GENETIC DIVERSITY AND TRAIT VARIABLITY IN SOME COFFEE [*Coffea arabica* L.] GENOTYPES FROM SOUTH ETHIOPIAN

PhD DISSERTATION

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HAWASSA UNIVERSITY, HAWASSA, ETHIOPIA

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(SPECIALIZATION: PLANT BIOTECHNOLOGY)

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This is to certify that the thesis entitled "Genetic Diversity and Trait Variablity in Some Coffee [*Coffea arabica* L.] Genotypes from South Ethiopian" submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy (PhD) with specialization in Plant Biotechnology, the Graduate Program of the School of Plant and Horticultural Sciences, College of Agriculture, and is a record of original research carried out by **Habtamu Gebreselassie Addo**, under my supervision and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged. Therefore, I recommend it be accepted as fulfilling the thesis requirements.

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I hereby declare that this PhD dissertation is my original work and has not been presented for a degree in any other university and all sources of material used for this dissertation have been duly acknowledged.

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BIOGRAPHICAL SKETCH

The author was born from his father Ato Gebreselassie Addo and his mother W/ro Maleche Bogale in southern Ethiopia, Keffa Zone, Decha District on Augest 15, 1985. He completed his elementary school (1992-1997), junior secondary school (1998-1999), and high school (2010-2003) at Dekya Elementary School, Wushwush Elementary and Junior Secondary School, and Bonga Comprehensive Secondary School, respectively. Then he joined Hawassa University in 2004 and graduated with a Bachelor of Science Degree in Food Science and Post-Harvest Technology in July 2006. After his graduation, he was employed by the Ministry of Agriculture in a different position in Kaffa Zone at Decha woreda. After serving for four years, he joined the Haramaya University for MSc study and graduated with a M.Sc. degree in horticulture in July 2013. Soon after his graduation, he was employed by SNNPRS Job opportunity creation agency, later hired by Assosa University, and served as a university instructor. In August 2014, he joined Ethiopian Institute of Agriculture Research and worked as assistant Researcher II, Associate Researcher I, and Researcher I in breeding and genetics at Wondo Genet Center, published 16 scientific articles in peer-reviewed journals. He also served as the Awada Agricultural Research Sub-Center Director for more than four years. Then, he joined Hawassa University in October 2019 to pursue his PhD degree in Plant Biotechnology Specialization.

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DEDICATION

This piece of work is dedicated to my late father Ato Gebreselassie Addo, my mother W/ro Maleche Bogale, my beloved wife Firmaye Tesfaye, and my children Betsegaw and Yilikal.

LIST OF ACRONYMS AND ABBREVIATION

AARSC	Awada Agricultural Research Sub-Center
AFLP	Amplified Fragment Length Polymorphism
AMMI	Additive main-effects and multiplicative interaction
AMOVA	Analysis of molecular variance
ASV	AMMI's stability value
BIC	Bayesian information criterion
CBD	Coffee Berry Disease
CLR	Coffee leaf Rust
CRD	completely randomized design
CSA	Central Statistical Agency
CTAB	Cetyltrimethylammonium bromide
DAPC	discriminate analysis of principal component
DNA	Deoxyribonucleic acid
ECTA	Ethiopian Coffee Tea and Spice Authority
ECX	Ethiopian commodity exchange
EIAR	Ethiopian institute of agricultural research
ENA	Ethiopian News Agency
FAO	Food and Agriculture Organization
GA	Genetic advance
GAM	Genetic advance as a percentage of the mean
GCA	General combining ability
GCV	Genotypic coefficient of variation,
GEI	genotype by environment interaction
GGE-Biplot	Genotype and genotype by environment interaction biplot
H'	Shannon-Weaver diversity index
Hb%	broad sense heritability
ICO	International Coffee Organization
IPGRI	International Plant Genetic Resources Board descriptor for coffee
ISSR	Inter Simple Sequence Repeats

ITC	International Trade Centre
JARC	Jimma Agricultural Research Center
LSD	Least significant difference
MAS	Marker Assisted Selection
PCA	Principal component analysis
PCoA	Principal coordinate analysis
PCR	Polymerase chain reactions
PCV	Phenotypic coefficient of variation
Pi	Cultivar superiority index
RADP	Randomly Amplified Polymorphic DNAs
REML	restricted maximum likelihood
RFLP	Restriction Fragment Length Polymorphism
SAS	Statistical Analysis System
SCA	Specific combining ability
SNNPRS	South Nations Nationalities and Peoples Region
SRAP	Sequence-related Amplified Polymorphism
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
USDA	United States Department of Agriculture
WCR	World Coffee Research
YSI	Yield Stability Index

MANUSCRIPTS TITLES/PUBLICATIONS DURING CANDIDATURE

Habtamu Gebreselassie, Bizuayehu Tesfaye, and Andargachewu Gedebo, (2024). Genetic Diversity of Arabica Coffee Genotypes Using Quantitative Agro-morphological Traits in South Ethiopia. *Genetic Resources and Crop Evolution*. 71(7): 3485-3506 https://doi.org/10.1007/s10722-023-01853-9

Habtamu Gebreselassie, Bizuayehu Tesfaye, Andargachewu Gedebo, and Kassaye Tolessa, (2023). Genotype by environment interaction and stability analysis using AMMI and GGE - biplot models for yield of Arabica coffee genotypes in south Ethiopia. Journal of Crop Science and Biotechnology.27(4):65-77 https://doi.org/10.1007/s12892-023-00213-4

Habtamu Gebreselassie, Bizuayehu Tesfaye, Andargachewu, Gedebo, and Kassaye Tolessa, (2024). Evaluation of physical and chemical characteristics of bean and cup quality of arabica coffee genotypes grown in Southern Ethiopia. Heliyon, 10(14): 1-16. https://doi.org/10.1016/j.heliyon.2024.e34378

Table of Contents

List of Tables

List of Figure

List of Table in Apendis

Thesis Abstract

DIVERSITY OF SOUTH ETHIOPIAN COFFEE [*Coffea arabica* L.]: AGRO-MORPHOLOGICAL, BIOCHEMICAL AND MOLECULAR ASPECTS

Ethiopia is the center of origin and genetic diversity for Coffea arabica, a crop that plays a central role in the country's economy, culture, and identity. Coffee provides a livelihood to about 25 million people. Despite Ethiopia's favorable climate, soil, and rich genetic resources, national coffee productivity remains low as compared to leading producer and world average. This is partly due to our incomplete knowledge of the wider adaptability and stability, morphological, biochemical, and molecular diversity among characteristics of the plant and the relevant agricultural traits of the plant in general, and South Ethiopian genotypes, in particular.

To address this gap, five integrated studies were conducted to evaluate the morphological, biochemical, and molecular diversity of South Ethiopian Arabica coffee genotypes and their relationship to yield and quality performance. These studies aimed to support genetic improvement efforts through better trait characterization, genotype selection, and understanding of genotype × environment interaction (GEI).

The first two studies involved the evaluation of 17 genotypes (14 selections and 3 released varieties) at Awada, Wonago, and Shebedino using a randomized complete block design (RCBD). A total of 26 quantitative and 15 qualitative traits were recorded. Results revealed wide phenotypic variation, with Shannon-Weaver diversity indices ranging from 0.22 to 1.12. Significant differences (p<0.05) were found for most qualitative traits, confirming high morphological diversity.

Quantitative trait analysis showed significant variation among genotypes, environments, and their interaction for several yield-related traits. Traits such as number of branches, canopy diameter, fruit size, and seed traits exhibited moderate-to-high heritability and genetic advance, indicating potential for improvement via selection. Principal component and cluster analyses grouped the genotypes based on trait similarities, highlighting key contributors to phenotypic diversity. Genotypes AW7705 and AW105 showed superior yield performance (1.37 t/ha), with AW7705 also demonstrating yield stability across environments.

The third study analyzed GEI effects using AMMI and GGE-biplot models across 12 environments. The interaction was significant, accounting for 17.5% of total variation. Genotype AW7705 (G16) was identified as both high-yielding and stable, making it a best candidate for *variety development in Sidama, Gedeo, and similar agro-ecologies. Conversely, AW105 (G7) was high-yielding but less stable, suggesting potential for targeted environments. These results emphasize the importance of multi-location trials in coffee breeding to capture GEI effects and ensure stability.*

In the fourth study, 30 genotypes were assessed for physical traits, cup quality, and biochemical composition using RCBd design. Significant variation was found among genotypes and environments almost all the studied traits. Most genotypes scored above 80%, qualifying them as specialty coffee. Biochemical diversity included a wide caffeine range (0.52%–1.53%), offering opportunities to develop low-caffeine varieties. PCA and cluster analyses identified key traits contributing to quality variation, including caffeine, trigonelline, chlorogenic acid, acidity, and flavor. Genotype AW9648 consistently exhibited high-quality scores, making it suitable for both quality breeding and specialty market promotion.

The fifth study, the genetic diversity of 40 accessions from Sidama, Guji, Amaro, and Jinka and 10 improved varieties analyzed using four ISSR markers. A total of 74 bands were generated, with private bands and higher polymorphism observed in improved varieties. Genetic diversity indices showed considerable variation among populations, with AMOVA revealing 67% of total variation occurring among populations. Principal coordinate analysis explained 43% of the genetic variation across the first three axes. UPGMA clustering grouped genotypes into four major clusters, often reflecting geographic origin. These findings confirm the rich genetic base in southern Ethiopian coffee and support its use in breeding and conservation programs.

The integrated results of these five studies confirm the presence of significant agro-morphological, quality, and molecular diversity among South Ethiopian coffee genotypes. Significant GEI effects further underline the need for multi-environment testing to develop widely adaptable and stable varieties. AW7705 and AW105 were high yielder and found to be promising candidate for yield competitive variety development; whereas, AW9648 was identified as superior quality. These results provide a foundation for selection, hybridization, and conservation strategies in Arabica coffee improvement. Future research should include more accessions and high-resolution molecular markers to enhance the precision of genetic diversity studies and guide sustainable crop improvement.

Key words: Arabica Coffee, Cup Quality, Genetic Diversity, Growing Environment, ISSR Markers, Population, Qualitative Traits, Quantitative Traits, Yield Stability

CHAPTER ONE

1. GENERAL INTRODUCTION

Coffee belongs to the family rubiaceae and the genus Coffea (Davis *et al*., 2011). The Rubiaceae is the fourth largest angiosperm family with 611 genera (Davis *et al*., 2009). According to Davis and Rokotonasolo (2021), 130 species have been identified in the genus coffea. Out of those species, only two species (*Coffea arabica* L. and *Coffea canephora*) are economically important species (Lachenmeier, 2023). *C. arabica* is the only tetraploid species in the genus and is self-fertile, while other species are diploid and generally self-incompatible (Lachenmeier, 2023).

