e is the number of environments. Quantitative data were being balanced in current study for which the REML based modification component estimates were similar with ANOVA (Shaw, 1987), for this reason the genotypic mean, phenotypic and genetic modifications from REML analysis were used to estimate genotypic and phenotypic coefficient of variation using the formula given by (Burton ,1952). The magnitude of the G x E interaction relative to the genetic modification was determined from REML variance component estimates of each trait using the ratio σ^2_{ge}/σ^2_g. Predictable genetic advance for each quantitative characters at 5% selection intensity was calculated using the methodology described by Burton. (1952). Where: GA = K * δP * H^2b Where: GA=the expected genetic improvement under selection; σ_p = the phenotypic standard deviation; H^2_b = heritability in broad sense and k is selection intensity.

CHAPTER FOUR.

RESULTS AND DISCUSSION

4.1 Determination of diversity among Arabica coffee cultivars in Burundi using agro- morphological characters.

4.1.1 Monthly rainfall, relative humidity and temperature data recorded during theexperimental period at Rukoba site.

The rainfall, temperature and relative humidity data recorded during the period of the current study are presented in Table 4.1. The data showed that, the highest and the lowest monthly mean temperatures were recorded in June (20.78°C) and November (18.79°C) respectively. The maximum monthly rainfall (285 mm) during the study period was recorded in December and the minimum monthly rainfall (0 mm) in June. The highest monthly relative humidity (89.83%) was recorded in November while the lowest relative humidity (64.21%) was recorded in October (Table 4.1).

Table 4.1: Total rainfall and mean monthly temperature and relative humidity recorded at Rukoba, Burundi during the experimental period in 2019/2020

Months	Monthly rainfall (mm)	Mean temperature (°C)	Relative humidity (%)
Oct-19	109.12	19.79	64.25
Nov-19	144.35	18.79	89.83
Dec-19	285.47	19.13	89.24
Jan-20	184.30	19.52	88.36
Feb-20	170.60	19.42	82.75
Mar-20	199.20	19.69	83.4
Apr-20	272.13	19.66	89
May-20	66.78	20.20	85.3

Jun-20	0.0	20.78	73.21

4.1.2 Variation in Qualitative Traits

Frequencies for the ten qualitative traits evaluated in the 20 Arabica coffee accessions are shown in Table 4.3. Overall, the frequency distributions of the ten qualitative traits presented a varied range of dissimilarity, although, some accessions differed from the main characteristics (Table 4.3). In terms of the angle of lateral insertion, most of the accessions (55%) were horizontal or spreading type, and the rest were semi-erect (45%) types. Likewise, greatest of the Arabica coffee accessions (60%) characterized were pyramidal, the rest are elongated conical (40%) in overall appearance, and no bushy types were distinguished (Table 4. 2 & Table 4.3).

Majority of the accessions (60%) had many primary branches with many secondary branches. For branching habit and (40%) of the accessions had many primary branches with few secondary branches. The accessions show variability in young leaf color, with green being the chief (45%) color (Table 4. 3).

The other young leaf colors that were detected in the Arabica coffee accessions assessed were bronze (35%), greenish (10 %), and brown green (10%). For plant height, majority of the genotypes (95%) were tall, only (5%) of the genotypes were short. For the rest of qualitative traits evaluated, there were minimal variations among all accessions in most of qualitative characters such us leaf shape, leaf apex shape, fruit color, fruit shape, beans shape. The leaf shape and fruit shape and bean shape from all accessions were elliptic, the leaf apex shape of all accessions were apiculate, the genotypes did not showed variability in fruit color, with light red being the dominant (100%) color in all accessions (Table 4. 2 & Table 4.3).

As a result, five of the ten morphological traits had moderate diversity indices ranging between 0.75–0.88 (Table 4.9). These were leaf apex shape (0.75), young leaf color

(0.77), fruit shape (0.85), disease reaction (0.80) and Bean shape (0.88). The other traits showed high phenotypic diversity index (H') (>0 .88). The angle of insertion of primary branches had the maximum calculated diversity index of 0.95 followed by branching habit (0.91), plant height (0.92), overall appearance and leaf shape (0.90) (Table 4.3 see Appendix 5)

Table 4.2: Variation of qualitative characters in the twenty cultivars

Accession name	Young leaf colour	Leaf shape	Leaf apex shape	Fruit colour	Fruit shape	Seed shape	Angle of insertion of primary branches	Branching habit	Plant height	Overall appearance	Disease reaction
MiK8914ET49	Green	Elliptic	Apiculate	Light red	Elliptic	Elliptic	Semi-erect	Many Primary branches with Few secondary branches	Tall	Conical	Absent
B71ET19	Green	Elliptic	Apiculate	Light red	Elliptic	Elliptic	Semi-erect	Many Primary branches with Few secondary branches	Tall	Conical	Absent
J2RS6	Green	Elliptic	Apiculate	Light red	Elliptic	Elliptic	Semi-erect	Many Primary branches with Few secondary branches	Tall	Conical	Absent
J2RS4	Green	Elliptic	Apiculate	Light red	Elliptic	Elliptic	Semi-erect	Many Primary branches with Few secondary branches	Tall	Pyramidal	Absent
MI49RS3	Green	Elliptic	Apiculate	Light red	Elliptic	Elliptic	Semi-erect	Many Primary branches with Few secondary branches	Tall	Conical	Absent
SL28RS	Green	Elliptic	Apiculate	Light red	Elliptic	Elliptic	Semi-erect	Many Primary branches with many secondary branches	Tall	Conical	Absent
J2RS3	Bronze	Elliptic	Apiculate	Light red	Elliptic	Elliptic	Horizontal	Many Primary branches with Few secondary branches	Tall	Pyramidal	Absent
S795ET55	Bronze	Elliptic	Apiculate	Light red	Elliptic	Elliptic	Horizontal	Many Primary branches with Few secondary branches	Tall	Pyramidal	Absent
M49RS2	Green	Elliptic	Apiculate	Light red	Elliptic	Elliptic	Semi-erect	Many Primary branches with Few secondary branches	Tall	Conical	Absent
Mik8914ET55	Bronze	Elliptic	Apiculate	Light red	Elliptic	Elliptic	Horizontal	Many Primary branches with many secondary branches	Tall	Pyramidal	Absent
B71RS	Bronze	Elliptic	Apiculate	Light red	Elliptic	Elliptic	Horizontal	Many Primary branches with many secondary branches	Tall	Pyramidal	Absent
J2(T)	Bronze	Elliptic	Apiculate	Light red	Elliptic	Elliptic	Horizontal	Many Primary branches with many secondary branches	Tall	Pyramidal	present
J2RS1	Greenish	Elliptic	Apiculate	Light red	Elliptic	Elliptic	Semi-erect	Many Primary branches with many secondary branches	Tall	Conical	Absent
S795ET49	Greenish	Elliptic	Apiculate	Light red	Roundish	Round	Horizontal	Many Primary branches with many secondary branches	Tall	Pyramidal	Absent
B71ET55	Brown green	Elliptic	Apiculate	Light red	Elliptic	Elliptic	Horizontal	Many Primary branches with Few secondary branches	Tall	Pyramidal	Absent
J2RS5	Brown green	Elliptic	Apiculate	Light red	Elliptic	Elliptic	Horizontal	Many Primary branches with Few secondary branches	Tall	Pyramidal	Absent
M49RS1	Bronze	Elliptic	Apiculate	Light red	Elliptic	Elliptic	Horizontal	Many Primary branches with Few secondary branches	Tall	Pyramidal	Absent
J2RS2	Bronze	Elliptic	Apiculate	Light red	Elliptic	Elliptic	Semi-erect	Many Primary branches with Few secondary branches	Tall	Conical	Absent
B139K9006ET49	Green	Elliptic	Apiculate	Light red	Elliptic	Elliptic	Horizontal	Many Primary branches with many secondary branches	Tall	Pyramidal	Absent

B139K900 6ET55	Green	Elliptic	Apiculate	Light red	Elliptic	Elliptic	Horizontal	Many Primary branches with many secondary branches	Short	Pyramidal	present

Table 4.3: Frequency distribution and Shannon-Weaver diversity indices (H') of eleven qualitative traits of coffee accessions in Rukoba, Burundi..

no	Quantitative Traits	Description	Frequency (%)	H' Value
1	young leaf color	1 Greenish	10	0.77
		2 Green	45	
		3 Bronze	35	
		4 Brown green	10	
2	leaf shape	1 Elliptic	100	0.9
3	leaf apex shape	1 Apiculate	100	0.75
4	fruit color	l 1ight red	100	0.62
5	fruit shape	1 Elliptic	100	0.85
6	Bean shape	1 Elliptic	100	0.88
7	Angle of insertion of Primary branches	1 horizontal or spreading	55	0.95
		2 semi-erect	45	
8	Branching habit	1 many primary branches with few secondary branches	60	0.91
		2 many primary branches with many secondary branches	40	
9	plant height	1 Short	5	0.92
		2 Tall	95	
10	Overall appearance	1 Elongated conical	40	0.9
		2 pyramidal	60	
11	Disease reaction (Rust)	1 Absent	90	0.8
		2 Present	10	

4.1.3 Variation in quantitative traits

Analysis of variance (ANOVA) for the 17 quantitative traits showed that variations among the accessions were highly significant ($P < 0.05$) for most of the traits except for

percent fruit bearing primary branches, fruit thickness and bean thickness which did not show any significant variation (Table 4.4).

Table 4.4: Mean values of 17 quantitative characters measured in the 20 coffee accessions evaluated at RUKOBA in 2019-2020.

Accession name	CODE	GIRTH	PH	NPB	PFBPB	LPB	ILB	NIB	NCB	LL	LW	F L	FW	FT	100CW	BL	BW	BT
B71ET55	B 1	3.4	3.3	61	66.8	55	2.89	18	5.2	12.6	4.7	15.4	12.7	11	161	12.5	8	4.5
B71ET19	B 2	3.9	3.1	59	63.5	48.6	3.04	15	4.5	12.2	4.6	16	13.3	11.5	161	12.8	8.3	4.8
B71RS	B 3	4.2	3.7	76	65.8	58	2.64	21	4.8	11.8	4.5	16.1	13.1	11.7	177	12.7	8	4.6
B139K9006ET49	B 4	4	2.5	69	57.8	50.4	2.52	19	9.2	13.4	5.2	15.3	12.5	10.6	175	12.1	7.9	4.6
B139K9006ET55	B 5	3.1	2.2	53	61.5	47.5	2.50	18	5.3	12.7	5	14.7	12.6	11.5	171	12.2	8	4.8
J2RS1	J 1	2.8	2.8	40	55.5	45.3	2.66	16	2.6	11.6	4.7	14.7	12.9	11.3	160	13.6	8.1	4.7
J2RS2	J 2	4	3.1	53	61.3	53.6	2.98	17	5	13.1	5.5	16	12	10.8	160	12.3	7.5	4.3
J2(T)	J 2(T)	3.9	3.1	61	56.8	55.3	2.91	18	2.9	11.5	5	15.6	12.7	11	183	12.5	8.2	4.7
J2RS3	J 3	4.1	3.6	68	69.4	55.2	2.76	19	4.3	11.9	4.7	15.9	13.4	11.8	175	12.6	8	4.6
J2RS4	J 4	3.8	3.2	60	64.8	43.2	2.27	18	3.4	13.1	5.3	16.2	12.8	11.2	146	12.6	7.6	4.6
J2RS5	J 5	4.1	3.3	52	63.5	55	3.06	17	5.5	11.9	4.8	15.6	12.8	11.3	167	12.4	7.9	4.6
J2RS6	J 6	3.2	3.2	60	58.3	43.8	2.58	16	4.2	12.2	5.7	15.3	12.3	10.5	162	12.6	7.7	4.6
MI49RS1	M 1	3.7	3.1	51	66	55.8	2.94	18	5.3	12.2	5	16.3	12.7	11.1	167	12.9	8	4.7
MI49RS2	M 2	4.6	3.5	78	79.5	47.2	2.48	18	4	12.7	4.9	16.7	13	11.3	158	13.1	7.9	4.9
MI49RS3	M 3	3.6	3.3	54	64.8	46.4	2.90	15	5	11.7	5	16.2	11.9	10.1	151	13.2	7.4	4.4
MIK8914ET49	MK1	3.3	2.8	52	87	44.5	2.47	17	3.4	11.5	5.1	15.3	12.6	10.8	157	12.3	7.6	4.6
MIK8914ET55	MK2	4.9	3.5	78	84	52.8	2.51	20	5.4	12.7	4.9	15.3	12.5	11.1	163	12.3	7.7	4.3
S795ET55	S 1	4.5	3.4	71	75	55.7	2.65	20	6.3	12	4.7	15.8	12.9	11.3	158	12.2	7.7	4.6
S795ET49	S 2	4.9	3.1	76	91.6	69.6	2.58	26	9.9	14	5.7	15.6	12.9	11.4	180	12.6	8.9	5.6
SL28RS	SL1	4.5	3.5	73	72.3	47.9	3.68	12	4.6	12.4	5.3	16.2	12.9	11.2	168	13.2	7.6	4.5
Mean		3.9	3.2	62	68.26	51.5	2.71	18	5	12.4	5	15.7	12.7	11.1	165	12.6	7.9	4.7
LSD (0.05)		0.24	0.14	6.27	ns	6.02	6.69	2.46	0.9	0.55	0.29	0.44	0.36	ns	5.06	0.37	0.24	ns

CV (%)	**10**	**7.1**	**16**	**23.9**	**18.8**	**23.2**	**22**	**27.9**	**7.1**	**9.2**	**4.5**	**4.6**	**4.8**	**4.9**	**4.8**	**4.9**	**6.8**

PH=Plant height in m, GIRTH=Stem girth in cm, NPB=Number of primary branches, PFBPB=percent fruit bearing primary branches per three ,LPB= length of primary branches in cm, ILB=Internode length of primary branches in cm, NIB=Number of internodes per branch ,NCB=Number of fruit per internode of primary branches ,LL=Leaf length in cm, LW=Leaf width in cm, FL=Fruit length in mm, FW=Fruit width in mm, FT=Fruit thickness in mm,100CW=Hundred cherries weight, BL=Bean length in mm, BW=Bean width in mm, BT=Bean thickness in mm

As shown in (Table 4. 4), the length of primary branches ranged from 43.2 to 69.6cm, averaging 51.5cm. Over half of the accessions (55%) had the length between 50 and 69.6cm. The internode length of primary branches averaged 2.71cm and ranged from 2.27 to 3.68 cm. Most of the accessions (85%) had 2.50 to 3.68 cm length of primary branches. The number of internode of primary branches averaged 18 and ranged from 12 to 26. A large proportion of the accessions (60%) had 18 to 26 number of internodes of primary branches. Leaf length ranged from 11.5 to 14 cm, averaging 12.4 cm. over the half of accessions (65%) had 12 to 14 cm. Leaf width ranged from 4.5 to 5.7 cm, and averaging 5 cm. over the half of accessions (55%) had 5 to 5. 7 cm. Fruit length ranged from 14.7 to 16.7mm, averaging 15.7mm. Majority of accessions (90%) had 15 to 16.7 mm fruit length. Most of the accessions (85%) had 12.5 to 13.4mm fruit width, averaged 12.7mm and ranged from 11.9 to 13.4mm fruit width. Fruit thickness averaged 11.1 mm, ranging from 10.1 to 11.7mm. Maximum of the accessions (75%) had 11 to 11.7 mm.

Weight of a hundred normal cherries ranged from 146 to 183.3g, averaging 164.8g. Most of accessions (70%) had 160 to 183.3 g. Stem girth ranged from 2.8 to 4.9 cm, averaging 3.9cm. over half of accessions (70%) had 3.25 to 4.9cm. Plant height averaged 3.2 m ranged from 2.2 to 3.6m. Most of the accessions (80%) had 3 to 3.6m plant height. Number of primary branches averaged 62, ranged from 40 to 78 number of primary branches. Majority of the accessions (95%) had 50 to 78 primary branches. Percentage of fruit bearing primary branches ranged from 55.5 to 91.6, averaging 68.26. Most of the accessions (80%) had 61.3 to 91.6 percentage of fruit bearing primary branches. Number of cherries per internodes averaged 5, ranging from 2.9 to 9.9 cherries per internode. Half of the accessions (50%) produced 5 to 9.9 cherries per internode. Beans length ranged from 12.1 to 13.6 mm and averaged 12.6 mm. A large proportion of the accessions (65%) had 12.5 to 13.6 mm. Beans width ranged from 7.4 to 8. 9 mm and averaged 7.9 mm. half of the accessions (50%) had 8 to 8.9 mm. Beans thickness averaged 4.7 mm, ranging from 4.3 to 5.6 mm. Maximum of the accessions (85%) had 4.5 to 5.6 mm (Table 4. 4).

4.1.4 Correlation among morphological traits

A highly significant ($p < 0.01$) positive correlation was noted between the following pairs of parameters (Table 4.5), stem girth and number of internodes per primary branch, stem girth and branch length, plant height and number of primary branches, plant height and fruit length. number of primary branches and number of internodes per branch, leaf length and leaf width, plant high and stem girth, internode length per branch and branch length. number of internodes per branch and internode length per branch, stem girth and number of primary branches. A highly negative ($p < 0.01$) correlation was recorded between bean length and internode length (Table 4.5).

Table 4.5: Correlations among 17 quantitative traits in 20 coffee accessions evaluated at Rukoba in 2019-2020.

Traits	GIRTH	PH	NPB	PFBPB	100CW	LL	LW	NCB	FL	FW	FT	NIB	ILB	LPB	SL	SW	ST
GIRTH	1																
PH	.429**	1															
NPB	.645**	.462**	1														
PFBPB	.590**	.163**	.666**	1													
100CW	.181**	-.088	.209**	.289**	1												
LL	.231**	-.120*	.182**	.320**	.050	1											
LW	.059	-.153**	-.036	-.057	.032	.523**	1										
NCB	.353**	-.179**	.193**	.581**	.211**	.346**	.127*	1									
FL	.249**	.331**	.176**	.106*	-.053	.057	.057	-.045	1								
FW	.123*	.134**	.155**	.098	.170**	-.047	-.119*	-.065	.122*	1							
FT	.140**	.087	.132**	.107*	.255**	-.021	-.142**	-.032	.075	.548**	1						
NIB	.329**	.097	.344**	.358**	.237**	.151**	.057	.280**	-.055	.035	.139**	1					
ILB	.261**	.114*	.175**	.240**	.219**	.138**	.097	.307**	-.061	.049	.120*	.817**	1				
LPB	.351**	.148**	.219**	.275**	.280**	.155**	.081	.277**	.034	.076	.103*	.376**	.440**	1			
SL	.001	.081	-.098	-.142**	-.123*	-.069	.006	-.197**	.186**	-.011	-.012	-.144**	-.152**	-.078	1		
SW	.170**	-.131**	.102*	.202**	.311**	.164**	.066	.189**	-.062	.242**	.237**	.211**	.243**	.257**	.123*	1	
ST	.147**	-.151**	.142**	.200**	.271**	.164**	.163**	.209**	-.039	.146**	.129*	.201**	.212**	.234**	.073	.453**	**1**

****. Correlation is significant at the 0.01 level (2-tailed).**

***. Correlation is significant at the 0.05 level (2-tailed).**

PH=Plant height in m, GIRTH=Stem girth in cm, NPB=Number of primary branches, PFBPB=percent fruit bearing primary branches per three ,LPB=Branch length in cm, ILB=Internode length of primary branches in cm, NIB=Number of internodes per branch ,NCB=Number of fruit per internode of primary branches ,LL=Leaf length in cm, LW=Leaf width in cm, FL=Fruit length in mm, FW=Fruit width in mm, FT=Fruit thickness in mm,100CW=Hundred cherries weight, BL=Bean length in mm, BW=Bean width in mm, BT=Bean thickness in mm.

4.1.5 Cluster Analysis

The 20 coffee germplasm accessions were classified into four distinct groups (Table 4.6, figure 4.1). Cluster II had nine accessions (45% of the total population). followed by cluster IV with eight accessions (40%). Clusters III had two accessions (10%), while I had one accession (5%) (Table 4. 6, Figure 4.1).

The cluster II includes accessions MIK8914ET49, J2RS6, J2RS4, J2RS2, J2RS1, MI49RS2, B71ET19, SL28RS and MI49RS3. These accessions contributed to the lowest mean value of fruit width number of cherries per internode, beans length, percent fruit bearing primary branches, hundred fresh cherries weight, number of primary branches, length of primary branches, internodes length of primary branches, moderate stem girth, plant high, number of internodes per branch, beans width, and high leaf length, fruit length, leaf width (Table 4.4).

Cluster I includes one accession S 795ET49 and it was characterized by the very high mean value of number of cherries per internode of primary branches, percent fruit bearing primary branches, number of internodes per branch, internode length per branch, length of primary branches. the high mean value of stem girth, leaf length, leaf width, hundred cherries weight, beans width. Moderate mean value of fruit width, fruit thickness. the low plant high, fruit length and bean length.

Cluster IV comprises accessions MIK8914ET55, S795ET55, B71RS, J2RS3, J2(T), MI49RS1, B71ET55 and J2RS5. This cluster grouped accessions with high mean value of stem girth, hundred cherries weight number of primary branches, length of primary branches, fruit width, internodes length of primary branches, number of internodes per branch. Moderate fruit length, fruit thickness, beans length and lowest mean value of leaf length, number of cherries per branches.

The cluster III includes two accessions B139K9006ET49 and B139K9006ET55. They were characterized by the high mean value of percent of fruit bearing primary

branches, number of cherries per internode of primary branches, leaf length. Moderate number of primary branches, number of internodes per branch, fruit width, beans width, length of primary branches (Table 4.4).

Table 4.6: Distribution of accessions in four clusters.

Cluster	Members	code	Abbreviation
I	1	S2	S795ET49
II	9	MK1	MIK8914ET49
		J6	J2RS6
		J4	J2RS4
		J2	J2RS2
		J1	J2RS1
		M2	MI49RS2
		B2	B71ET19
		SL1	SL28RS
		M3	MI49RS3
III	2	B4	B139K9006ET49
		B5	B139K9006ET55
IV	8	S1	S795ET55
		MK2	MIK8914ET55
		M1	MI49RS1
		J5	J2RS5
		B1	B71ET55
		J2(T)	J2 (T)
		J3	J2RS3
		B3	B71RS