Ethiopia is the origin and center of genetic diversity for the Arabica coffee species (Vavilov 1951). The country has collected over 12,452 accessions from various coffee growing ecologies and maintained them in *in-situ* and *ex-situ* gene banks (Yirga *et al*., 2021). Different researchers witnessed the existence of abundant genetic diversity among Ethiopian coffee genotypes (Abdulfeta 2018; Argaw & Taye 2018; Beksisa *et al*. 2018; Dawit 2018; Desalegn 2018; Beksisa 2021; Merga *et al*., 2021). The Ethiopian coffee genepool represents the most important and diversified genepool of this species (Bakala *et al*. 2022).

Coffee is the most widely traded commodity next to petroleum in the world providing a livelihood to 125 million people (Tran *et al.,* 2016). Millions of people around the world treasure its beverage and over 2.25 billion cups are consumed every day in the Globe (ICO, 2014). About 56 developing countries that produce and export coffee generate a total of more than US$42.3 billion in 2023 (ICR, 2024), and employ over 125 million people (Tran *et al*., 2016). Its popularity and volume of consumption are growing every year, and coffee shops are the fastest growing part of the restaurant business.

Coffee is well known pillar of Ethiopian economy. Until the 1990s, it was fetching about 60% of foreign exchange for the nation (Yilma, 2017). Since then, with gradual diversification of export commodities, its export share has declined; but, still, it plays a pivotal economic role by accounting on average, 32% of the total government's export revenue, 35% of agricultural export share (USDA, 2023), and its exports generated 1.4 billion USD in the 2022 fiscal year for the country (ECTA 2023; ENA, 2023). Furthermore, the crop provides livelihoods to about 25 million smallholder farmers and other actors directly or indirectly (Yirga *et al*., 2021). In addition to economic importance, coffee is also the defining feature and sphere of Ethiopian culture, politics, social life, and identity. An invitation to drink coffee is considered a symbol of friendship, respect, and great hospitality (USDA, 2023). The cultural ceremony of Ethiopian coffee beverage preparation and drinking is unique and stunning. Ethiopia is one of the world's largest coffee consumer with about 55 % of the production consumed domestically (USDA, 2021).

Ethiopia is the leading producer in Africa, and the world's fifth largest exporter, following Brazil, Vietnam, Colombia and Indonesia contributing about 4.2% of the total world coffee production (USDA, 2023). The total productive coffee area in Ethiopia is estimated at 856,591.99 hectares with annual average production of 5.85 million tons and productivity of 0.68 ton/ha (Motebayenore, 2022). According to ICO statistics, the production of Ethiopian coffee has been constantly increasing since 2000/01 harvest season. The annual production stood at the highest level of 8.10 million bags (= 486 thousand tons) in 2012/13 compared to 3.11 million bags (= 186.6 thousand tons) in 2000/01 cropping season. The productivity has also reportedly reached 748 kg/ha in 2014 compared to only less than 300 – 500 kg/ha before a decade (CSA, 2015). It is believed that the surge in production is largely attributed to the increase in cultivated area from 400,000 ha in the early 2000s to roughly 560,000 ha in 2015 (CSA, 2015; Bayetta, 2015). Ethiopia also has the largest highland area suitable for production and, hence has the potential to be a leading producer of coffee in both quality and quantity (CSA, 2015; Bayetta, 2015). Nearly all coffees produced in Ethiopia are shade grown, with 40-60% canopy cover, except few home garden systems in Eastern Ethiopia. The coffee plants are also mainly either local varieties/ land races or of wild origin (Bayetta, 2015).

Preliminary coffee improvement work in Ethiopia was started in 1952 by the then called Jimma Agricultural Technical School (JATS) with the help of P.G. Sylvain, FAO coffee specialist, who provided seeds of 50 Arabica varieties, and 6 varieties of other species (Belachew, 1968). Subsequently, the JATS had made additional collections of indigenous coffee types and international varieties. In 1965, the FAO coffee collection mission to Ethiopia (FAO, 1968) handed over 433 additional accessions to the technical school. Under the Institute of Agricultural Research (IAR), a comprehensive research work on coffee was started after the establishment of Jima Agricultural Research Center (JARC) in late 1967. Following JARC establishment, Sub-Centers including Awada Agricultural Sub-Center was established in1990. Long and short term breeding programs were immediately launched with the major objectives of collecting and conserving coffee germplasm, develop cultivars that combine high yield, disease resistance and good quality, and multiply and supply improved seeds (Bayeta, 1998). The first breeding activity was started with 76 accessions obtained from French Coffee Collection Mission to Ethiopia in 1966 and 123 Arabica varieties introduced from different countries (Bayeta, 1998). Since then, a number of research achievements were made in germplasm collection, finding a number of CBD resistant cultivars and heterotic hybrids in conventional breeding approach. Following the standard steps over 6,700 germplasm has been collected, 6 hybrid and 40 pure line varieties were developed and further steps are underway to release high yielder, best quality and disease resistant/tolerant hybrids and pure-line varieties (EIAR. 2017; Benti, 2017). However, given the wealth of coffee genetic resources Ethiopia has and its diverse agro-ecologies, these achievements are far short of what is needed to support the growing coffee industry in Ethiopia and coffee production still faces various challenges in terms of development of high yielder, major disease resistant, and wider agro-ecology adaptable varities.

Studying the genetic diversity of *Coffea arabica* is a fundamental component of crop improvement programs, as it provides the basis for effective selection and hybridization strategies. Understanding the extent and pattern of genetic variation enables breeders to identify genotypes with desirable traits, assess the potential for selection, and determine suitable parental lines for crossing. Such analyses help reveal which traits contribute most significantly to the observed diversity, offering valuable insights into their potential utilization in breeding programs aimed at improving yield, quality, disease resistance, and adaptability to environmental stresses.

Different genetic diversity assessment methods, viz., molecular, biochemical, and morphological markers are used to analyze genetic diversity. Of these, the morphological method is direct, simple to score, and easy to apply, and its estimations were perceived as the determinant of the plant's agronomic value and taxonomic classification (Bhandari *et al.* 2017). Plant breeders can differentiate and identify the promising genotypes for cultivar development by assessing morphological characters (Akpertey *et al.*, 2019). In Ethiopia, until today, coffee genetic diversity assessment through morphological traits has been the main tool that has been successfully used to analyze genetic diversity for improved cultivar development (Benti 2017).

Green bean physical character and beverage quality assessment by a professional coffee cupper is crucial to examine the coffee quality and identify characteristic variations of the genotype. In the Ethiopian coffee research program, a coffee-cupping protocol was developed for the evaluation of coffee genotypes with specific evaluation criteria for the physical characteristics of green beans and cup quality (Sualeh & Mekonnen, 2015). The main bean physical characteristics are bean odor, color, and shape, whereas cup quality attributes are flavor, acidity, body, aromatic quality, aromatic intensity, astringency, bitterness, and overall cup quality.

In conventional coffee breeding and selection program, it takes long time to develop a new coffee cultivar (Benti, 2017). Use of molecular markers associated with important traits can make coffee breeding more precise, rapid, allow the detection of variations in DNA level, overcome environmental factors and growth stage of the plant, and probably cost effective in comparison to phenotypic selection (Henry, 1997; Ni *et al*., 2008). Some of the DNA based markers that have been extensively used in coffee genetic diversity studies includes Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Randomly Amplified Polymorphic DNAs (RADP), Sequence-related Amplified Polymorphism (SRAP) and Simple Sequence Repeats (SSR), Inter Simple Sequence Repeat (ISSR), single-nucleotide polymorphisms (SNPs) markers (Maluf *et al.*, 2005; Mishra *et al*., 2011; Oktavioni *et al*., 2020; Benti *et al*., 2021).

1.1 Problem Statement

Under Ethiopian Institute of Agricultural Research in coffee commodity research program, there are five national coffee research projects viz., South Western Ethiopia, South Ethiopia, Western Ethiopia, Eastern Ethiopia and New Potential Coffee Growing Areas, which are mandated to run a comprehensive research activity on Arabica coffee (EIAR, 2017). Awada Agricultural Research Sub center in collaboration with Jimma Agricultural Research Center mandated to run research activities on South Ethiopia coffee research project mainly on Sidama and Yirgachefe coffee (AARSC, 2000). But, no research achievement is reported on Gujji, Amaro, Jinka, and Gamogofa coffee, which are source of Arabica coffee germplasm having immense genetic diversity for coffee improvement. Therefore, this study broadened the scope of the south coffee research project and addressed unaddressed but high potential and immense diverse coffee types of Amaro, Gujji, Gamogofa and Jinka.

Despite the importance of coffee in Ethiopia's economy and the presence of huge genetic resources, a favorable climate, and soil types for coffee production and productivity, the crop yield is by far very low (0.68) as compared to the yield recorded in Vietnam (2.4 ton/ha), Brazil (1.4 ton/ha), Colombia (0.9 ton/ha), and the world average (0.8 ton/ha) (WEF 2021).

Many factors contributed to low level of production and productivity; lack of high-yielding improved varieties with consistent performance in wide ranges of environments, biotic, abiotic (low soil fertility, poor agronomic management), and failure to use appropriate technology (improved variety, fertilizer) (Ayele *et al*., 2021). As compared to the extent of genetic diversity available, the effort made to efficiently utilize them is still very little (EIAR, 2017). In this regard, this study focused on the promotion of promising selection for development of yield competitive variety to increase production and productivity of the nation.

Environmental conditions strongly influence agricultural production, leading to considerable variations in yield and quality. Such influence is discriminated when yield experiments are performed in various locations and different years (Beksisa 2021; Merga 2021; Ndikumana 2022). South Ethiopia accounts for more than 46% of the Ethiopian coffee market (NBE, 2011). Most of the bean yield performance and stability studies over location were done in southwest Ethiopian growing ecologies, but little information was generated for wider adaptability and stability in south Ethiopian and other ecologies. Thus, investigation of southern Ethiopian genotypes in the main south Ethiopian coffee growing ecologies is an urgent task. Thus, conducting a study that targets the evaluation of existence and extent of the effect of GEI on Arabica coffee bean yield across the diverse agro-ecologies of south Ethiopia is necessary.