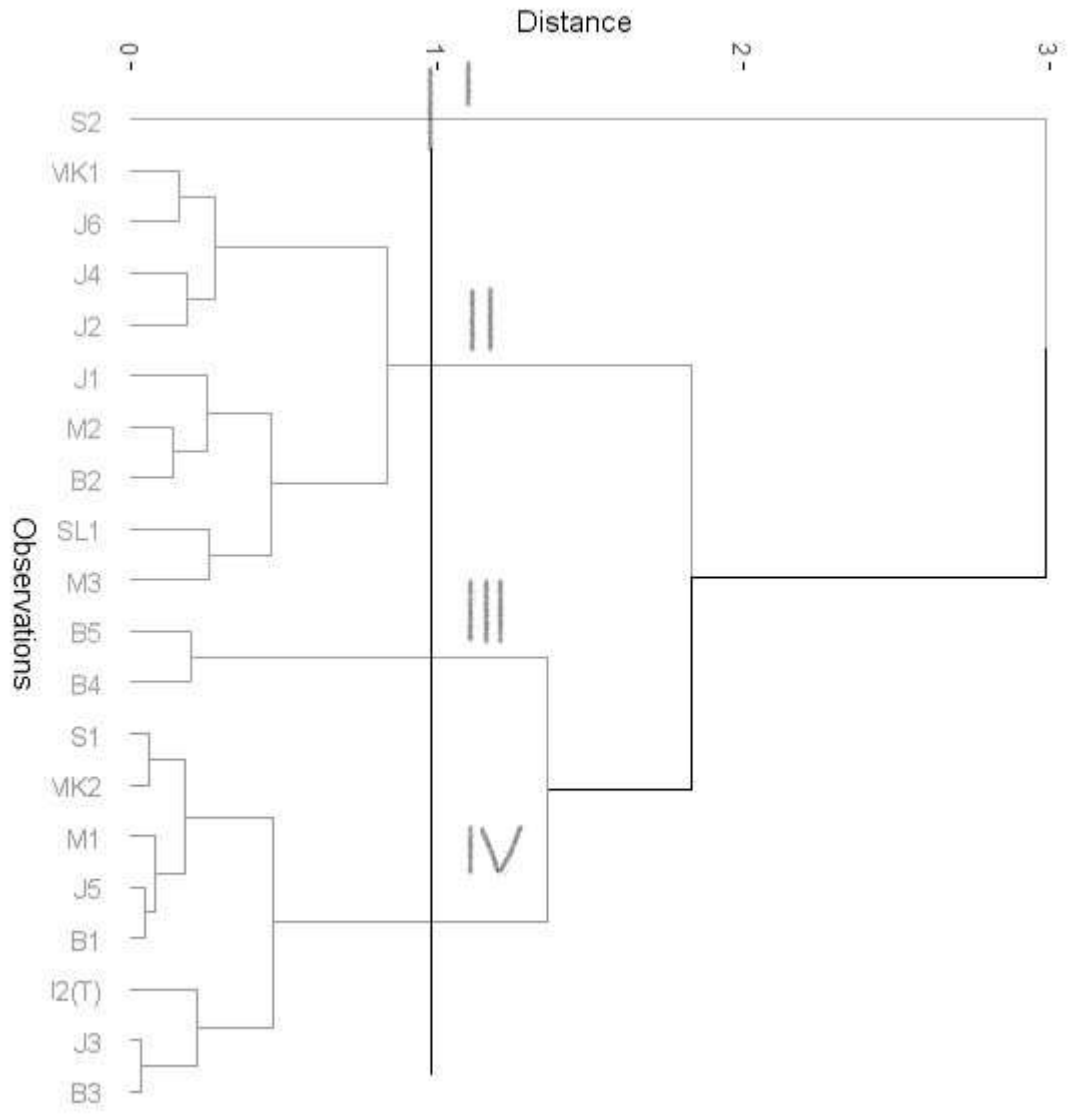

Figure 4.1: **UPGMA dendrogram depicting the genetic relationship of coffee germplasm based on 17 quantitative characters evaluated at RUKOBA. The symbols used are indicated in the Table 4.6.**

4.6 Principal Component Analysis

The presence of phenotypic variation between the accessions was further described by diverse groups across the PCA biplot (Figure 4.2). The PCA grouped the accessions into

four clades centered on their comparisons and variances in terms of the quantitative traits. The results presented that 4 PCs accounted for 84.56% of the total dissimilarity in the population. The relative selective control of the PCA as exposed by the Eigen values was high in PC1 (5.1) and lower in PC4 (1.03). The first component (PC1) accounted for 44.79% of the total disparity. The highest contributors to the variations were: LPB (0.9), NIB (0.89), PFBPB (0.78), ILB (0.72%), NCB (0.75), NPB (0.63), and GIRTH (0.69), 100CW (0.63), SW (0.66%). The trait SL (-0.38) contributed negatively in PC1. All other characters contributed a little to the PC1 (Table 4.7).

The PC2 contributed by 19.61% of the total dissimilarity. Characters that contributed to this component were: PH (0.88), FL (0.68), GIRTH (0.46), NPB (0.46), FW (0.55). However, NCB (−0.35), LL (−0.38) and LW (-0.54) had the highest negative contributions to the PC2. The PC3 accounted for 10.75% of the total variation (Table 4.7).

The traits FW (-0.53), 100CW (-0.43) contributed negatively to the PC3, however, the LW (0.55), FL (0.49) had the highest contributions followed by GIRTH (0.43), NPB (0.37) which contributed well to the variations. The PC4 contributed 9.41% of the total variation. Most of characters of PC4 had low contribution to the variation except SL (0.68), SW (0.4), LL (0.34), LW (0.35) which contributed well to the variation (Table 4.7).

Thus, these traits were the key source of the distinction in all accessions studied. The accessions which were widely dispersed had larger genetic variability for the characters studied, while accessions which are less distributed in the principal component axes had substantial comparisons in the traits evaluated. The pair-wise genetic distances founded on the phenotypic characters presented various genetic distances for the 20 coffee accessions (Table 4.8).

Table 4.7: Eigenvalue, factor scores and contribution of the first four principal component axes to variation in the coffee accessions.

Variable	PC.1	PC.2	PC.3	PC.4
PH	0.14	0.88	0.19	-0.18
GIRTH	0.69	0.46	0.43	-0.03
NPB	0.63	0.44	0.37	-0.06
PFBPB	0.78	0.12	-0.17	-0.21
NIB	0.89	-0.1	-0.09	-0.17
ILB	0.82	-0.04	-0.23	-0.11
LPB	0.9	0.08	-0.18	-0.08
NCB	0.75	-0.35	0.26	0.06
LL	0.57	-0.38	0.5	0.34
LW	0.19	-0.54	0.55	0.35
FL	0.08	0.68	0.49	0.19
FW	0.28	0.55	-0.53	0.2
FT	0.41	0.36	-0.24	0.03
100BW	0.63	-0.12	-0.43	0.02
SL	-0.38	0.45	-0.15	0.68
SW	0.66	-0.06	-0.59	0.4
ST	0.58	-0.19	0.25	-1.1
Eigenvalue	5.14	2.75	2.15	1.03
Variance (%)	44.79	19.61	10.75	9.41
Cumulative variance (%)	44.79	64.41	75.15	84.56

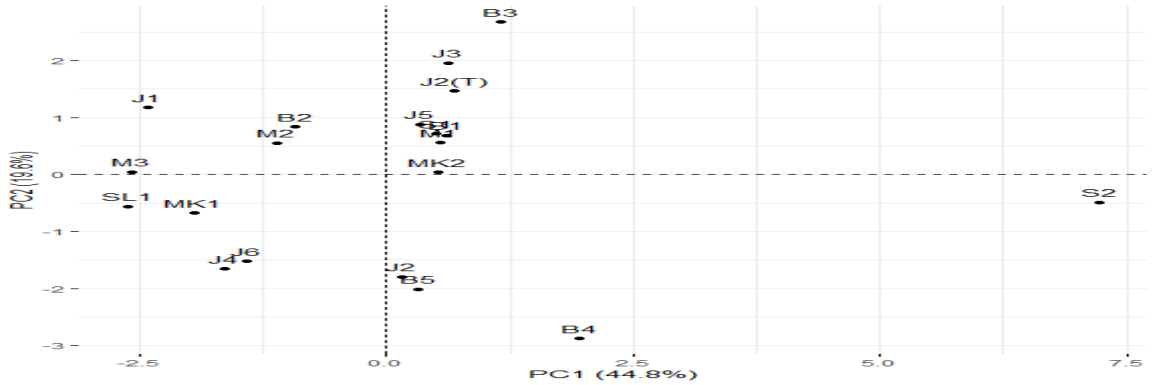

Figure 4.2: Principal component score plot of PC 1 and PC 2 describing the overall variation among coffee accessions of 17 quantitative traits.

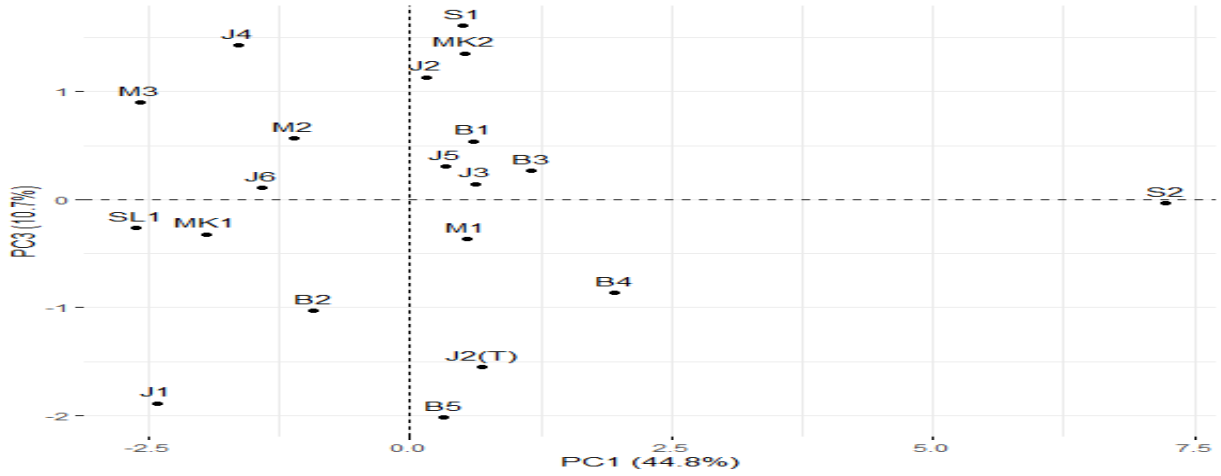

Figure 4.3: Principal component score plot of PC 1 and PC 3 describing the overall variation among coffee accessions of 17 quantitative traits.

Genetic distances ranging from 1.15 to 9.97 were recorded in the pair-wise combinations. The minimum genetic distance of 1.15 was noted between the J2R3 and B71RS accessions while the highest genetic distance of 9.97 was noted between S795ET49 and SL28RS accessions (Table 4.8).

Table 4.8: Estimates of genetic distance based on quantitative characters for all pair-wise comparisons of 20 coffee accessions.

Trait	B1	B2	B3	B4	B5	J1	J2	J2(T)	J3	J4	J5	J6	M1	M2	M3	MK1	MK2	S1	S2	SL1
B1	0																			
B2	2.37	0																		
B3	2.71	3.79	0																	
B4	4.37	5.11	5.73	0																
B5	3.89	3.89	5.35	2.73	0															
J1	4.34	2.74	4.99	6.57	4.97	0														
J2	3.02	4.16	4.83	3.72	3.68	5.37	0													
J2(T)	3.34	3.61	2.85	5.31	4.11	4.5	4.38	0												
J3	2.04	3.08	1.15	5.24	4.64	4.5	4.01	2.2	0											
J4	3.69	3.97	5.58	5.21	4.44	4.59	2.64	5.41	4.79	0										
J5	1.3	2.64	2.39	4.41	3.86	4.33	3.08	2.69	1.55	4.08	0									
J6	3.91	3.79	5.19	4.7	3.9	4.28	2.5	4.19	4.28	2.55	3.6	0								
M1	1.68	2.46	2.67	4.13	3.56	3.58	3.03	2.7	2.12	3.91	1.6	3.42	0							
M2	2.5	2.31	3.67	5.34	4.63	3.06	3.44	4.17	3.08	2.71	2.91	3.14	2.46	0						
M3	3.78	3.45	4.94	5.93	5.17	3.31	3.83	5.11	4.31	3.11	3.54	3.04	3.47	2.56	0					
MK1	3.6	3.35	4.87	5.06	3.38	3.75	3.42	3.95	4.01	3.03	3.16	2.43	3.39	3.45	2.9	0				
MK2	1.68	3.45	2.93	4.09	3.96	5.07	2.44	3.67	2.32	3.26	1.79	3.46	2.33	2.74	3.79	3.41	0			
S1	1.93	3.72	2.88	4.71	4.55	5.25	3.35	4.03	2.52	4.07	1.7	4.24	2.62	3.4	3.78	3.59	1.52	0		
S2	7.07	8.45	7.22	6.61	7.93	9.78	7.48	7.5	7.39	9.05	7.43	8.74	6.82	8.36	9.79	9.51	7.28	7.61	0	
SL1	4.75	3.89	5.55	5.85	5.28	4.07	4.16	5.13	4.85	4.17	4.47	3.06	4.19	3.37	3.11	4.1	4.63	5.26	9.97	0

B1:B71ET55, B2:B71ET19, B3:B71RS, B4:B139K9006ET49, B5:B139K9006ET55, J1:J2RS1, J2:J2RS2, J2(T): , J3:J2RS3, J4:J2RS4, J5:J2RS5, J6:J2RS6, M1:MI49RS1, M2:MI49RS2, M3:MI49RS3, MK1:MIK8914ET49, MK2:MIK8914ET55, S1:S795ET55, S2:S795ET49, SL1:SL28RS