Substantial variation was observed in coffee -green bean caffeine, chlorogenic acids, sucrose and trigonelline contents (Silvarolla *et al*., 2004), bean size, shape and color and cup quality (Wondimu, 1998). However, in most of collection maintained in gene bank including all south coffee collections, biochemical variability analysis is yet to be done (EIAR, 2017).

Many researchers (Marie *et al*., 2020; Beksisa 2021; Merga 2021; Legesse *et al*., 2022) have reported the existence of high agro mophological diversity among coffee germplasm collections in Ethiopia. This high variation is yet to be confirmed with molecular characterization. Until now, no molecular genetic diversity has been conducted to determine the variation in south Ethiopian coffee gerrmplasm (EIAR, 2017; Benti, 2017). Hence, in conventional breeding achieving a precise result is too long, there is urgent need to supplement conventional breeding with molecular breeding techniques. Taking these recurring situations, this research work was conducted to address the following research objectives to improve coffee production and productivity in near future.

1.2 Research Objectives

1.2.1 General Objective

To assess diversity and trait variation in South Ethiopian *Coffea arabica* using morphological, biochemical, and ISSR markers for yield and quality improvement

1.2.2 Specific Objectives

1. To characterize and evaluate south Ethiopian coffee genotypes based on qualitative and quantitative agro-morphology traits
2. To determine the extent and nature of GEI on coffee bean yield and to identify the stable genotypes that exhibit wide adaptation over south Ethiopian agroecologies
3. To investigate biochemical and cup quality variability among south Ethiopian coffee genotype
4. To analyze the genetic diversity and population structure of South Ethiopian coffee genotypes using ISSR markers

1.3. Thesis Organization

This thesis work contains separate chapters that address the above specific objectives. The chapters were inter-related and prepared as distinct research papers; therefore, overlaps might occur in terms of contents and references. The chapters are organized as follows:

List of Chapters	Title of Chapters
Chapter 1	General Introduction
Chapter 2	Phenotypic Diversity of Arabica Coffee (*Coffea arabica* L.) Genotypes for Qualitative Characteristics at Awada, Ethiopia
Chapter 3	Genetic Diversity of Arabica Coffee Genotypes in South Ethiopia Using Quantitative Agro-morphological Traits
Chapter 4	Genotype by Environment Interaction and Stability Analysis Using AMMI and GGE-Biplot Models for Yield of Arabica Coffee Genotypes in South Ethiopia Abstract
Chapter 5	Evaluation of Physical and Chemical Characteristics of Bean and Cup Quality of Arabica Coffee Genotypes Grown in Southern Ethiopia
Chapter 6	Genetic Diversity and Population Structure Analysis of South Ethiopian Coffee [*Coffea arabica* L.] Genotypes Using ISSR Marker
Chapter 7	General Summary, Conclusion and Recommendations

1.3 Reference

AARSC (Awada Agricultural Research Sub-center), (2000). Annual research progress report of the year 2000. Awada, Ethiopia.

Akpertey, A., Anim-kwapong, E., & Ofori, A, (2019). Assessment of Genetic Diversity in Robusta Coffee Using Morphological Characters Assessment of Genetic Diversity in Robusta Coffee Using Morphological Characters. International Journal of Fruit Science, 19(3), 276–299. https://doi.org/10.1080/15538362.2018.1502723

Argaw, T., & Taye, G. (2018). Genotype by Environment Interaction Analysis of Arabica Coffee Bean Yield. 6(October), 509–519. https://doi.org/10.14662/ARJASR2018.090

Ayele, A., Worku, M., & Bekele, Y. (2021). Heliyon Trend , instability and decomposition analysis of coffee production in Ethiopia (1993 – 2019). Heliyon, 7(September), e08022. https://doi.org/10.1016/j.heliyon.2021.e08022

Bakala, N., Bersisa, H., Tilehun, A., & Misgana, Z. (2022). Assessment on the Current Status of Coffee Arabica (*Coffea arabica* L) Landraces Diversity in Coffee Belt Areas of Western Oromia , Ethiopia. 10(1), 24–30. https://doi.org/10.11648/j.ijbmr.20221001.12

Bayeta Belachew, (1998). Coffee Breeding (unpublished). Jima agricultural research station, IAR, 36p.p

Bayetta Belachew, (2015). ACRN activity report for the period 2013 – 2015. IACO Abidjan, 2015.

Beksisa, L. (2021). GGE Biplot Analysis of Genotype x Environment Interaction and Bean Yield Stability of Arabica Coffee (Coffee arabica L .) Genotypes in Southwestern Ethiopia. 9(3), 110–115. https://doi.org/10.11648/j.ajbio.20210903.16

Beksisa, L., Alamerew, S., Ayano, A., & Daba, G. (2018). Genotype × environment interaction and yield stability of Arabica coffee (*Coffea arabica* L .) genotypes. 13(4), 210–219. https://doi.org/10.5897/AJAR2017.12788

Benti, T. (2017). Progress in Arabica Coffee Breeding in Ethiopia: Achievements, Challenges and Prospects. International Journal of Sciences: Basic and Applied Research, 33(2), 15–25. http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Benti, T., Gebre, E., Tesfaye, K., Berecha, G., Kyallo, M., & Yao, N. K. (2021). Genetic diversity among commercial arabica coffee (*Coffea arabica* L .) varieties in Ethiopia using simple sequence repeat markers. Journal of Crop Improvement, 35(2), 147–168. https://doi.org/10.1080/15427528.2020.1803169

Bhandari HR, Bhanu AN, Srivastava K, *et al*., (2017). Assessment of genetic diversity in crop plants - an overview. Adv Plants Agric Res. 7(3):279-286. https://doi.org/10.15406/apar.2017.07.00255

CSA (Central Statistical Authority), (2015). Federal Republic of Ethiopia, Central Statistical Agency, Agricultural Sample Survey Report on Area and Production of Major Crops, 2014/2015, Vol. 1, Statistical Bulletin 578, May 2015, Addis Ababa, 121P.

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

Davis, A. P., & Rakotonasolo, F. (2021). Six new species of coffee (Coffea) from northern Madagascar. Kew Bulletin, 76(3), 497–511. https://doi.org/10.1007/s12225-021-09952-5

Davis, A. P., Govaerts, R., Bridson, D. M., Ruhsam, M., Moat, J., & Brummitt, N. A. (2009). A Global assessment of distribution, diversity, endemism, and taxonomic effort in the rubiaceae. Annals of the Missouri Botanical Garden, 96(1), 68–78. https://doi.org/10.3417/2006205

Dawit, (2018). Genetic Diversity Analysis among Promising Wollega Coffee (Coffea arabica l.) Selections in Western Ethiopia. MSc. Thesis of Hawassa University. Thesis Submitted to Graduate Studies of in partial fulfillment of Hawassa University, Hawassa Ethiopia. P38.

Desalegn A., (2018). Genetic Variability and Character Association of Amaro Coffee (Coffea arabica l.) Accessions at Awada, Southern Ethiopia. Thesis Submitted to Graduate Studies of Jimma University, Jimma, Ethiopia. P1-38

ECTA (Ethiopian Coffee Tea and Spice Authority), (2023). available at https://borkena.com/2022/09/15/ethiopia-coffee-export-earned-1-4-billion/

EIAR (Ethiopian Institute of Agricultural Research), (2017). National Coffee Commodity Research Strategy of Fifteen Years (2016 -2030). Addis Ababa, Ethiopia. 124: 213–221.

ENA (Ethiopian News Agency), (2023). Ethiopia Generates More Than Half Billion USD From Coffee Export in Four Months of Fiscal Year. https://www.ena.et/web/amh/ Retrieved on January, 2023.

FAO (Food and Agriculture Organization), (1968). FAO coffee collection mission to Ethiopian 1964-1965. Food and Agriculture Organization of the United Nations, Rome, Italy.

Gebreselassie Habtamu. (2023). Estimation of Variability , Heritability , and Genetic

Advance Among Released Varieties and Local Cultivar of Potato (Solanum tuberosum L .) in Eastern Ethiopia. *1*(3), 38–44. https://doi.org/10.11648/j.wjast.20230103.11

Henry R.J., (1997). Molekular markers in plant improvement. In: Practical application of plant molecular biology. Chapman and Hall, New York, pp 101-134.

ICO (International Coffee Organization), (2021). Coffee market report. https:// www. ico. org/ news/ cmr- 0121-e. pdf

ICO (International Coffee Organization), (2014). World coffee trade (1963 – 2013): A review of the markets, challenges and opportunities facing the sector. www.ico.org

ICO (International Coffee Organization), (2015). International Coffee Organization, Annual Review 2015/2016

ICR (Industrial Coffee Roasters), (2024). Coffee Roaster for Businesses. (Accessed 6.1.2025). https://www.worldstopexports.com/coffee-exports-country/

Lachenmeier, D. W., (2023). Identification of Coffee Species, Varieties, Origins, and Processing and Preparation Methods—A Status Report. 9. https://doi.org/10.3390/icc2023-14824

Legesse A., Alamrew S., Tesfaye A., (2022). Stability analysis of coffee (Coffea arabica L.) bean yield using GGE biplot in South Western Ethiopia. Am J Biol Environ Stat 8(1):36–42. https://doi.org/10.11648/j.ajbes.20220 801.15

Maluf M.P, Silvestrini M., Ruggiero L.C.M., Guerreiro Filho O., Colombo C.A., (2005). Genetic diversity of cultivated Coffea arabica inbred lines assessed by RAPD, AFLP and SSR marker systems. Sci. Agric. 62:366-373.

Merga D., Mohammed H., Ayano A., (2021). Estimation of genetic variability, heritability and genetic advance of some wollega coffee (Coffea arabica L.) landrace in Western Ethiopia using quantitative traits. J Plant Sci 9(4):182–191. https://www.org/10.11648/j. jps.20210904.18

Merga W., (2021). An overview of genotype x environment interaction and yield stability analysis in applied plant breeding: great emphasis given to coffee (Coffea arabica L.). Innov Technol 11(2):117–123

Mishra M.K., Tornincasa P., De Nardi B., Asquini E., Dreos R., Del Terra L., Rathinavelu R., Rovelli P., Pallavicini A. and Graziosi G., (2011). Genome organization in coffee as revealed by EST PCR-RFLP, SNP and SSR analysis. J. Crop Sci. Biotech. 14: 17-24.