4.2 Effect of locations on agro-morphological diversity among cultivated Arabica coffee cultivars in Burundi

4.2.1 Weather data recorded during the experimental period at Kayanza ,Rukoba and Nyange site.

The rainfall, temperature and relative humidity data noted during the period of the existing study are presented in Table 4.9. The results exposed that the highest monthly mean and the lowest monthly mean temperature were noted in October and December respectively in all the three locations. The maximum monthly rainfall during the study period was noted in April and the minimum monthly rainfall in June. The highest monthly relative humidity was noted in April while the lowest relative humidity was noted in October in three study areas (table 4.9)

Table 4.9: Monthly rainfall, relative humidity and temperature data recorded during the experimental period at Kayanza, Rukoba and Nyange sites

Months	Monthly rainfall (mm)			Relative humidity in %			Mean temperature (ºC)		
	Kayanza	Rukoba	Nyange	Kayanza	Rukoba	Nyange	Kayanza	Rukoba	Nyange
October /2019	124.14	109.30	112.61	71.25	64.25	61.36	17.79	19.79	22.36
November /2019	195.23	144.57	169.15	89.62	89.83	72.68	17.28	18.79	20.14
December/2019	374.34	285.29	249.28	90.43	89.24	88.12	17.73	19.13	19.73
January/2020	161.89	184.33	117.93	85.81	88.36	84.15	18.18	19.52	20.62
February/2020	205.48	170.44	148.21	89	82.75	79.39	18.63	19.42	21.6 4
March/2020	247.51	199.36	147.45	87.82	83.40	80.59	18.47	19.69	21.46
April/2020	368.62	272.63	265.50	93.27	89	89.39	18.06	19.66	20.18
May/2020	107.42	66.22	64.60	89	85.3	84.06	18.66	20.20	20.72
June/2020	12.91	0.0	0.0	78.28	73.21	70.04	18.96	20.78	21.7

4.2.2 Analysis of variance and mean agronomic performances

There were significant phenotypic differences (P <0.05) to high significant (P <0.01) among the accessions in most of quantitative traits studied. Leaf length, leaf area, fruit length, fruit width, hundred fruit weight, bean length, bean width, bean thickness and hundred bean weight (Table 4.10), confirming the presence of variability between the accessions for these significant characters offering a well scope for further enhancement of the crop. There was also significant P <0.05) to high significant differences P <0.01 between environments for some important quantitative traits, fruit length, hundred fruit weight, and hundred beans weight (Table 4.10). This could be attributed to microclimate variances (like, moisture, temperature, humidity) present among the three sites, specifically at early growth stages of the coffee trees (Table 4.9).

Analysis of variance for genotypes and environment interaction was not significant for most of the traits except hundred fruit weight, fruit width, beans width and hundred beans weight (Table 4. 10). Indicating that the genotypes were influenced by environment for some of traits. The significant interaction effect of genotypes across the different environments suggests that different genotypes perform differently at different environments (Tables 4.10).

Mean performance results showed that genotypes SL 28, Mysore and S795 got the highest mean value in most of morphological traits studied, especially significantly highest for leaf length, hundred fruit weight, hundred beans weight. On the other hand, the commercial variety checks MI 49, MI68, MI TV got moderate mean value in most of traits studies. However, genotypes S288, ABK56 and ABK 57 recorded lower mean value in all traits studied except fruit width and beans width (Table 4.11).

Table 4.10: Mean square values for fruit, bean and leaf traits of fifteen Arabica coffee genotypes evaluated at Nyange, Rukoba and Kayanza in Burundi.

Variable	Environment (E)	Rep/E	Genotype (G)	G x E interaction	Error	CV (%)
LL	12.49	1.45*	1.11**	0.15	0.18	5.09
LW	2	0.54	2.79	0.14	0.23	12.15
LA	1000.86	112.56**	535.3**	26.5	2.42	15.3
FL	2.91*	2.47	2.01**	0.29	0.36	4.37
FW	0.03	2.58	2.46**	0.19*	0.206	5.39
100CW	1384.4**	739.7*	614.7**	44.21*	23.7	6.37
SL	3.78	0.08	1.28**	0.27	0.15	4.8
SW	0.68*	0.2	0.56*	0.21**	0.07	5.01
ST	0.19	0.02	0.12**	0.04	0.02	4.26
100BW	143.15**	9.42**	10.67**	1.33**	0.67	11.28

* and ** Significant at the 0.05 and 0.01 probability levels, respectively;

FL=fruit length in mm; FW=fruit width in mm; 100CW= hundred cherries weight in gm; SL=bean length in mm; SW=bean width in mm; ST=bean thickness in mm; 100BW= hundred bean weight in gm; LL=leaf length in cm; LW=leaf width in cm; LA=leaf area in cm^2.

Table 4.11: Mean values for leaf, fruit, and bean traits of fifteen coffee genotypes evaluated at Nyange, Rukoba and Kayanza in Burundi

Genotypes	Code	L L	L W	LA	F L	F W	100CW	B L	B W	B_T	100BW
ABK 5691	A1	12.43	4.13	45.26	14.96	13.39	151.81	12	7.94	4.61	**16.2**
ABK 5718	A2	12.44	4.23	46.36	15.05	13.29	150.9	11.99	8.02	4.47	**16.32**
B 71	B1	12.95	5.14	58.5	15.55	12.22	161.16	12.65	7.95	4.65	**16.88**
B 139	B2	13.13	5.44	62.82	16.13	12.17	165.13	13.04	7.87	4.6	**17.51**
Blue Mountain	B3	13.12	5.35	61.9	16.14	12.04	163.49	12.81	7.77	4.5	**17.7**
Mi TV	B4	12.88	5.4	61.29	16.29	11.6	161.99	13.21	7.48	4.36	**17.88**
J2/1257	J1	12.92	5.32	60.6	15.76	12.17	165.78	12.44	7.86	4.58	**17.14**
K7	K	13.41	5.44	63.98	15.45	12.42	163.16	12.3	8.1	4.66	**17.8**
Mi68/1589	M1	12.91	5.41	61.47	16.34	11.8	167.13	13.13	7.75	4.54	**18.02**
Mi49/1848	M2	13.32	5.47	64.07	16.27	11.91	164.77	12.78	7.62	4.51	**17.52**
Mysore	M3	13.56	5.84	69.78	15.78	12.41	176.84	12.43	7.9	4.65	**19.54**
S 795	S1	13.14	5.78	66.92	15.62	12.68	177.2	12.41	8.52	4.89	**19.77**
S 288	S2	12.51	4.27	47.02	15.01	13.01	151.73	12.19	8.26	4.56	**16.59**
SL 28	S3	13.35	5.17	67.11	16.02	12.42	175.23	12.5	8.01	4.67	**19.04**
Tekisic	T	13.27	5.49	64.08	15.53	12.03	165.25	12.71	7.99	4.56	**16.94**
Mean		13.02	5.23	60.08	15.73	12.37	164.24	12.57	7.94	4.59	**17.66**
LSD (0.05)		0.62	0.46	5.88	0.72	0.69	7.88	0.61	0.43	0.23	**1.32**

FL=fruit length in mm; FW=fruit width in mm; 100CW= hundred cherries weight in gm; BL=bean length in mm; BW=bean width in mm; BT=bean thickness in mm; 100BW= hundred bean weight in gm; LL=leaf length in cm; LW=leaf width in cm; LA=leaf area in cm^2.

4.2.3 Phenotypic variations

Phenotypic variations for 10 quantitative traits of coffee were estimated and are presented in Table (4.12). Relatively low values of variability were recorded across genotypes (1.5 % for fruit width to 5.3% for hundred fruit weight) than across environments (0.2% for fruit length to 10.1% for hundred bean weight) .However, the range in fruit, bean and leaf traits (fruit length, fruit width, bean length, bean width, bean thickness ,leaf length and leaf area) were relatively larger across accessions than across environments demonstrating the phenotypic variability of these traits were under strong influence of genetic than environment (Table 4.12). High ranges occurred across environments than accessions for hundred fruit weight and hundred bean weight traits demonstrating more of the current variations were under control of environment than genetic (Table 4.12).

Table 4.12: Genotypic and environmental range and mean values for ten agronomic traits recorded on fifteen coffee genotypes at Nyange, Rukoba and Kayanza in Burundi.

Traits	Genotypes	CV%	Environment	CV%	Mean	CV%
	n=15		n=3		N=28	
LL	12.41-13.86	4	12.61-13.60	1.81	13.02	3.1
LW	4.13-5.83	3.71	5-5.42	4.2	5.23	5.5
LA	45.26-69.78	7.8	55.59-65	6.47	60.08	15.36
FL	14.96-16.34	1.69	15.44-15.90	1.6	15.73	3.1
FW	11.60-13.39	1.5	12.35-12.40	0.2	12.37	3.8
100CW	150.94-177.20	5.3	160.72-170.63	9.4	164.24	4.28
BL	12.05-13.81	2.06	12.25-12.82	2.3	12.57	3.4
BT	4.36-4.89	2.14	4.52- 4.65	1.5	4.59	3.2
BW	7.48-8.52	1.80	7.81-8.06	1.4	7.94	3.3
100 BW	16.20-19.77	5.12	16.96-19.52	10.1	17.66	11.33

FL=fruit length in mm; FW=fruit width in mm; 100CW= hundred cherries weight in gm; BL=bean length in mm; BW=bean width in mm; BT=bean thickness in mm;

100BW= hundred bean weight in gm; LL=leaf length in cm; LW=leaf width in cm; LA=leaf area in cm^2.

4.2.4 Genetic variation and heritability of morphological traits

Estimates of the genotypic and phenotypic variability indicated that coffee genotypes in this study expressed different level of variations in the morphological traits measured. Genotypic coefficient of variation (GCV) values varied from 0.39 to 23.58% (Table 4.13). The trait with high GCV value was leaf area with 23.58%, followed closely by hundred cherries weight with 13.78%, hundred beans weight with 6.107% and leaf width 5.340%. The values recorded for the phenotypic coefficient of variation (PCV) ranged between 1.932 to 26.70% with leaf area scoring the highest value (Table 4. 13). The broad sense heritability values ranged from 0.248 to 0.82 within the traits. The quantitative traits that showed a higher broad sense heritability values (>0.50) were leaf width, leaf area, fruit length and leaf length which scored 0.798, 0.82, 0.55 and 0.502 respectively (Table 4.13). Beans thickness scored a low broad sense heritability of 0.248, followed by hundred beans weight and fruit width which scored 0.341 and 0.419 respectively.

Table 4.13: Variance components, genotypic and phenotypic coefficients of variation, and heritability (its standard error) estimates of fruit, bean and leaf traits of fifteen coffee genotypes.

				PCV	GCV	h^2_{bs}	$\sigma^2 ge/\sigma^2 g$
Traits	**$\sigma^2 g$**	**$\sigma^2 ge$**	**$\sigma^2 e$**	**%**	**%**	**%**	
LL	0.248	0.125	0.593	1.944	0.976	0.502(0.12)	0.490
LW	0.343	0.074	0.107	6.702	5.340	0.798(0.16)	0.215
LA	15.40	6.50	20.371	26.70	23.58	0.82(0.13)	0.422
FL	0.53	0.06	0.162	1.96	1.085	0.55(0.20)	0.113
FW	0.389	0.293	0.581	2.44	1.026	0.419(0.18)	0.753
100CW	0.19	0.37	0.983	18.37	13.78	0.48(0.14)	1.94
BL	0.419	0.197	0.173	3.305	1.555	0.470(0.12)	0.470
BW	0.093	0.078	0.114	1.932	0.39	0.418(0.16)	0.838
BT	0.25	0.062	0.091	3	0.75	0.248(0.16)	0.248
100BW	1.42	1.53	0.894	11.88	6.107	0.341(0.12)	1.080

FL=fruit length in mm; FW=fruit width in mm; 100CW= hundred cherries weight in gm; BL=bean length in mm; BW=bean width in mm; BT=bean thickness in mm;

100BW= hundred bean weight in gm; LL=leaf length in cm; LW=leaf width in cm; LA=leaf area in cm^2.

4.2.5 Estimate of regression coefficients (β1) and deviation mean squares (σ2 di) for fruits,beans and leaves of coffee genotypes.

The regression coefficients and deviation mean squares for different traits are presented in (Table 4.14). It is evident from the table that most of the traits had low regression coefficient (≤ 1) except bean thickness, hundred cherries weight and hundred beans weight for which the regression coefficients were greater than unity. The deviation means squares from regression of stability parameters showed lack of differentiation in their adaptation patterns and only two traits, showed specific adaptability to unfavorable environments LL and FL with predictable response (σ^2 di =0) and indicating that they performed better in unfavorable conditions.

Table 4.14: Estimates of regression coefficient (bi) and deviation mean squares (S^2 di) for fruits, beans and leaves of coffee accessions.

Traits	Bi	S^2 di
LL	0.75*	0.83
LW	0.68	2.84**
LA	0.87*	5.54**
FL	0.78*	0.84
FW	0.74	1.34**
100CW	1.81	2.45**
BL	0.96*	0.79**
BW	0.79*	0.54*
BT	1.21*	0.46**
100BW	1.73	2.24**

FL=fruit length in mm; FW=fruit width in mm; 100CW= hundred cherries weight in gm; BL=bean length in mm; BW=bean width in mm; BT=bean thickness in mm; 100BW= hundred bean weight in gm; LL=leaf length in cm; LW=leaf width in cm; LA=leaf area in cm^2

* indicate significant difference from 1.00 at the 0.05 level.

* and ** indicate significant difference from 0 at the 0.05 and 0.01 levels, respectively.

4.2.6 Correlations among morphological traits

The genotypic (Cg) and phenotypic (Cp) correlation coefficients among leaf, fruit and bean traits across the three environments/locations are shown in (Table 4.15). All leaf, fruit and bean traits showed significant and positive correlation among themselves, both at genetic and phenotypic level, except in case of leaf length and bean weight, between leaf width and bean width and thickness, between leaf area and bean width and bean thickness, between fruit length and bean thickness which showed non-significant correlations (Table 4.15).Most leaf traits showed significant and positive genetic correlation with fruit and bean traits except for bean width and bean thickness. The correlation among all four leaf traits and fruit traits were significant and positive at both genetic (C g= 0.57 to 1.00) as well as at phenotypic level Cp= 0.32 to 0.95), except leaf length and fruit length which were not significant and leaf length and fruit width, leaf width and fruit width which were correlated negatively. Similar significant positive association exhibited between fruit traits with leaf traits except fruit width. Fruit and bean traits also exhibited significant and positive correlation at genotypic level in most of the cases except bean thickness and fruit length, bean length and fruit width. Low magnitude of association at phenotypic level suggesting the association of these traits were under genetic control (Table 4.15).

Table 4.15: Genotypic (above diagonal) and phenotypic (below diagonal) correlations among leaf, fruit and bean traits based on the means of fifteen coffee accessions across locations.

Variables	LL	LW	LA	FL	FW	100CW	BL	BW	BT	100BW
LL	1	0.9**	0.94**	0.55	-0.56*	0.82**	0.36	-0.07	0.32	0.7**
LW	0.539**	1	1**	0.74*	-0.72*	0.93**	0.58*	-0.12	0.31	0.87**
LA	0.754**	0.958**	1	0.7*	0.69*	0.92**	0.54*	-0.11	0.32	0.81**
FL	0.323**	0.427**	0.437**	1	-0.88**	0.57*	0.89**	0.63*	-0.24	0.49
FW	-0.203*	-0.466**	-0.420**	-0.311**	1	-0.47	-0.91**	0.62*	0.31	0.3*
100CW	0.617**	0.730**	0.776**	0.360**	-0.274**	1	0.36	0.1	0.54*	0.91**
BL	0.179*	0.299**	0.284**	0.583**	-0.294**	0.210*	1	_0.62*	-0.35	0.25
BW	-0.078	0.164	-0.148	-0.134	0.376**	-0.005	-0.129	1	0.8**	0.19
BT	0.203*	0.143	0.174*	-0.087	0.171*	0.334**	0.012	0.472**	1	0.52*
100BW	0.658**	0.528**	0.634**	0.241**	0.115*	0.663**	0.023	0.005	0.297**	1

* and ** Significant at the 0.05 and 0.01 probability levels, respectively;

FL=fruit length in mm; FW=fruit width in mm; 100CW= hundred cherries weight in gm; BL=bean length in mm; BW=bean width in mm; BT=bean thickness in mm; 100BW= hundred bean weight in gm; LL=leaf length in cm; LW=leaf width in cm; LA=leaf area in cm^2.

4.2.7 Genetic divergence analyses

Genetic divergence among the pair of component genotypes in the fifteen genotypes including five commercial cultivars quantified using Euclidian distance as dissimilarity/similarity measure showed moderately high degree of variation ranging from 1.46 (between K7 and B71) to 8.42 (between S795 and ABK57), thus, indicating a high genetic diversity among the genotypes (Table 4.16). Cultivars Mi68, Mi49, B71, B139 and J2 were found to be the most dissimilar genotypes, with a Euclidian genetic distance from 6.04 to 8.42 followed by the pair combinations coffee genotype S795 and ABK5691, S795 and ABK5718, S795 and B71, S795 and B139, S795 and Blue mountain, S795 and MiTV, S795 and J2, S795 and Mi68, S795 and M68, S795 and Mi49, SL28 and ABK5691, SL28 and ABK5718, which also showed high genetic distances. The wide genetic distance (8.42) between the two categories of genotypes specify a high genetic variance for exploiting necessary genes.

A cluster dendrogram based on morphological distance was used to assess the genetic relationship among the fifteen coffee genotypes. The 15 accessions were classified into three distinct clusters (Figure 4.4). Cluster III had the highest number of accessions with nine accessions (60% of the total population). cluster I and II had the same number of accessions with 3 accessions (each had 20% of the total population). Cluster I included the accessions ABK5691 and ABK5718 from Abyssinie via Rubona (RWANDA) while S 288 from India. These Accessions contributed to the lowest mean value of leaf length. moderate hundred fruit weight, leaf width, bean width, hundred beans weight and the high fruit width. (Table 4.11). Cluster II included accessions S 795 from India, Mysore from ISAR RWANDA and SL 28 from KENYA. These accessions were characterized by the highest mean value of, leaf width, beans width, beans thickness, hundred fruit weight and hundred beans weight. moderate fruit length (Table 4.11). Cluster III comprised of accessions J2/1257, B71, B139, Mi TV, Mi49/1848, Mi68/1589 all from ISAR RWANDA, Blue mountain and K7 from KENYA and Tekisic from San Salvador (Table 3.4). This cluster grouped accessions with highest mean value of traits like fruit length, leaf length and lowest fruit width and leaf width (Table 4.11)

Table 4.16: Estimates of genetic distance based on quantitative characters for all pair-wise comparisons of 15 coffee accessions

Distance	A1	A2	B1	B2	B3	B4	J1	K	M1	M2	M3	S1	S2	S3	T
A1	0														
A2	3.07	0													
B1	5.73	5.76	0												
B2	5.79	5.86	2.53	0											
B3	5.81	5.97	2.38	2.3	0										
B4	5.82	4.61	4.61	3.79	3.67	0									
J1	5.94	5.86	2.76	2.99	2.1	3.46	0								
K	6.43	6.66	1.46	2.85	2.44	5.09	2.95	0							
M1	5.93	5.17	4.27	2.85	3.65	2.49	3.41	4.63	0						
M2	5.73	5.1	3.06	2.24	3.3	2.78	3.04	3.71	1.96	0					
M3	6.42	6.48	4.18	4.07	3.69	4.83	3.88	3.57	4.55	4.61	0				
S1	8	8.42	6.13	6.58	5.76	8.03	6.04	5.83	7.31	7.63	4.97	0			
S2	4.82	5.12	7.08	6.3	5.98	6.08	6.54	7.57	6.19	6.97	6.87	7.29	0		
S3	7.28	7.94	4.55	4.07	4.41	6.62	5.02	3.74	5.61	5.53	2.98	4.73	7.61	0	
T	4.77	4.73	2.41	3.25	2.7	3.84	2.03	3.17	3.9	3.2	4.4	6.12	6.08	5.47	0

evaluated at Nyange, Rukoba and Kayanza in Burundi.

:ABK5691,A2:ABK5718, ,B1: B71, B2: B139, B3: Blue Mountain, B4:Mi TV, J1: J2/1257, K:K7ure M1:Mi 68/1589 ,M2:Mi 49/1848, M3: Mysore ,S1 : S795, S2: S288 , S3: SL28,T:Tekisic 3

.

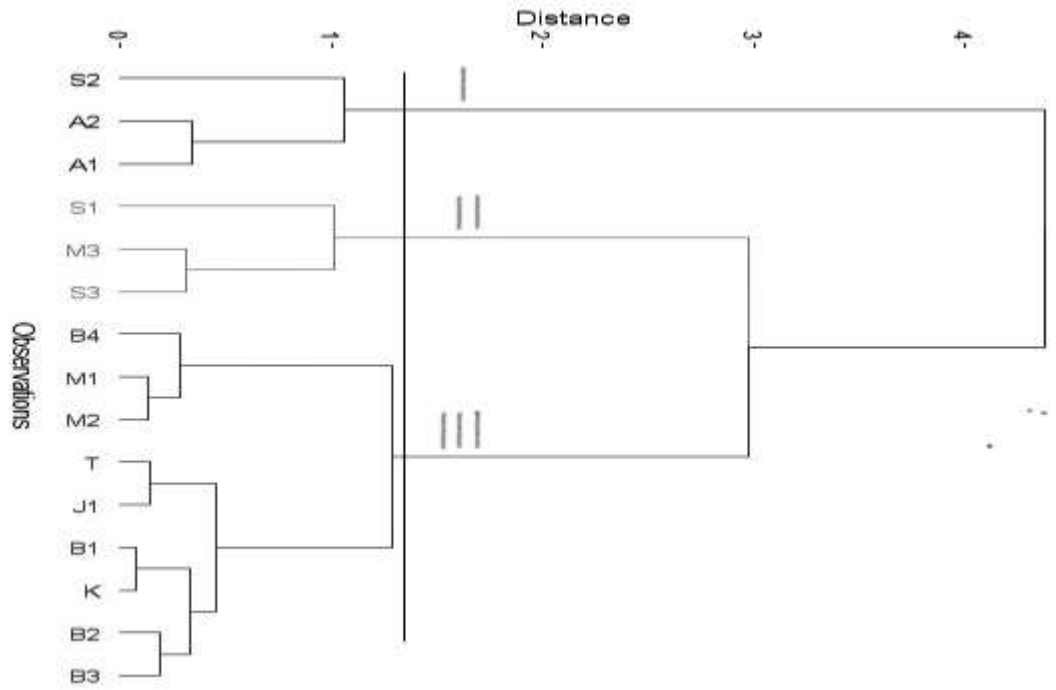

Figure 4.4: Dendogram of fifteen coffee genotypes considering 10 significant quantitative morphological traits evaluated at Nyange, Rukoba and Kayanza in Burundi.

A two-dimensional PCA plot (Figure 4.5) supported the results found with cluster analysis in which 40.49% and 27.08 % of the total variation was explained by PC1 and PC2, respectively. Genotypes ABK5691, ABK5718 and S288 separated clearly from the others and were located in the far right hand side while genotypes J2/1257, B71, B139, Mi TV, Mi49/1848, Mi68/1589, Blue mountain, K7, Tekisic were placed in opposite most left k and side while the genotypes SL28, Mysore and S795 assembled in the middle position of the PCA graph and categorized by average recital for most of traits studied.