NBE (National Bank of Ethiopia), (2011). Annual report of National Bank of Ethiopia for Ethiopian 2011 fisical year.

Ndikumana, J., (2022). Agro-morphological Characterization of Arabica Coffee Cultivars in Burundi. A Thesis Submitted in Partial Fulfilment of the Requirements for the degree of Master of Science in Plant Breeding of the Jomo Kenyatta University of Agriculture and Technology. P1-135

Ni S., Yaho M., Chen L., Zhao L. and Wang X.C., (2008). Germplasm and breeding research of tea plant based on DNA marker approaches. Front. Agric. China. 2, 200-2007.

Oktavioni, M., Mada, U. G., Irawati, I., Andalas, U., Syukriani, L., Andalas, U., Setiawan, M. A., & Andalas, U., (2020). Analysis of genetic diversity of Arabica coffee [Coffea arabica L .] in Solok Regency by SRAP molecular markers

Silvarolla M.B., Mazzafera P. and Fazuoli L.C., (2004). A naturally decaffeinated arabica coffee. Nature 429: 826.

Sualeh, A., and Mekonnen, N., (2015). Manual for Coffee. Quality Laboratory. ISBN: 978999446605410-8 . PP. 1-24. https://doi.org/10.13140/RG.2.2.18203.49441

Tran H.T,, Lee LS., Furtado A., Smyth H. and Henry RJ., (2016). Advances in genomics for the improvement of quality in coffee. J Sci Food Agric 96:3300–3312

USDA (The United States Department of Agriculture), (2021). Ethiopia Coffee Annual Report, GAIN Report Number: ET2021-0013, 6p.

USDA (The United States Department of Agriculture), (2023). Ethiopia Coffee Annual Report, GAIN Report Number: ET2023-0014 (2023), 1-7.

Vavilov, N.I., (1951). The origin, variation, immunity and breeding of cultivated plants. (Translated by S. K. Chestitee). Chronica Botonica, 13, 1-366

WEF (World Economic Forum), (2021). Which country produced the most coffee in 2020? Retrieved on 20 Mar 2023 and available at https://www.weforum.org/agenda/

Woyesa, T., & Kumar, S., (2021). Potential of coffee tourism for rural development in Ethiopia: a sustainable livelihood approach. *Environment, Development and Sustainability*, *23*(1), 815–832. https://doi.org/10.1007/s10668-020-00610-7

Yilma Yemane Teferaa, (2017). A guide to coffee production in Ethiopia. Addis Ababa Ethiopia. P 1-280.

Yirga, M., Gebreselassie, W., & Tesfaye, A. (2021). Correlation and Path Coefficient Analysis in Coffee (Coffea arabica L .) Germplasm Accessions in Ethiopia. 9(2), 27–34. https://doi.org/10.11648/j.sr.20210902.12

CHAPTER TWO

2 Phenotypic Diversity of Arabica Coffee (*Coffea arabica L.*) Genotypes for Qualitative Characteristics at Awada, Ethiopia

Abstract

Understanding the amount and distribution of genetic diversity is crucial in managing germplasm and using it efficiently in breeding programs. This study aimed to assess the variation in qualitative morphological traits among 17 genotypes of Arabica coffee to promote strategic conservation and effective utilization. The characterization of Arabica coffee genotypes for qualitative traits displayed a wide range of phenotypic variation, with the Shannon-Weaver diversity index ranging from 0.22 (least polymorphic) to 1.12 (most polymorphic) and an overall mean of 0.67. The chi-square test revealed significant differences for the tarits of branching habit, stipule shape, young leaf colour, leaf shape, leaf apex shape, leaf petiole colour, young shoot colour, overall appearance, canopy diameter, fruit colour, fruit shape, pulp thickness, seed shape, suggesting dominant phenotypic variation among the evaluated traits. Path coefficient analysis showed a positive direct effect of angle of insertion of primary branches (0.485), canopy diameter (0.264), overall appearance (0.101), and leaf apex shape (0.014) on branching habit. According to the PCA, leaf shape (-0.41) and fruit color (-0.36) from the first PCA and leaf apex shape (-0.43) from the second PCA were the important variables contributing more to the variations. The genotypes were classified into five clusters using cluster analysis. The pairwise generalized squared distance among the clusters based on Mahalanobis's D^2 statistics showed significant ($P<0.05$) divergence between most of the clusters. In conclusion, the present study confirmed the existence of qualitative morphological trait variation among evaluated south Ethiopian coffee genotypes. It is recommended that the studied genotypes be properly conserved and utilized for the coffee genetic improvement program through selection and hybridization.

Key words: Cluster Analysis, Genetic diversity, Principal Component Analysis, Qualitative Traits, and Shannon Diversity Index

2.1 Introduction

Coffee is one of the most widely traded commodities in the world and the second most consumed non-alcoholic beverage after water (Samper *et al.*, 2017). Its popularity and volume of consumption are growing every year, and coffee shops are the fastest-growing part of business. Among the 130 species of the Coffea genus in the Rubiaceae family, only Arabica coffee (*Coffea arabica* L.) and Robusta coffee (*Coffea canephora* P.) are the two most economically important species (Lachenmeier, 2023), with Arabica accounting for about 60% of global coffee production and Robusta accounting for approximately 40% (ICO, 2018). Coffee is produced by over 80 tropical countries and exported by 50 countries, with an annual production of about 9 million tons (Sarada, 2017).

Ethiopia is the birthplace of Arabica coffee (*Coffea arabica* L.), which has the largest genetic diversity and abundance of genetic resources in the world (Vavilov, 1951). Arabica coffee is a crucial component of the Ethiopian economy and is deeply intertwined with the social, economic, political, cultural, and spiritual aspects of the country. Ethiopia's unique climate and environment provide ideal conditions for producing high-quality coffee (Tadesse *et al.*, 2020). As of the 2022/23 fiscal year, the total area covered by coffee in Ethiopia is estimated to be 590,000 hectares, with a production and productivity of 496,000 tons and 0.84 tons per hectare, respectively (USDA, 2023).

Genetic diversity refers to the amount of genetic variability present among individuals within a population or variety of a species (Minter *et al.*, 2021). It can be observed at three different levels: diversity between species, diversity between populations within one species, and diversity between individuals within one population (Salgotra & Chauhan, 2023). This diversity is the result of genetic material (DNA) recombination during the inheritance process, mutations, gene flow, and genetic drift, which lead to variations in DNA sequence, protein structure or isoenzymes, epigenetic profiles, physiological properties, and morphological properties (Salgotra & Chauhan, 2023).

Genetic diversity is essential for maintaining a healthy population since it enables individuals to adapt to various biotic and abiotic stresses and promotes resistance to pests, diseases, and other stress conditions (Salgotra & Chauhan, 2023). Under environmental changes, different crop varieties survive due to the presence of genetic variation, which enables the varieties to adapt (Salgotra & Chauhan, 2023).

The wealth of diversity in a species offers ample opportunities for genetic improvement of the crop to develop varieties suitable for different agro-ecologies, cropping systems, and purposes. Genetic diversity aids breeders in maintaining crossbred varieties, resulting in the conservation of desired characteristics in the variety (Temesgen, 2021). The more diverse the breeding materials, the higher the probability that they will contain desirable genes and the higher the probability of improving the crop for traits of interest (Temesgen, 2021). Therefore, diverse lines are necessary for commercial variety defect correction, novel variety development, identification of diverse lines, creation of diversity, and subsequent utilization. Information about the amount and distribution of genetic diversity among the germplasm is essential for its efficient management and effective utilization in the breeding program. Plant genetic resource genetic diversity analysis can be conducted using phenotypic or morphological traits and cytological, biochemical, and molecular assessment techniques (Zhu *et al*., 2022). Morphological markers were initially used for the analysis of diversity, and they are still in use. Morphological evaluation and characterization of genotypes using qualitative traits are the most direct methods of identifying genotype identity and studying genetic diversity among genotypes (Tatari and Abdollahi, 2021). This technique is easy, simple, cost-effective, time-efficient, and does not require complicated equipment or a high level of specialist knowledge for scoring (Tesfa, 2022).

Several studies have been conducted to estimate the genetic diversity of Arabica coffee using qualitative traits. Different scholars from different countries reported the presence of high variability among Arabica coffee genotypes using qualitative morphological traits (Ameha, 1986; Montagnon & Bouharmont, 1996; Selvakumar & Sreenivasan, 1989; Asegid, 2019; Weldemichael, 2020; Yirga, 2021; Gebreselassie *et al.,* 2024). Morphological traits are still successfully used to analyze the genetic diversity required for germplasm conservation, reduce accession numbers by identifying and eliminating duplicates, and enhance crop breeding through selection.

Indigenous genotypes play a key role in sustainable production and have an inherent value as the storehouse of wild coffee genetic resources (Davis *et al*., 2019). They also have high functional diversity in terms of disease resistance (Davis *et al*., 2023) and pest and drought tolerance (Burkhardt *et al*., 2007; Hein and Gatzweiler, 2006; Carvalho *et al*., 2017). As part of a future-proofing resource, and especially for providing genetic potential for mitigating climate change, indigenous populations are perceived as a key resource for the medium- to long-term sustainability of Arabica coffee production (Hein and Gatzweiler, 2006). However, indigenous coffee genetic resources are being lost at a rapid pace due to varied threats, such as the conversion of natural forest to agricultural land in the center of origin, deforestation, land degradation, climate change, leading to an increased incidence of pests and diseases, a higher incidence of drought, and unpredictable rainfall patterns (Sarada, 2017).

Hence, indigenous coffee genetic resources are being lost at a rapid pace, an urgent strategy has to be sated in coffee research program to collect, conserve, and utilize indigenous coffee genetic resources. Accordingly, coffee accessions were collected in the major coffee-growing areas of southern Ethiopia (Sidama, Gedeo, Amaro, Gamo Gofa, Jinka, and Gujji), and over 700 coffee accessions have been collected and maintained at the Awada field bank. Among the southern collection, 14 promising accessions were selected for variety development after wider adaptability studies at different testing locations. Although these accessions were selected for their high yield potential, coffee berry disease resistance, and coffee leaf rust resistance, they have not yet been systematically characterized for qualitative morphological traits. Therefore, this study was conducted to assess the variation in qualitative morphological diversity among these Arabica coffee genotypes for effective utilization and strategic conservation.