Table 4.17: Eigenvalue, factor scores and contribution of the first four principal component axes to variation in the coffee accessions evaluated at Nyange, Rukoba and Kayanza in Burundi

Variable	PC.1	PC.2	PC.3	PC.4
LL	0.54	0.78	0.09	-0.1
LW	0.26	-0.88	-0.16	0.3
LA	0.71	-0.31	-0.2	0.36
FL	0.35	0.38	-0.75	0.16
FW	0.54	-0.68	0.16	0.11
100BW	0.69	0.01	0.27	0.56
SL	0.35	0.57	-0.68	-0.01
SW	0.67	-0.49	0.3	-0.29
ST	0.78	-0.39	0.23	-0.1
100BW	0.6	0.37	0.06	0.43
Eigenvalue	5.45	5.2	1.91	1.56
Variance (%)	40.49	27.08	11.53	7.17
Cumulative (%)	40.49	67.57	79.10	86.27

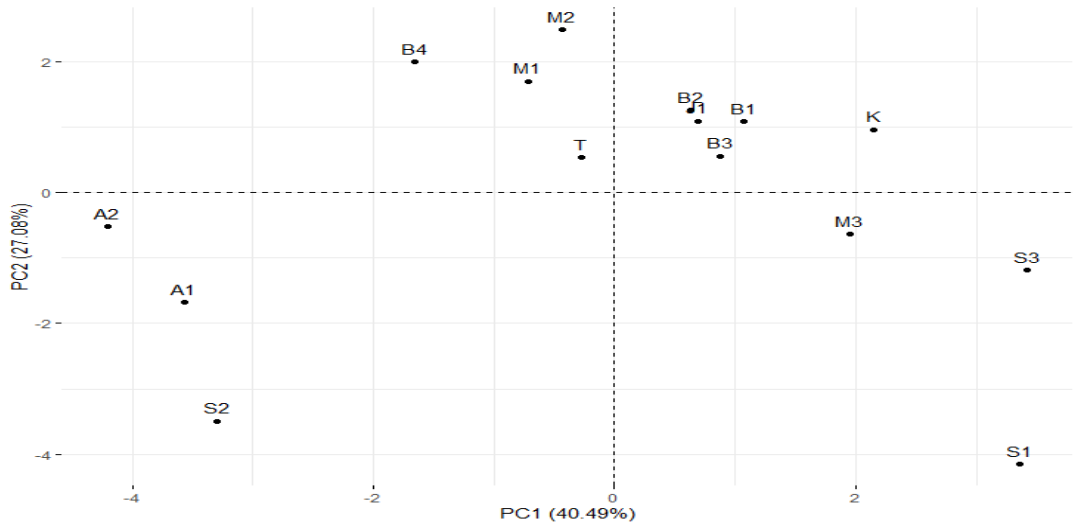

Figure 4.5: Principle Component Analysis plots illustrating variation among genotype

4.3 Discussion

4.3.1 Morphological diversity among Arabica coffee cultivars

Agro-morphological description is an important step towards actual exploitation of assortment in a crop species. (N'Da *et al.*, 2014;Rakshit *et al* ., 2012). Variation in agro -morphological traits is a standard process used to differentiate amongst accessions based on differences in plant size, color of the shoot tip, characteristics of the fruits, shape of the leaf and the plant, angle of branching and length of the internodes (Rakshit *et al* , 2012). Dissimilarity in Arabica coffee using agro-morphological traits has previously been conducted by various authors including (Kebede, 2003; N'Da *et al*., 2014; Rakshit *et al*., 2012).

Classification of the Arabica coffee genotypes based on dissimilarity in qualitative characters showed that there were only minor differences among all Cultivars with respect to fruit color, fruit shape, leaf apex shape, leaf shape, beans shape. Significant variations were recorded with respect to branching habit, plant shape, young leaf color, overall appearance and angle of insertion of primary branches. In case of branching habit, genotypes with numerous primary branches as well as well

as those with several secondary and tertiary branches occurred at a frequency of only 60%. Such accessions are expectable to bear extra leaves that would increase the photosynthetic efficiency thereby donate to possible yield increases. Also, accessions with horizontal or spreading angle of lateral insertion occurred at a relatively high occurrence (55%) in the set of accessions studied. The accessions with a horizontal or spreading angle of lateral insertion would have maximum of its leaves exposed to complete sunlight, thereby increasing the rate of photosynthesis of such accessions, and consequently their yield performance (Abubakar & Bubuche, 2013).

Results on quantitative traits studied showed highly significant ($P < 0.01$) and significant ($P < 0.05$) differences among the coffee cultivars for most of the quantitative traits evaluated. Therefore, most of traits were variable in the multivariate examination like cluster analysis and PCA. Such variability clearly exposed that there is a huge potential for increasing coffee fruit and bean characteristics through selection and crossing (Bhandari et al., 2017; Hedrick,2000). Variability between coffee genotypes may be attributed to the outcrossing nature of the species (Tran, 2005) or to the evolutionary improvements and normal mutations happening in the population (Kebede & Bellachew, 2008). The existence of wide morphological difference between Arabica coffee accessions has also been reported by many authors (Avise & Hamrick, 1997; Bhandari *et al* , 2017; Ermias , 2005; Gichimu & Omondi, 2010; Kebede,2003).

Significantly, positive correlations among quantitative characters indicated that such traits could be enhanced concurrently or could be used for indirect selection of desired traits which might be difficult to choice and are highly valued by breeders (Tran, 2005). In this study, significantly positive correlations were noted among several combinations of traits. for example, stem diameter, which is naturally used as a selection standard for vigor (Padi *et al*, 2012; Zec *et al*, 2013) was significantly and highly correlated with other architectural characters such as height, number of primary branches, number of internodes, length of primary branches.

Significantly negative correlations were also noted between some combinations of traits. Characters with negative correlations need vigilant selection considerations since an enhancement of one trait leads to a deterioration of the other trait (Tran,

2005). Correlation examination in our study exposed some significant relations among the quantitative characters considered.

Cluster analysis based on coffee quantitative traits clustered the coffee accessions into four different clusters. Coffee accessions in the same cluster group were morphologically similar with respect to some traits (Bayetta, 1997; Tounekti *et al*, 2017).

In terms of performance, the accessions in cluster I were the most interesting with respect to number of cherries per internode of primary branches, number of internodes per branch, percent fruit bearing primary branches, length of primary branches, hundred cherries weight, leaf length, leaf width, characteristics. The accessions clustered under cluster III gave the best characters of interest (number of cherries per internode, leaf length, number of primary branches, number of internodes per branch, fruit width, percent of fruit bearing primary branches, beans width, length of primary branches). This cluster was followed by the cluster IV in terms of stem girth, number of primary branches, length of primary branches, number of internodes per branch, internodes length of primary branches, fruit width, hundred cherries weight. However, most of the accessions in cluster II displayed poor agronomic performance except fruit length and beans length, thus, improvement program should target upgrading these accessions. These results are in agreement with those of (Muvunyi *et al*, 2017; Tikader *et al*, 1999; Tounekti *et al*, 2017) who indicated that Arabica coffee cultivars show dissimilarities for numerous morphological traits.

The PCA showed that number of primary branches per tree, percentage of fruits bearing primary branches per tree, hundred cherries weight, plant height, and stem girth, length of primary branches, leaf length, number of cherries per internode and internode length was the key source of variation in the 20 coffee accessions. The first 2 principal components PC1 and PC2 with values of 44.79% and 19.61% respectively contributed extra to total disparities among coffee germplasm accessions. According to (Gessese *et al*, 2015) , characters with largest absolute values closer to the unity inside the first principal components influence the grouping of accessions that are superior for some traits. So, morphological chiefly those

contributed to the PC1 and PC2 played a key role in categorizing coffee genotypes into diverse groups and should be used in selecting diverse parents, in crossing program. The key traits described in this study agree with those of (Chahal & Gosal , 2002; Gichimu & Omondi, 2010a), who stated various morphological traits such as leaf length and leaf width, hundred bean weight, and bean length as the highest donors to the variation among coffee genotypes. The pair-wise genetic distances based on the phenotypic traits also showed that there is a huge potential of exploiting heterosis from crosses among the accessions from different groups which could allow extension of the range of variability in segregating generations, as it has been reported in numerous studies (Olika et al, 2011; Tikader *et al.*, 1999).

4.3.2 Assessment of the affect of locations on agro-morphological diversity

The presence of significant mean differences among genotypes for leaf, fruit and bean parameters at the different locations indicates that there is a true genetic difference among genotypes and improving these traits by selection is possible. Similar results were reported by Yonas *et al*. (2014) who showed that different genotypes exhibit differential performance when grown across environments (Yonas *et al*, 2014). Phenotypic variations for 10 quantitative traits of the tested genotypes showed that high ranges occurred across environments than cultivars for some of traits, especially hundred fruit weight, fruit width, beans width and hundred bean weight indicating the phenotypic variability of these traits were under strong influence of environment than genetic. Such major environmental influence of these agronomic characters of Arabica coffee genotypes has been reported by previous workers (Mesfin &Bayetta, 1987; Walyaro, 1983; Yonas & Tarekegn, 2015).

The significant environment effect between the accessions in most of quantitative traits studied, confirmed the presence of variance between the genotypes for these characters offering a possibility for further enhancement of the crop. This is predictable as the climatic and edaphic elements at the different locations in Burundi are diverse and recital of different genotypes is variance to these factors. Comparable results were reported by diverse authors who showed that different genotypes exhibited differential performance when grown across environments (Abrar *et al* 2014b; Yonas *et al,* 2014). In addition, frequently measured development traits were

also subjected to the time related environment difference that cause variations in their size. Similarly, large ecological effects on growth traits have been stated (Walyaro, 1983; Yonas & Tarekegn, 2015). This may specify the fact that a genotype which is larger in performance at one established of settings for one agronomic trait may not be greater at a diverse set and consequently its performance at the diverse environments essential be examined before it is suggested for profitable use. The outcomes are partly in agreement with the conclusion of (Getachew *et al*. 2017; Mistro *et al*., 2008; Olika *et al*.,2011) who reported the existence of important variations among Arabica coffee genotypes for most agronomic traits studied across different environment.

Analysis of variance revealed that the effects of environments and Genotype by Environment Interaction were highly significant to significant for some important traits studied, indicating that the genotypes for these less environmentally influenced traits. (Wamatu & Thomas 2001) for yield and (Gichimu *et al*., 2010a) for plant and agronomic traits has reported similar result. However, (Walyaro ,1983; Yonas & Tarekegn, 2015) reported significant effects of environments and Genotype by Environment Interaction for plant and agronomic traits.

The existence of significant effects of environments and Genotype by Environment Interaction for hundred bean weight, hundred fruit weight, fruit and bean width and across the diverse environments suggested that diverse coffee quantitative traits performed differently at diverse environments. The highest significant effects of environments and Genotype by Environment Interaction for hundred bean weight, hundred fruit weight, should be attributed to the favorable climate as the flowering which are different in three locations especially in December, January, February, March and April (Table 4.9).The adequate rainfall amount was adequate for luxurious exocarb and endocarb expansion which are prerequisite, for complete development of endosperm to result in fruit and bean which are grown to their full genetic limits apart from the optimum edaphic factors present at the particular location (Tesfaye *et al.* 2013a).

The rainfall amount received at Kayanza starting from November 2019 to May 2020, on wards until maturity was also adequate for better growth of fruits and beans as

shown in (Table 4.9*)* can be contributed to the effects of environments and Genotype by Environment Interaction for fruits and beans characters. Similar results were reported by (Tesfaye *et al.* 2008; (Tesfaye & Ismail, 2008) that moisture quantity received during fruit growths had a significant influence on beans physical quality. In conformity to the outcome of the present study. (Cavaco Bicho *et al*, 2010) also stated that moisture amount obtainable during fruit growths affect berry and bean growth.