2.2 Materials and Methods

2.2.1 Description of study area

The experiment took place during the 2021 cropping season at Awada agricultural research sub-center, located in Sidama Regional State, Ethiopia. The center is situated at a latitude of $6^0$45'46"N and a longitude of $38^0$22'36"E, with an elevation of 1740 meter above sea level (a.s.l.) The agro ecology of the experimental site is characterized by a semi-bimodal rainfall pattern with double wet and dry seasons, as described by Ajema & Nigussie (2021). According to NASA (2023), the average wind speed, precipitation, root zone moisture, maximum temperature, and minimum temperature of the experimental site over the past forty-one years (01/01/1981-12/31/2021) were 1.46 m/s, 1399.20mm, 0.68, 34.11^0C, and 7.61^0C, respectively. The experimental site has eutric-nitosol and chromotic-cambisol soil type, which is ideal for coffee production (Kebede & Bellachew, 2008).

2.2.2 Planting materials

For this study, a total of 17 Arabica coffee genotypes were used, consisting of 14 accessions from the south Ethiopian coffee gene pool and three standard reference varieties. The genotypes were originally collected from various agroecologies in south Ethiopia and have been maintained at Awada Sub Center (Gebreselassie *et al.,* 2024). The genotypes and their sources are listed in Table 2.1.

Table 2. 1 Description of experimental materials

Serial No.	Genotypes	Status	Source
1	AW105	Promising Selection	ARSC
2	AW1777	Promising Selection	ARSC
3	AW1995	Promising Selection	ARSC
4	AW3106	Promising Selection	ARSC
5	AW4994	Promising Selection	ARSC
6	AW5994	Promising Selection	ARSC
7	AW7494	Promising Selection	ARSC
8	AW7705	Promising Selection	ARSC
9	AW9622	Promising Selection	ARSC
10	AW9623	Promising Selection	ARSC
11	AW9628	Promising Selection	ARSC
12	AW9641	Promising Selection	ARSC
13	AW9644	Promising Selection	ARSC
14	AW9662	Promising Selection	ARSC
15	Feyate	Released Variety	ARSC
16	Angafa	Released Variety	ARSC
17	74112	Released Variety	JARC

ARSC= Awada Agricultural Research Sub-center; JARC=Jima Agricultural Research Center

2.2.3 Experimental design

The experimental materials were planted in August 2015, utilizing a randomized complete block design with three blocks (Gomez and Gomez 1984). Each experimental plot comprised eight coffee trees, planted in a single row for each genotype, with a spacing of 2 meters between plants and rows. The distance between replications was 4 meters.

2.2.4Experimental Procedures

Seed preparation, seedling media preparation, seed sowing, seedling management, land preparation, planting, and tree management (weeding, nutrient management, permanent and temporary shedding, soil conservation, and sucker management) were carried out according to Endale *et al*., (2008).

2.2.5 Data collection

The data on qualitative traits were evaluated using the International Plant Genetic Resources Board descriptor for coffee (IPGRI, 1996). The genotypes were assessed for 15 qualitative traits, which are listed in Table 2.2 below. The qualitative traits were assessed and coded by an three experienced team of breeders' subjective judgment (appendix 1).

Table 2. 2 Recorded Qualitative traits based on descriptors for each Arabica coffee genotype

No.	**Genotype**	**FC**	**FS**	**PT**	**SS**	**PH**	**BH**	**AIPB**	**ST**	**YL**	**LS**	**LAS**	**LPC**	**YS**	**OA**	**CD**
1	AW1777	5	1	5	2	7	3	2	3	1	3	5	1	1	2	3
2	AW105	5	1	5	2	7	4	2	4	5	3	4	1	3	2	3
3	AW1995	5	2	4	2	7	3	1	3	5	3	5	1	3	2	2
4	AW3106	5	2	5	2	7	4	2	3	5	3	4	1	3	2	3
5	AW4994	5	2	6	4	9	3	1	3	5	3	4	1	3	2	2
6	AW5994	4	3	6	3	7	4	1	4	5	3	4	1	3	2	3
7	AW7494	5	2	5	3	7	4	2	4	2	3	5	1	1	2	2
8	AW7705	5	2	6	4	7	4	2	3	5	3	5	1	3	2	3
9	AW9622	5	2	6	3	7	3	1	3	5	3	4	1	3	2	3
10	AW9623	5	1	5	1	7	3	1	5	5	4	1	3	3	2	2
11	AW9628	5	1	5	2	7	4	2	3	5	3	4	1	3	2	3
12	AW9641	5	2	6	2	7	3	1	4	5	3	4	1	3	2	2
13	AW9644	5	2	5	2	7	3	1	4	5	3	5	1	3	1	1
14	AW9662	5	1	5	1	9	4	1	3	2	3	5	1	1	2	3
15	74112	6	1	5	2	3	3	2	3	2	4	4	1	1	1	1
16	Angafa	5	2	5	2	7	4	2	3	5	3	5	1	3	2	3
17	Feyate	5	2	5	2	7	3	2	3	2	3	5	1	1	2	3

Where: No.= number, FC=Fruit colour; FS=Fruit shape; PT=Pulp thickness; SS=Seed shape; PH=Plant height; BH=Branching habit; AIPB=Angle of insertion of primary branches; ST=Stipule shape; YL=Young leaf color; LS=Leaf shape; LAS=Leaf apex shape; LPC=Leaf petiole color; YS=Young shoot color; OA=Overall appearance; CD=Canopy Diameter

2.2.5 Data Analysis

Descriptive statistics such as frequency distribution and percentage of various categories of the 15 phenotypic qualitative traits were conducted for all genotypes using Microsoft Excel version 2010.

A chi-square test was conducted to test the homogeneity of the populations for the 15 qualitative traits by using Minitab version 21.4 statistical software.

The Shannon diversity index was computed for each of the 15 qualitative morphological characters using the formula described by Shannon and Weaver (1949) as follows:

$$H\,' = -\sum_{i=0}^{n} \left(\left| pi \right| x \left(lnpi\text{¿} \right) \right) \text{¿}$$

Where H’= Shannon diversity index; n=total number of phenotypic classes for a character; pi= is the proportion of genotypes that have the character in the i^{th} class for a character; ln = natural logarithm.

Path coefficient analysis was conducted to study independent traits direct and indirect effects (Dewey & Lu, 1959). For path coefficient analysis, coffee branching habit was taken as a dependent variable, while the rest of the variables were considered contributory factors. The direct and indirect effects of the independent characters on branching habit were studied using the formula suggested by Dewey and Lu (1959). Principal component analysis was done using SAS software to determine the relative importance of the traits responsible for variation among the coffee genotypes. Pearson’s correlation coefficients were calculated among qualitative agro-morphology traits to estimate their relationships by using SAS. The cluster analysis was executed, and the dendrogram was constructed based on the complete linkage method with Euclidean distance by using Minitab version 21.4 software. All 15 qualitative trait data were standardized before clustering.

Inter-cluster distances were calculated based on the Mahalanobis's D^2 statistics as:

$$D^2\, ij = (xi - xj)'\, cov - 1(xi - xj)$$

Where, D^2ij = the distance between genotype i and j; xi and xj = vectors of the values of the variables for cases i and j; and cov-1 = the pooled within groups variance-covariance matrix. The significance of D^2 values was tested by comparing the D^2 values between any two clusters against tabulated chi-square (χ^2) values at p-1 degrees of freedom, where p refers to the number of qualitative characters considered. Inter-cluster distances based on the Mahalanobis's D^2 statistics were computed using Minitab.

2.3 Results and Discussions

2.3.1 Frequency Distribution of Qualitative Traits

In this study, 15 qualitative traits were used to characterize the genotypes of south Ethiopian coffee genotypes (Table 2.3). According to the percentage of the frequency distribution, the predominant traits among the majority of the studied genotypes were green leaf petiole color (94.12%), elliptic leaf shape (88.24%), pyramidal overall appearance (88.24%), tall plant height (82.35%), red fruit color (88.24%), bronz young leaf color (70.59%), triangular stipule shape (64.71%), intermediate pulp thickness (64.71%), obovate fruit shape (58.82%), obovate seed shape (58.82%), many primary branches (52.94%), and horizontal angle of insertion of primary branch (52.94%). The variability observed in these phenotypic characters indicates the presence of genetically diverse genotypes, which can be used as markers for future selection and hybridization programs. Previous research has also reported a wide variability in percent frequency distribution for qualitative traits among Arabica coffee genotypes (Asegid, 2019; Weldemichael, 2020; Degefa, 2021; Yirga, 2021; Ndikumana, 2022).

The number of branches is an important qualitative trait that influences crop yield. An increase in the number of branches of the coffee plant helps to increase productivity of the crop. In this study, the frequency of the studied genotypes for branching habits showed that 52.94% of genotypes had many primary branches, whereas 47.06% had many primary, secondary, and tertiary branches. Hence, the 47.06% of genotypes having many primary, secondary, and tertiary branches could be used for future yield improvement programs via selection and/or hybridization due to their branching habit.

The predominance of the open growth habit is a manifestation of the suitability of such traits for ease of management practice (Yirga, 2021). It permits uniform exposure and better interception of sunlight for all leaves and other vegetative parts, creating a less favorable environment for disease development as compared to compact types (Yirga, 2021). In the current investigation, the canopy class of the genotypes was open (58.92%), intermediate (29.41%), and compact (11.76%). In addition to the canopy class, the angle of insertion of primary branches recorded was horizontal (52.94%) and drooping (47.06%). Accordingly, most of the studied genotypes showed an open growth habit and were suitable for ease of management practice, permitting uniform exposure and better interception of sunlight.

2.3.2 Chi Square

The chi-square test values for the 15 qualitative traits were evaluated among different genotypes and are presented in Table 2.3. The results showed that there is a significant difference among all qualitative traits, except for branching habit, young shoot color and angle of insertion of primary branch, which exhibit dominant phenotypic variation among the evaluated traits. These significant differences among genotypes based on the chi-square test suggest that there is high variation within the south Ethiopian coffee genotypes for the studied qualitative traits. Previous studies have also reported significant chi-square test values for qualitative traits among Ethiopian Arabica coffee genotypes (Adem, 2009).