.The character leaf area, hundred cherries weight, hundred beans weight, leaf width had relatively highest GCV compared with the others with respective value of 23.58%, 13.78%, 6.107 % and 5.340% with corresponding PCV of 26.70%, 16.37%, 9.88% and 6.702) demonstrating the relative importance of these traits for enhancement of these coffees. Other studies have obtained partly similar results (Dharmaraj &Gopal, 1986; Olika *et al*., 2011) though have assessed in single environments. (Fekadu *et al* ,2020; Olika *et al*., 2011) stated maximum phenotypic and genotypic coefficients of variation for coffee traits across location. However, it is not possible to determine the amount of the variation that was heritable from only the genotypic coefficient of variation (Khaliq *et al*., 2009). The narrow gap among PCV and GCV for leaf length, leaf width, fruit length, bean length suggesting that the influence of weather in phenotypic performance is minimal. The current result is in agreement with conclusions of (Olika *et al*., 2011; Seyoum & Bayetta, 2007) who indicated high PCV and GCV values for yield and leaves, beans, fruits traits. Lemi and Ashenafi, 2016 also described high standards of phenotypic coefficient of dissimilarity but medium genotypic coefficient of inequality for yield and with higher GCV values for number of main stem nodes. Low σ^2ge/σ^2g ratio as shown by most of traits (σ^2 ge/σ^2 g ratio < 1.0) suggested that the GEI was minimal for these traits except for hundred fruit weight and hundred beans weight. Heritability indicate the effectiveness with which selection of accessions can be based on phenotypic presentation. Estimations of broad sense heritability (h^2 bs) in this study ranged from 0.248 to 0.82 for beans thickness and leaf area. According to (Verma & Agarwal, 1982), heritability values >50% are measured as high, whereas values less than 20% are detected as low and values between 20 and 50% are moderate. For this study, high heritability (> 50%) was noted for leaf area (0.82), leaf width (0.798), fruit

length (0.55) and leaf length (0.502). The predictable high heritability for these characters, suggest the superior effectiveness of assortment and development to be expected for these characters in future breeding program.

Characters such as fruit width, hundred fruits weight, beans length, bean width, hundred beans weight, bean thickness showed moderate heritability. The current result partly agrees with findings of (Cilas et al. 1998; Getachew *et al*.,2017; Yigzaw, 2005) who stated high heritability for coffee growth traits and low heritability for coffee beans and fruits characteristics traits. In contrast of this result, (Bayetta, 2001) stated high heritability estimates among 71.43 and 97.32 for all characters measured, suggesting that the effect of environment on phenotypic appearance of the characters was small. This is probably due to the modifications in test materials and the climate.

Since significant part of the interaction is linear in nature, stability analysis was done to identify traits which exhibit stable performance across the different environments. The regression coefficients and deviation mean squares from regression of stability parameters are presented in Table 4.14. It is evident from the table that some traits showed the regression coefficients lower than unit. As result, most of the traits showed lack of differentiation in their adaptation patterns and only two traits, showed specific adaptability to unfavorable environments, FL and LL with predictable response (σ^2 di =0) indicating that they performed better in unfavorable conditions. The pooled deviations were significantly different from the pooled error for most of traits. Similarly, Wamatu *et al.* (2003) reported that in the same manner, reported that with significant l low stability, the relationships between Arabica coffee genotypes and environments was not strictly linear (Sharma, 1998). For countries like Ethiopia where the larger share of the coffee is produced by small-scale farmers where the environments are not well defined such genotypes may help to avoid crop failure that may arise from uncertain environmental conditions. However, their significant deviation means squares from regression (s^2 di) impose a restriction to use them across such environments (Yonas, 2014).

Correlations observed between characters might be used to categorize imperious characters that are preferred by coffee investigators (Tran, 2005). A significantly

positive genetic and phenotypic correlation coefficient was detected between most of the traits evaluated. Positive correlation showed that both characters share some common genetic substantial, while traits with negative correlations necessity careful assortment considerations (Tran, 2005). A large positive genetic correlation indicates that selection for one trait will have a correlated response to selection in other traits, which enable either indirect selection or rapid gain in multiple-trait selection. (Fekadu *et al*., 2019). Apart from this, all significant correlations combination at both genetic and phenotypic level exhibited the same sign indicates lack of contrary action among effects. The significant positive genetic associations between leaf, fruit and bean traits agrees with previous findings in Arabica coffee of (Fekadu *et al*., 2019). Similarly, (Abrar *et al.,* 2014b) found correlations between yield and vegetative characters of arabica coffee. PCA was done to evaluate the relative significance of each trait for classification of cultivars (figure 4.5). The results showed that about 67.57% of the dissimilarities present among genotypes were explained by two principal components (PC). Yan & Rajcan, 2002 reported that the PC biplot is used to display genotype by trait combination to classify genotypes that are chief for some traits. According to (Chahal *et al* ., 2002), characters with major absolute values closer to the unity in the first principal component (PC1) influence the grouping more than those with lesser absolute values nearer to zero. Therefore, morphological traits with superior donations to the PC1 and PC2 played an imperative role in categorizing coffee accessions into different groups and should be used in choosing various performance of accessions, in crossing programs.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION.

5.1 Conclusion

Two trials were conducted to evaluate two sets of coffee cultivars. In the first trial, twenty coffee Cultivars including, one commercial check variety were evaluated at one location over one season, while in the second trial, fifteen cultivars were evaluated at three sites over one season. Result of genetic variability studies in 17 quantitative-morphological traits showed that significant genotypic variation among the tested coffee accessions for most of the traits in the first set and 10 traits in the second set indicating the presence of large amount of variability for those significant traits for potential improvement. Qualitative description and variety studies is one of the main criteria used to classify significant traits that are lacking in germplasm assortment to direct future outlines. In the current study, significant variations were seen in branching habit, plant shape, young leaf color, overall appearance and angle of insertion of primary branches that may have significant influence on yield of Arabica coffee and should be the focus of future germplasm conservation in Burundi. In term of quantitative traits, one accession S795ET49 was characterized by high mean values of most quantitative traits, followed by two accessions, B139K9006ET49 and B139K9006ET55 show that diverse accessions have varied background value and accessions conservation that permit coffee researchers to increase different traits and, therefore potential selection should be produced on the relative evidences of each group for each trait depending on the objectives of the researcher program.

GEI analysis in second study for leaves, fruits and beans using various statistical models clearly showed that all genotypes tested across environments, displayed larger GEI variation. The study indicates the presence of variation across location for fruit and bean traits and low variation for leaf traits among the tested genotypes. Therefore, environmental conditions could be the major source of GEI observed in the traits evaluated in these studies. The Plot based broad sense heritability for most of traits were moderate and high indicating the regularity of the superiority of the

individuals among the measurements and/ or environments, which demonstrate that selection based on those traits is a reliable strategy. The present study identified also genotypes such as S795, Mysore and SL28 exhibited higher mean value for most of traits evaluated across location except fruit length and leaf length where the highest were observed in the genotypes: J2/1257, B71, B139, Mi TV, Mi49/1848, Mi68/1589, Blue mountain, K7, Tekisic The genotypes: S795, Mysore, B2, A1 and SL28 exhibited the top five mean values for hundred fruit weight and three of these genotypes: S795, Mysore and SL 28 were also shown to be top in overall performance for hundred beans weight.These studies contribute additional information on the role of genetic and environmental influence in the enhancement of vital traits in Arabica coffee. The full potential for improvement of the traits need to be assessed for each program population/materials based on target environments.

5.2 Recommendation

On the basis of the findings from this study, the following recommendations are made:

- The cultivars S795ET49, B139K9006ET49 and B139K9006ET55 showed high mean value for most of traits studied especially the components of yield and can be recommended to the farmers in Mumirwa Region of Burundi.
- It is important to evaluate these accessions in Burundi's three agro-ecological zones.
- The separation of the accessions into distinct groups based on morphological descriptors presents an opportunity for integration of these genotypes into our breeding program in efforts to improve Arabica coffee in Burundi.
- The study indicates the presence of variation across location for fruit and bean traits and low variation for leaf traits among the tested genotypes, should respond to phenotypic selection in target environments.
- The present study identified also accessions such as S795, Mysore and SL28 exhibited higher mean value for most of traits evaluated across location, should show high response to selection in coffee improvement programs in the country.

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APPENDICES

Appendix I: ANOVA of 17 quantitative traits studied at RUKOBA

Hundred cherries weight (100CW)

Source of variation	d.f.	s.s	m.s	v.r	F pr
Varieties	19	35164.69	1850.77	27.87	<.001
Residual	380	25236.25	66.41		
Total	399	60400.94			

Length of primary branches (LPB)

Source of variation	d.f.	s.s	m.s	v.r	F pr
Varieties	19	15248.09	802.53	8.54	<.001
Residual	380	35717.85	93.99		
Total	399	50965.94			

Fruit length (FL)

Source of variation	d.f.	s.s	m.s	v.r	F pr
Varieties	19	107.6811	5.6674	11.11	<.001
Residual	380	193.8740	0.5102		
Total	399	301.5551			

Fruit thickness (FT)

Source of variation	d.f.	s.s	m.s	v.r	F pr
Varieties	19	60.7083	3.1952	11.27	0.191
Residual	380	107.7055	0.2834		
Total	399		168.4138		

Fruit width (FW)

Source of variation	d.f.	s.s	m.s	v.r	F pr
Varieties	19	52.4959	8.02	8.02	<.001
Residual	380	0.3443	0.3443		
Total		399	183.3439		

Stem girth (GIRTH)

Source of variation	d.f.	s.s	m.s	v.r	F pr
Varieties	19	126.6719	6.6669	43.73	<.001
Residual	380	57.9375	0.1525		
Total		399	184.6094		

Internode length of primary branches (ILB)

Source of variation	d.f.	s.s	m.s	v.r	F pr
Varieties	19	27391.7	1441.7		<.001
Residual	380	44069.8	116.0	12.43	
Total	399	71461.5			

Leaf length (LL)

Source of variation	d.f.	s.s	m.s	v.r	F pr
Varieties	19	176.4793	9.2884	12.01	<.001
Residual	380	293.8995	0.7734		
Total	399	470.3788			

Leaf width(LW)

Source of variation	d.f.	s.s	m.s	v.r	F pr

Varieties	19	44.4935	2.3418	11.12	<.001
Residual	380	79.9945	0.2105		
Total	399	124.4880			

Number of internodes per branch (NIB)

Source of variation	d.f.	s.s	m.s	v.r	F pr
Varieties	19	2717.43	143.02	9.12	<.001
Residual	380	5960.65	15.69		
Total		399	8678.08		

Number of fruit per internode of primary branches (NCB)

Source of variation	d.f.	s.s	m.s	v.r	F pr
Varieties	19	296.974	15.630	12.50	<.001
Residual	380	475.193	1.251		
Total	399	772.167			

Number of primary branches (NPB)

Source of variation	d.f.	s.s	m.s	v.r	F pr
Varieties	19	45702.3	2405.4	23.68	001
Residual	380	38597.5	101.6		
Total	399	84299.8			

Percent of fruit bearing primary branches (PFBPB)

Source of variation	d.f.	s.s	m.s	v.r	F pr
Varieties	19	59355.69	3123.98	61.61	0.007
Residual	380	19268.75	50.71		
Total	399	78624.44			

Plant high (PH)

Source of variation	d.f.	s.s	m.s	v.r	F pr
Varieties	19	50.62547	2.66450	53.49	<.001
Residual	380	18.92787	0.04981		
Total	399	69.55334			

Bean length (BL)

Source of variation	d.f.	s.s	m.s	v.r	F pr
Varieties	3.2038	8.85	<.001		
Residual	380	137.5705	0.3620		
Total	399	198.4420			

Bean thickness (BT)

Source of variation	d.f.	s.s	m.s	v.r	F pr
Varieties	19	28.1164	1.4798	14.77	0.140
Residual	380	38.0620	0.1002		
Total	399	66.1784			

Bean width (BW)

Source of variation	d.f.	s.s	m.s	v.r	F pr
Varieties	19	43.1921	2.2733	14.95	<.001
Residual	380	57.7830	0.1521		
Total	399	100.9751			

Appendix II: Mean values of 10 quantitative traits evaluated at each environment

Appendix 2.1: Mean values of fruit, bean and leaf traits measured in 15 coffee accessions evaluated at Kayanza

Genotypes	Code	LL	LW	LA	FL	FW	100CW	BL	BW	BT	100BW
ABK 5691	A1	11.36	5.48	54.76	14.69	13.28	158.27	12.11	10.19	4.78	17.87
ABK 5718	A2	11.53	5.4	54.81	14.77	13.37	156.33	12.22	10.11	4.77	18.33
B 71	B1	13.58	5.62	67.2	15.71	13.07	167.14	12.36	8.92	4.7	19.69
B 139	B2	12.78	5.45	61.27	16.41	12.38	167.2	12.97	7.92	4.61	19.4
Blue Mountain	B3	12.61	5.29	58.64	16.97	12.14	162.84	13.36	7.66	4.43	19.23
Mi TV	B4	12.32	5.83	63.24	16.84	11.5	175.29	13.96	7.61	4.38	19.49
J2/1257	J1	12.53	5.53	61.05	15.97	12.35	171.22	12.41	8.04	4.73	19.24
K7	K	13.57	5.39	64.41	15.55	13.1	176.22	12.38	9.04	4.66	19.39
Mi68/1589	M1	12.83	5.38	60.7	16.39	11.66	170.93	12.99	7.88	4.63	19.56
Mi49/1848	M2	13.46	5.35	63.32	16.45	12.73	171.1	12.64	8.49	4.54	19.16
Mysore	M3	13.76	5.51	66.68	15.96	13.12	185.9	12.44	8.92	4.63	22.26
S 795	S1	13.75	5.54	66.99	16.1	13.09	185.21	12.51	9	4.81	22.44
S 288	S2	11.94	5.33	56.04	15.03	13.17	155.56	12.51	9.97	4.6	18.14
SL 28	S3	13.73	5.75	69.51	16.3	12.98	182.02	12.24	8.91	4.77	21.95
Tekisic	T	12.28	5.42	58.56	15.35	11.94	174.29	12.78	8.05	4.72	19.17
Mean		12.8	5.48	61.81	15.9	12.66	170.63	12.66	8.71	4.65	19.69
LSD (0.05)		0.86	ns	5.5	0.82	0.54	5.79	0.78	0.38	0.32	0.88

FL=fruit length in mm; FW=fruit width in mm; 100CW= hundred cherries weight in gm; BL=bean length in mm; BW=bean width in mm; BT=bean thickness in mm; 100BW= hundred bean weight in gm; LL=leaf length in cm; LW=leaf width in cm; LA=leaf area in cm^2.