2.3.3 Shannon-Weaver Diversity Index

The estimated Shannon-Weaver diversity index (H') is presented in Table 2.3. The studied traits contributed to the phenotypic diversity at various levels and the diversity index (H') ranged from 0.22 for leaf petiole color (lowest polymorphism) to 1.12 for seed shape (highest polymorphism), with an overall mean of 0.67 (Table 2.3). The highest diversity was found for seed shape (1.12), canopy diameter (0.924), leaf apex shape (0.88), fruit shape (0.85), stipule shape and pulp thickness (0.81), young leaf color (0.75), branching habit and angle of insertion of the primary branch (0.69), young shoot color (0.61), and plant height (0.58). The higher shannon-weaver diversity index implies the presence of adequate variability for these traits among the evaluated genotypes. Accordingly, the genotypes were highly diverse for seed shape, canopy diameter, leaf apex shape, fruit shape, stipule shape, pulp thickness, and young leaf color. On the other side, the lowest diversity was found for leaf petiole color (0.22), overall appearance (0.36), leaf shape (0.36), and fruit color (0.44), signifying the possibility of a close association between coffee genotypes for those traits. Many studies reported wider range of Shannon-Weaver diversity values among Arabica coffee qualitative traits. 0.67 for branching habit to 0.98 for leaf shape among 124 accessions (Atinafu and Mohammed, 2017); 1.22 for stipule shape to 0.24 for calyx limb persistence among 137 accessions (Asegid, 2019); 0.92 for growth habit to 0.13 for calyx limb persistence among 104 accessions (Degefa, 2021); 1.22 for fruit color to 0.35 stem habit (Yirga, 2021); 1.08 for angle of insertion of primary branches to 0.17 for screen size among 49 accessions (Weldemichael, 2021).

Table 2. 3 Frequency distribution, proportion, Shannon-waver diversity index, and chi square of 15 Qualitative traits of 17 south Ethiopian coffee genotypes

Trait	Code		Frequency	% contribution	Contribution to Chi-Square	Chi-Sq	
Plant height	3	Short	1	5.88	3.84	18.47**	0.578
	7	Tall	14	82.35	12.25		
	9	Very tall	2	11.76	2.37		
Branching habit	3	Many branches (primary)	9	52.94	0.03	0.06^{ns}	0.691
	4	Many branches (primary) with many secondary and tertiary	8	47.06	0.03		
Angle of insertion of primary brunch	1	Drooping	8	47.06	0.03	0.07^{ns}	0.691
	2	Horizontal	9	52.94	0.03		
Stipule shape	3	Triangular	11	64.71	5.02	8.94*	0.808
	4	Deltate (equilaterally triangular)	5	29.41	0.08		
	5	Trapeziform,	1	5.88	3.84		
Young leaf color	1	Greenish	1	5.88	3.84	11.41**	0.753
	2	Green	4	23.53	0.49		
	5	Bronze	12	70.59	7.08		
Leaf shape	3	Elliptic	15	88.24	4.97	9.94*	0.362
	4	Lanceolate	2	11.76	4.97		
Leaf apex shape	1	Round	1	5.88	3.84	5.76*	0.876
	4	Acuminate	8	47.06	0.96		
	5	Apiculate, spatulate	8	47.06	0.96		
Leaf petiole color	1	Green	16	94.12	6.62	13.24**	0.224
	3	Other	1	5.88	6.62		
Young shoot color	1	Green	5	29.41	1.44	2.88^{ns}	0.606
	3	Dark brown	12	70.59	1.44		
Overall appearance	1	Elongated conical	2	11.76	4.97	9.94*	0.362
	2	Pyramidal	15	88.24	4.97		
Canopy Diameter	1	Compact	2	11.76	2.37	5.76*	0.924
	2	Intermediate	5	29.41	0.08		
	3	Open	10	58.82	3.31		
Fruit colour	4	Orange-red	1	5.88	3.84	23.06**	0.444
	5	Red	15	88.24	15.37		
	6	Red – purple	1	5.88	3.84		
Fruit shape	1	Roundish	6	35.29	0.02	7.18*	0.846
	2	Obovate	10	58.82	3.31		
	3	Ovate	1	5.88	3.84		
Pulp thickness	4	Thin	1	5.88	3.84	8.94*	0.808
	5	Intermediate	11	64.71	5.02		
	6	Thick	5	29.41	0.08		
Seed shape	1	Round	2	11.76	1.19	10.53*	1.122
	2	Obovate	10	58.82	7.78		
	3	Ovate	3	17.65	0.37		
	4	Elliptic	2	11.76	1.19		

2.3.4 Correlation

The correlation analyses for 15 qualitative morphological characteristics were assessed, and the results are given in Table 2.4. Branching habit was significantly and positively correlated with the angle of insertion of the primary branch (r = 0.417), overall appearance (r = 0.344), and canopy diameter (r = 0.548), whereas, it was negatively correlated with leaf shape (r = -0.344) and leaf apex shape (r = -0.203) (Table 2.4). Pulp thickness was significantly and positively associated with fruit shape (r = 0.412), seed shape (r = 0.632), young leaf color (r = 0.275), and young shoot color (r = 0.278), whereas it was negatively correlated with fruit color (r = -0.577). Seed shape was positively correlated with fruit shape (r = 0.56) and negatively correlated with leaf shape (-0.352) and leaf petiole color (r = -0.393). Canopy diameter was significantly and positively correlated with overall appearance (r = 0.772), branching habit (r = 0,548), plant height (r = 0.427), and angle of insertion of the primary branch (r = 0.299), whereas it was negatively correlated with leaf shape (r = -0.509), fruit color (r = -0.493), and stipule shape (r = -0.323) (Table 2.4).

The correlation analysis provides important insights for plant breeding by identifying trait relationships that can guide selection. The positive correlations between branching habit, canopy diameter, and overall appearance suggest that selecting for better branching may improve plant structure and yield potential. The strong association of pulp thickness with seed and fruit shape indicates these traits can be used as indirect selection criteria for improving fruit quality. Negative correlations between pulp thickness and fruit color or canopy diameter and leaf shape, highlight potential trade-offs that breeders should consider. Overall, understanding these trait relationships helps in designing more efficient breeding strategies to develop superior coffee genotypes with desirable qualitative morphological traits.

Table 2. 4 Correlation coefficients among 15 qualitative morphological characters

	FC	FS	PT	SS	PH	BH	AIPB	SH	YLC	LS	LAS	LPC	YSC	OA	CD
FC	1	-0.601**	-0.314*	-0.208^{ns}	-0.577**	-0.144ns	0.344*	-0.286*	-0.349*	0.532**	0.000^{ns}	0.000^{ns}	-0.376*	-0.532**	-0.493**
FS		1	0.412**	0.560**	0.174^{ns}	0.073^{ns}	-0.280*	0.010^{ns}	0.370*	-0.452**	0.267*	-0.309*	0.346*	0.13 ^{ns}	0.052^{ns}
PT			1	0.632**	0.182^{ns}	0.02 ^{ns}	-0.241^{ns}	0.063^{ns}	0.275 *	-0.158^{ns}	-0.133^{ns}	-0.108^{ns}	0.278*	0.158^{ns}	0.173^{ns}
SS				1	0.120^{ns}	0.093^{ns}	0.05 ^{ns}	-0.245^{ns}	0.228^{ns}	-0.352*	0.264^{ns}	-0.393**	0.231^{ns}	0.130^{ns}	0.066^{ns}
PH					1	0.198^{ns}	-0.397**	0.000^{ns}	0.202^{ns}	-0.615**	0.104^{ns}	0.000^{ns}	0.217^{ns}	0.615**	0.427**
BH						1	0.417**	-0.058^{ns}	0.122^{ns}	-0.344*	0.203*	-0.236^{ns}	0.091^{ns}	0.344*	0.548**
AIPB							1	-0.335*	-0.362*	-0.022^{ns}	0.290*	-0.265*	-0.350*	0.022^{ns}	0.299*
SH								1	0.239^{ns}	0.358*	-0.622**	0.662**	0.228^{ns}	-0.054^{ns}	-0.323*
YLC									1	-0.138^{ns}	-0.346*	0.160^{ns}	0.989**	0.138^{ns}	0.030^{ns}
LS										1	-0.685**	0.685**	-0.165^{ns}	-0.433**	-0.509**
LAS											1	-0.862**	-0.342*	-0.079^{ns}	0.146^{ns}
LPC												1	0.161^{ns}	0.091^{ns}	-0.169^{ns}
YSC													1	0.165^{ns}	0.065^{ns}
OA														1	0.772**
CD															1

Where: FC= Fruit colour, FS=Fruit shape, PT=Pulp thickness, SS=Seed shape, PH=Plant height, BH=Branching habit, AIPB=Angle of insertion of primary branches, SH=Stipule shape, YLC=Young leaf color , LS=Leaf shape, LAS=Leaf apex shape, LPC=Leaf petiole color, YSC=Young shoot color, OA=Overall appearance, CD=Canopy Diameter.

2.3.5 Path analysis

To assess the magnitude of direct and indirect contributions of qualitative characters to Arabica coffee branching habit, path coefficient analysis was performed using five significantly associated traits with branching habit. Among the 5 significantly correlated characters with branching habit, an angle of insertion of primary branches (0.485), followed by canopy diameter (0.264), overall appearance (0.101), and leaf apex shape (0.014), exerted a positive direct effect, whereas leaf shape (-0.003) had a negative direct influence on branching habit (Table 2.5). An angle of insertion of primary branches and canopy diameter had the highest positive direct effect on branching habit; they are found to be important components, and direct selection for this trait may be rewarding for the improvement of Arabica coffee branching habit.

Table 2. 5 Path coefficient analysis (bold and diagonal value indicating direct effect; above and below the diagonal value indicating indirect effect) of 5 qualitative morphological traits on branching habit

	AIPB	LS	LAS	OA	CD
AIPB	**0.485**	0.000	0.004	-0.002	0.079
LS	-0.011	**-0.003**	-0.009	0.044	-0.134
LAS	0.141	0.002	**0.014**	0.008	0.039
OA	0.011	0.001	-0.001	**0.101**	0.204
CD	0.145	0.001	0.002	-0.078	**0.264**

Where: AIPB=Angle of insertion of primary branches, LS=Leaf shape, LAS=Leaf apex shape, OA=Overall appearance, CD=Canopy Diameter.

2.3.6 Principal Component Analysis

The principal component analysis (PCA) of 17 Arabica coffee genotypes for 15 qualitative morphological traits were performed to estimate the relative contribution of each attribute to the observed variability, and the results are presented in Table 2.6. The PCA analysis generated the fifteen eigenvalues and eigenvectors. However, according to Kaiser (1960), as cited by Amare *et al*. (2018), factors to be retained should have more than 1 eigenvalue, at least 5% of the variance explained for each component, and/or more than 70% of the cumulative proportion of variance explained. Accordingly, the first five components that explained 83.26 percent of total variation were used for displaying characters (Table 2.6).