Appendix 2.2: Mean values of fruit, bean and leaf traits measured in 15 coffee accessions evaluated at Rukoba.

Genotypes	Code	LL	LW	LA	FL	FW	100CW	BL	BW	BT	100BW
ABK 5691	A1	11.42	5.11	51.34	13.52	12.99	147.89	10.27	9.34	5.35	16.72
ABK 5718	A2	11.27	5.41	53.61	13.53	12.89	151.13	10.54	9.54	5.28	16.73
B 71	B1	13.4	5.39	63.57	15.29	12.69	162.37	12.53	8.53	4.58	18.53
B 139	B2	12.73	5.52	61.86	15.51	11.87	166.54	12.65	7.64	4.58	17.81
Blue Mountain	B3	12.62	5.41	60.15	15.57	12.1	161.58	12.26	7.68	4.49	17.95
Mi TV	B4	12.62	5.21	57.87	16.03	11.35	154.17	12.17	7.33	4.37	17.76
J2/1257	J1	12.58	5.53	61.13	15.44	12.1	162.11	12.1	7.53	4.42	18.2
K7	K	13.38	5.33	62.77	15.24	12.8	155.01	12.26	8.23	4.64	17.79
Mi68/1589	M1	12.64	5.42	60.23	16.34	11.85	163.54	12.78	7.57	4.39	18.15
Mi49/1848	M2	13.32	5.63	66.04	16.61	12.01	163.97	12.61	7.82	4.48	17.79
Mysore	M3	13.86	5.54	67.55	15.87	12.91	174.13	12.02	8.5	4.67	20.52
S 795	S1	13.77	5.48	66.44	15.26	12.72	177.32	12.51	8.51	4.79	19.5
S 288	S2	11.55	5.7	57.92	14.73	12.43	148.2	12.3	9.41	4.72	16.69
SL 28	S3	13.74	5.83	70.48	15.4	12.64	172.77	12.03	8.44	4.47	20.08
Tekisic	T	12.17	5.58	59.79	15.65	12.56	159.78	12.15	7.79	4.48	18.26
Mean		12.74	5.47	61.39	15.33	12.39	161.37	12.08	8.26	4.65	18.18
LSD (0.05)		0.52	ns	5.19	0.87	0.76	10.3	0.69	0.35	0.3	1.14

FL=fruit length in mm; FW=fruit width in mm; 100CW= hundred cherries weight in gm; BL=bean length in mm; BW=bean width in mm; BT=bean thickness in mm; 100BW= hundred bean weight in gm; LL=leaf length in cm; LW=leaf width in cm; LA=leaf area in cm^2.

Appendix 2.3; Mean values of fruit, bean and leaf traits measured in 15 coffee accessions evaluated at Nyange.

Genotypes	Code	LL	LW	LA	FL	FW	100CW	BL	BW	BT	100BW
ABK 5691	A1	11.55	5.8	58.99	15.27	12.64	149.27	11.95	8.76	4.6	15.04
ABK 5718	A2	11.37	5.43	54.28	15.39	12.61	145.37	12.04	8.7	4.38	15.56
B 71	B1	13.4	5.07	59.77	15.65	12.18	159.99	13.05	8.1	4.68	17.68
B 139	B2	12.62	5.34	59.34	16.46	12.27	161.66	13.49	7.52	4.62	17.6
Blue Mountain	B3	12.79	5.36	60.44	15.86	12.21	166.04	12.79	7.97	4.57	17.44
Mi TV	B4	12.68	5.47	61.1	16	11.84	156.51	13.23	7.49	4.34	17.47
J2/1257	J1	12.64	5.18	57.6	15.88	12.06	164	12.81	7.63	4.59	17.05
K7	K	13.59	5.2	62.16	15.56	12.28	158.23	12.27	8.18	4.68	17.13
Mi68/1589	M1	12.4	5.34	58.29	16.84	11.88	166.9	13.62	7.82	4.6	17.77
Mi49/1848	M2	13.28	5.42	63.36	16.23	11.98	159.26	13.08	7.88	4.51	16.92
Mysore	M3	13.84	5.47	66.63	15.83	12.27	170.5	12.83	7.92	4.64	19.33
S 795	S1	13.83	5.63	68.52	15.5	12.29	169.08	12.44	8.13	5.06	19.09
S 288	S2	11.42	4.93	49.51	15.26	12.47	151.44	12.44	8.65	4.37	15.99
SL 28	S3	13.8	5.41	65.67	16.38	12.32	170.9	13.24	8.19	4.78	19.21
Tekisic	T	12.55	5.47	60.37	15.61	11.96	161.68	12.86	7.66	4.49	17.54
Mean		12.78	5.37	60.4	15.85	12.22	160.72	12.81	8.04	4.59	17.39
LSD (0.05)		0.43	0.57	6.37	0.65	0.37	7.68	0.58	0.44	0.21	0.72

FL=fruit length in mm; FW=fruit width in mm; 100CW= hundred cherries weight in gm; BL=bean length in mm; BW=bean width in mm; BT=bean thickness in mm; 100BW= hundred bean weight in gm; LL=leaf length in cm; LW=leaf width in cm; LA=leaf area in cm^2.

Appendix 3: ANOVA summary for variance components studied at each environment for 100 CW

Mean square at each environment

S V	DF	KAYANZA	NYANGE	RUKOBA
Replication	2	3.28	53.31	3.51
Genotype	14	281.07**	177.38**	244.70**
Error	28	12.7	18.96	40.68

Appendix 4: ANOVA summary for variance components studied at each environment for 100 BW

Mean square at each environment				
S V	DF	KAYANZA	NYANGE	RUKOBA
Replication	2	0.1630	0.5534	0.9559
Genotype	14	6.01**	4.6116**	3.8486**
Error	28	0.2875	0.1614	0.4394

Appendix 5: Coffee Descriptor Used in the Study

Plant descriptors

Vegetative

Plant habit

- Bush (<5 m - without distinct trunk)
- Shrub or small tree (<5 m - one or more trunks)
- Tree (>5 m - single trunk)
- Plant height

Visual estimation

- Very short
- Short
- Tall
- Very tall

Overall appearance

Specify age of plant

- Elongated conical
- Pyramidal
- Bushy

Branch-ramification number

Average of ramifications scored on five well-developed branches

Branching habit

- Very few branches (primary)
- Many branches (primary) with few secondary branches
- Many branches (primary) with many secondary branches
- Many branches (primary) with many secondary and tertiary branches

Angle of insertion of primary branches

(On the main stem)

- Drooping
- Horizontal or spreading
- Semi-erect

Young leaf colour

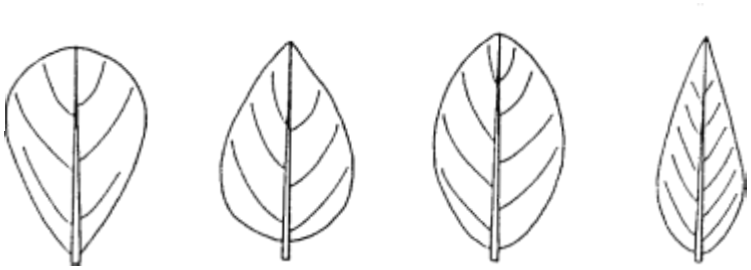
- Greenish
- Green
- Brownish
- Reddish brown
- Bronze
- Other (specify in descriptor

Notes)

Leaf shape (See Figure 4)

- Obovate
- Ovate
- Elliptic
- Lanceolate
- Other (specify in descriptor

Notes)



1 2 3 4

Figure 4 Leaf shape

Leaf apex shape

(See Fig. 5)

Round

Obtuse

Acute

Acuminate

Apiculate

Spatulate

Other (specify in descriptor

Notes)

Figure 5 Leaf apex shape

Leaf length [mm]

Average of five mature (> node 3 from the terminal bud) leaves, measured from petiole end to apex

Leaf width [mm]

Average of five mature (> node 3 from the terminal bud) leaves, measured at the widest part

Young shoot colour

Green

Dark brown

Other (specify in descriptor

Notes

Fruit

Fruit colour

Observed on mature fruits

Yellow

Yellow-orange

Orange

Orange-red

Red

Red-purple

Purple

Purple-violet

Violet

Black

Other (specify in descriptor

Fruit shape

Average of five normal (not caracoli) mature fruits. (See Fig. 6)

Roundish

Obovate

Ovate

Elliptic

Oblong

Other (specify in descriptor 6.5 Notes)

Figure 6 Fruit shape

Fruit length [mm]

Average of five normal mature green fruits, measured at the largest part

Fruit width [mm]

Average of five normal mature green fruits, measured at the widest part

Fruit thickness [mm]

Average of five normal mature green fruits, measured at the thickest part

Seed

Seed length [mm]

Maximum length average of five normal mature seeds

Seed width [mm]

Average of five normal mature seeds, measured at the widest part

Seed thickness [mm]

Average of five normal mature seeds, measured at the thickest part

Seed colour

(At 11% humidity)

Yellow

Brown-purple

Other (specify in descriptors 6.5 Notes)

Seed shape (3.13)

Round

Obovate

Ovate

Elliptic

Oblong

Other (specify in descriptor

Notes

Any additional information, especially in the category of ‘other’ under various descriptors above, may be specified here

Plant descriptors

Vegetative

Trunk diameter [cm]

Measured at 5 cm above ground level in seedling and cutting trees, or 10 cm above graft union in grafted tree. Specify approximate tree age

Trunk height [m]

Measured on the highest trunk, from ground level to top. Specify approximate tree age

Number of primary branches

Total number of primary branches counted per tree

Length of primary branches (cm)

Average of five primary branches at the middle of the stem, measured from point of attachment to main stem to apex of branch

Internode length on primary branches (cm)

Average of five primary branches at the middle of the stem per tree, calculated as length divided by the number of nodes

Number of internodes per branch

Average of five primary branches nodes counted per tree

Yield characteristics

Number of cherries per internode

Number of cherries per internode on five primary branches, obtained by the ration between the total number of cherries bearing node of primary branch and the number of node bearing per each primary branch selected

Percent of fruit bearing primary branches per tree

Percent fruit bearing primary branches obtained by the ratio between the number primary branches produced and the number of fruiting branches per tree

100 fruits weight (gm)

Hundreds of normal cherries of each coffee tree weight using sensitive balance

100-bean weight [g]

Calculated at (11% moisture) content as follows: (“Bean weight at 0% moisture content” x 100) / (“Bean number” x 0.89)

Appendix 6: Publication 1 Abstract