The PCA analysis might be useful for deducing the nature of attributes and reducing the complexity of data collection (Al-Sayed *et al*., 2012). The first PCA explained 29.26% of the total variations (Table 2.6). According to Jeffers (1967), the relative weight given to the variables in each component is determined by the importance of the variables, which possess a high positive and negative weight. Based on this suggestion, the most important characters contributing more to the variation were leaf shape (-0.41), fruit color (-0.36), plant height (0.31), canopy diameter (0.31), fruit shape (0.30), and overall appearance (0.3). The sign indicates the direction of the relationship between the components and the characters (Tsehaye & Kebebew, 2002). Accordingly, in the first principal component, all traits listed except leaf shape and fruit color were positively associated.

The second PCA explained 23.42% of the variation, and leaf apex shape (-0.43), angle of insertion of primary branches (-0.34), stipule shape (0.39), leaf petiole color (0.39), young leaf color (0.36), and young shoot color (0.36) mainly accredited this variation. The third PCA explained 8.33% of the total variation, and this variation is mainly contributed by canopy diameter (0.42), overall appearance (0.41), seed shape (-0.38), and fruit shape (-0.35) in decreasing order (Table 2.6).

The fourth and fifth principal components explained 8.95% and 7.37%, respectively. High variation in the fourth PC is attributed to angle of insertion of primary branches (0.52), plant height (-0.42), branching habit (0.4), young shoot color (0.29), seed shape (0.24), while high variation in the fifth PC is attributed to pulp thickness (0.59), young leaf color (-0.41), and seed shape (0.38) (Table 2.6).

From the PCA result of the present study, it may be concluded that important variables in Arabica coffee genotype with respect to qualitative morphological characters were leaf shape, leaf apex shape, canopy diameter, overall appearance, angle of insertion of primary branches, plant height, branching habit, pulp thickness, and young leaf color. These variables might be taken into consideration for the effective selection of parents.

Table 2. 6 Eigen values and Eigenvectors of the first 5 principal components (PCA) for 15 qualitative morphological characters

	Eigenvectors				
	PCA1	PCA2	PCA3	PCA4	PCA5
Fruit colour	-0.36	-0.2	-0.12	0.17	-0.03
Fruit shape	0.30	0.09	-0.35	-0.06	0.04
Pulp thickness	0.22	0.15	-0.25	0.16	0.59
Seed shape	0.26	-0.04	-0.38	0.24	0.38
Plant height	0.31	0.10	0.22	-0.42	-0.01
Branching habit	0.22	-0.11	0.29	0.40	-0.15
Angle of insertion of primary branches	-0.05	-0.34	0.18	0.52	0.04
Stipule shape	-0.11	0.39	0.09	-0.06	0.06
Young leaf color	0.19	0.36	-0.15	0.3	-0.41
Leaf shape	-0.41	0.18	0.02	0.2	0.21
Leaf apex shape	0.18	-0.43	-0.18	-0.19	-0.19
Leaf petiole color	-0.21	0.39	0.27	0.00	0.15
Young shoot color	0.20	0.36	-0.13	0.29	-0.4
Overall appearance	0.30	0.06	0.41	-0.02	0.20
Canopy Diameter	0.31	-0.1	0.42	0.16	0.13
EV	4.39	3.51	2.14	1.34	1.11
PVE	29.26 %	23.42%	14.26 %	8.95%	7.37%
CPVE	29.26 %	52.68%	66.94 %	75.89%	83.26%

Where; EV = Eigen value, PVE = Present variation explained. and CPVE = Cumulative present variation explained

2.3.7 Cluster Analysis

The classification of the 15 qualitative traits using complete linkage hierarchical clustering with Euclidean distance revealed the presence of five distinct clusters. Figure 2.1 and Table 2.7 illustrate the distribution of genotypes, with 7 genotypes (41.18%) in Cluster-V, 6 genotypes (35.29%) in Cluster-III, 2 genotypes (11.76%) in Cluster-IV, and 1 genotype, 74112, in Cluster-I

(5.88%) and AW9623 in Cluster-II (5.88%). Similarity was considered within a cluster, while dissimilarity was considered between different clusters. The distribution of genotypes into different clusters indicates the presence of genetic variation among coffee genotypes.

The presence of such distinct clusters is crucial for practical breeding applications, especially in selection, hybridization, and trait introgression programs. The significant intra-cluster similarity and inter-cluster dissimilarity suggest that genotypes within the same cluster are more phenotypically similar, while those from different clusters show marked trait divergence. This implies that selecting parents from different clusters especially from the most distinct clusters such as Cluster-I and Cluster-V can enhance heterosis (hybrid vigor) and increase the chances of obtaining superior recombinants in segregating populations. Moreover, clustering helps to prioritize and conserve genetic resources by identifying redundant types (within the same cluster) versus unique germplasms (in single-genotype clusters). This information is vital for core collection development, minimizing resource duplication in conservation programs, and focusing efforts on preserving diverse and unique genotypes.

The observed clustering pattern not only confirms the genetic diversity among coffee genotypes but also provides a strategic framework for parental selection, trait-based breeding, and germplasm conservation. By combining morphological clustering results with molecular and biochemical analyses, breeders can make more informed decisions that accelerate the development of improved *Coffea arabica* varieties.Many studies reported the clustering of Arabica coffee accessions into distinct groups based on qualitative traits. Accordingly, 10 distinct clusters among 124 accessions based on 7 qualitative traits were reported by Atinafu and Mohamed (2017); 5 distinct clusters among 137 based on 12 qualitative traits were reported by Asegid (2019); 5 distinct groups among 104 accessions based on nine qualitative traits were reported by Degefa (2021); 6 clusters among 64 accessions based on 13 qualitative traits were reported by Desalegn (2018); and 5 distinct groups among 62 accessions based on 12 qualitative traits were reported by Yirga (2021).

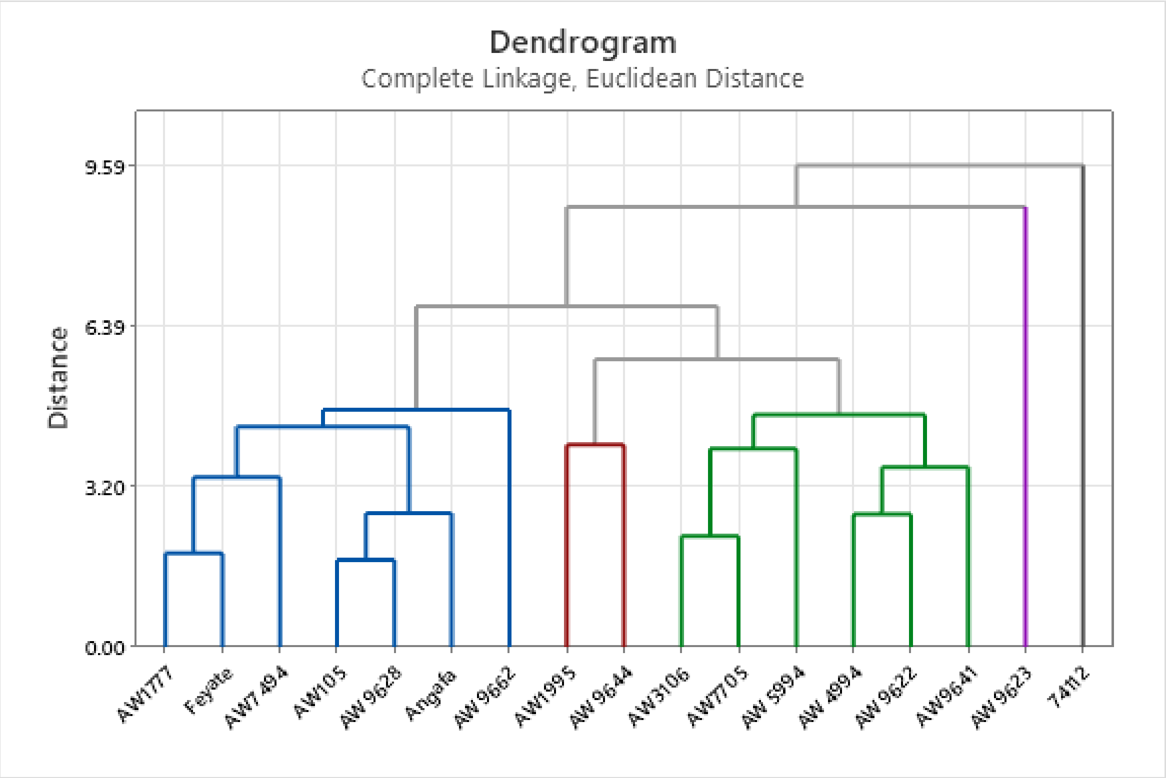

Fig Figure 2. 1 Cluster dendrogram describing variation among 17 genotypes of Arabica coffee for 15 qualitative phenotypic traits

Table 2. 7 Clustering patterns of 17 coffee genotypes based on 12 qualitative characters

Cluster No.	No.of Genotype	Percent (%)	List of genotype
I	1	5.88	74112
II	1	5.88	AW9623
III	6	35.29	AW3106, AWAW7705, AW5994, AW4994, AW9622, AW9641
IV	2	11.76	AW1995, AW9644
V	7	41.18	AW1777, Feyate, AW7494, AW105, AW9628, Angafa, AW9662

2.3.8 Distance b/n cluster centroid

The pairwise generalized squared distance (D^2) among the five clusters based on the Mahalanobis's D^2 statistics revealed significant ($P \leq 0.05$) divergence between most of the clusters (Table 2.8). The maximum genetic distance was observed between clusters C-IV and C-

V (D^2=9.086*), followed by C-I and C-IV (D^2=8.166*), C-II and C-IV (D^2=7.768*), C-II and C-V (D^2=7.570*), C-III and C-V (D^2=7.501*), C-III and C-IV (D^2=7.290*), C-I and C-V (D^2 =7.032*), and C-I and C-II (D^2= 4.023*) indicating divergence of genotypes in these clusters. Non-significant inter-cluster distances were observed between clusters C-I and C-II (D^2 = 3.332^{ns}) and clusters C-II and C-III (D^2=3.135^{ns}) indicating that genotypes in these clusters share similar genetic backgrounds (Table 2.8). The higher inter-cluster distance value showed the existence of wider genetic variability among the tested groups of genotypes (Asegid, 2019). According to Singh *et al*. (1987), the maximum genetic recombination is expected from the hybridization of the parents selected from divergent cluster groups. Accordingly, the maximum genetic recombination and higher heterotic F1 offspring are expected from crosses involving parents selected from clusters I and V, followed by I and IV, II and IV, II and V, III and V, III and IV, I and V, and I and II in decreasing order. However, the selection of parents should also consider the special advantages of each cluster and each genotype within a cluster depending on the specific objectives of hybridization (Singh, 2001).

Table 2. 8 Inter cluster distance for 15 qualitative traits of south Ethiopian coffee genotypes

	I	II	III	IV	V
I	0	3.332^{ns}	4.023*	8.166*	7.032*
II		0	3.135^{ns}	7.768*	7.570*
III			0	7.290*	7.501*
IV				0	9.086*
V					0

2.4 CONCLUSION

Estimates of frequency distribution, Shannon index, and cluster analysis based on 15 qualitative morphological traits revealed the existence of genetic variation among Arabica coffee genotypes. The frequency distribution of traits showed a wide range of phenotypic variation, with the maximum Shannon index (H') found for seed shape, canopy diameter, leaf apex shape, fruit shape, stipule shape, pulp thickness, and young leaf colour. Results of the chi-square test showed significant differences for most of the traits indicating dominant phenotypic variation among the evaluated traits.

Path coefficient analysis showed a positive direct effect of angle of insertion of primary branches, canopy diameter), overall appearance, and leaf apex shape on branching habit. From the PCA, important variables in Arabica coffee genotype with respect to qualitative morphological characters were leaf shape, leaf apex shape, canopy diameter, overall appearance, angle of insertion of primary branches, plant height, branching habit, pulp thickness, and young leaf color. These variables might be taken into consideration for the effective selection of parents.

The genotypes were classified into five groups based on the results of the cluster analysis. Cluster-V had the maximum number of genotypes (41.18%), followed by cluster-III (35.29%), and cluster-IV (11.76%). 74112 is the only variety found in cluster I and this genotype had a chance to develop hybrid vigor through crossing diverged parents found in different clusters. The pairwise generalized squared distance among the clusters showed significant divergence between the clusters. However, the maximum genetic recombination and higher heterotic F1 offspring are expected from crosses involving parents selected from clusters 1 genotype 74112 and clusters 5 genotype AW1777, Feyate, AW7494, AW105, AW9628, Angafa, AW9662 .

The study confirmed the existence of significant genetic variability among the Arabica coffee genotypes for most of the studied qualitative traits, which provides excellent opportunities for genetic gain through selection, hybridization, and strategic conservation. However, the morphological diversity observed in this study needs to be further confirmed using molecular techniques of characterization.

2.5 REFERENCES

Adem, A., (2009). Agro-morphological characterization of coffee (Coffea arabica L.) landrace collected from Mesela, West Harerge, Ethiopia. M.Sc. Thesis Submitted to Graduate Studies of Hawassa University, Hawassa, Ethiopia. 88 pp.

Ajema, L., & Nigussie, A. (2021). Yield and Yield Advantage of the Component Crops as Affected by Strip Intercropping of Coffee (*Coffea arabica* L.) with Pineapple (*Ananas comosus L*.). American Journal of BioScience, 9(4), 116. https://doi.org/10.11648/j.ajbio.20210904.11

Al-Sayed, H.M., Fateh, H.S., Fares, W.M., Attaya, A.S., 2012. Multivariate analysis of sugar yield factors in sugarcane. *Am.-Eurasian J. Sustain. Agric*. (AEJSA) 6 (1), 44–50.

Ameha, M., 1986. The significance of Ethiopian coffee genetic resources to coffee improvement. In the proceedings of an international symposium on the conservation and utilization of Ethiopian germplasm, pp. 247-253. Addis Ababa, Ethiopia, 13-16 October, 1986.

Asegid, A. (2019). Morphological Characterization of Bale and West Arsi Coffee (Coffea arabica L.) Collections at Gera, Southwest Ethiopia. A thesis Submitted for the Partial Fulfillment of the Degree of Masters of Science in Plant Breeding of Jimma University. PP 1-91.

Atinafu, G., & Mohammed, H. (2017). Agro-Morphological Characterization of Sidama Coffee (Coffea Arabica L .) Germplasm Accession under its Specialty Coffee Growing Area, Awada , Southern Ethiopia. *International Journal of Research Studies in Science, Engineering and Technology*. 4(12) 11-23

Bicho, N. C. C., J. F. S. Oliveira, A. E. Leitão, J. C. Ramalho and F. C. Lidon, (2011). Coffee – Origins, Production, Processing and Quality Definition. Livraria Escolar Editora. P. 170, Lisboa, Portugal.

Burkhardt J, Kufa T, Beining A, Goldbach HE, Fetene M (2007). Drought adaptation strategies of Coffea arabica populations along a rainfall gradient in Ethiopia. 21st International Conference on Coffee Science, Montpellier, France, 11–15 September: 1032–1036.

Carvalho, F. G., Sera, G. H., Andreazi, E., Sera, T., de Barista Fonseca, I. C. B., Carducci, C., *et al,*. (2017). Drought tolerance in seedlings of coffee genotypes carrying genes of different species. Coffee Sci. 12, 9–16.

CSA (Central Statistical Authority), 2015. Federal Republic of Ethiopia, Central Statistical Agency, Agricultural Sample Survey Report on Area and Production of Major Crops, 2014/2015, Vol. 1, Statistical Bulletin 578, May 2015, Addis Ababa, 121P.

Davis AP, Kiwuka C, Faruk A, Mulumba J and Kalema J (2023) A review of the indigenous coffee resources of Uganda and their potential for coffee sector sustainability and development. Frontiers in Plant Science. 1-14. https://doi.org/10.3389/fpls.2022.1057317

Davis AP., (2011). Psilanthusmannii, the type species of Psilanthus, transferred to Coffea. Nordic Journal of Botany,29:471–472. https://doi.org/10.1111/j.1756-1051.2011.01113.x

Davis, A. P., Chadburn, H., Moat, J., O'Sullivan, R., Hargreaves, S., and Nic Lughadha, E. (2019). High extinction risk for wild coffee species and implications for coffee sector sustainability. Sci. Adv. 5, eaav3473. https://doi.org/10.1126/sciadv.aav3473

Degefa, M. (2021). Phenotypic Classification of Coffee (Coffea arabica L.) Germplasm in Southern Ethiopia. American Journal of Agriculture and Forestry, 9(6), 358. https://doi.org/10.11648/j.ajaf.20210906.15

Desalegn A., 2018. Genetic Variability and Character Association of Amaro Coffee (Coffea arabica l.) Accessions at Awada, Southern Ethiopia. Thesis Submitted to Graduate Studies of Jimma University, Jimma, Ethiopia. P1-38

Dewey, D. R., & Lu, K. H. (1959). A correlation and path-coefficient analysis of components of crested wheatgrass seed production. Agronomy Journal, 51(9), 515–518. https://doi.org/10.2134/agronj1959.00021962005100090002x

Endale, T., T. Kufa, A. Nestre, T. Shimber, A. Yilma and T. Ayano, 2008. Research on coffee field management. Proceedings of the workshop on four decades of coffee research and development in Ethiopia: A National workshop, Addis Ababa, Ethiopia. pp.187-195.

Gebreselassie, H., Tesfaye, B. & Gedebo, A. 2024. Genetic diversity of Arabica coffee genotypes in south Ethiopia using quantitative agro-morphological traits. Genet Resour Crop Evol 71, 3485–3506 . https://doi.org/10.1007/s10722-023-01853-9

Gomez, K. A., & Gomez, A. A. (1984). Statistical Procedures for Agricultural Research (2nd ed.). New York: John Wiley & Sons.

Hein L., Gatzweiler, F. (2006). The economic value of coffee (Coffea arabica) genetic resources. Ecol Econ 60: 176–185.

ICO (International Coffee Organization) (2018). Coffee market report. Available at http://www.ico.org/

IPGRI (International Plant Genetic Resources Board descriptor for coffee) (1996). Descriptions of coffea spp. and Psilanthus spp. International Plant Genetics Resource Institute, Rome. Johnson, H.W., Robinson, H.F. and Comstooc, R.E (1955). Estimates of genetic and environmental variability in soybeans.Agronomy Journal 47:314-318.

Jeffers JNR., (1967). Two case studies in the application of principal component analysis. Appl. Stat. 16: 225-36.

Kebede, M. and Bellachew, B. (2008). Phenotyoic Diversity in the Hararge Coffee (Coffee arabica L) Germplasm for Quantitative Traits. East African Journal of Sciences, 2, 13-18. https://doi.org/10.4314/eajsci.v2i1.40358

Lachenmeier, D. W. (2023). Identification of Coffee Species, Varieties, Origins, and Processing and Preparation Methods—A Status Report. 9. https://doi.org/10.3390/icc2023-14824

Minter, M., Nielsen, E. S., Blyth, C., Bertola, L. D., Kantar, M. B., Morales, H. E., Orland, C., Segelbacher, G., & Leigh, D. M. (2021). What Is Genetic Diversity and Why Does it Matter? Frontiers for Young Minds, 9:.656168 https://doi.org/10.3389/frym.2021.656168

Montagnon, C. and Bouharmont, P. (1996). Multivariate analysis of phenotypic diversity of Coffea arabica . Genetic. Resour. Crop. Evol. 43: 221 – 227.

NASA (National Aeronautics and Space Administration) (2023) ArcGIS World Geocoding Service Power data access viewer. Retrieved from https://power.larc.nasa.gov/data-access-viewer/. Accessed 13 June 2023

Ndikumana, J. (2022). Agro-morphological Characterization of Arabica Coffee Cultivars in Burundi. A Thesis Submitted in Partial Fulfilment of the Requirements for the degree of Master of Science in Plant Breeding of the Jomo Kenyatta University of Agriculture and Technology. P1-135

Rain, R. (2023). "Shannon Diversity Index Calculator". Available at: https://www.omnicalculator.com/ecology/shannon-index. Accessed: 10 May 2023.

Salgotra, R. K., & Chauhan, B. S. (2023). Genetic Diversity, Conservation, and Utilization of Plant Genetic Resources. Genes, 14(1). https://doi.org/10.3390/genes14010174

Samper L, D. Giovannucci, L. Marques-Vieira, (2017). The Powerful Role of Intangibles in the Coffee Value Chain. WIPO Economic Research Working Paper No. 39. Geneva: WIPO

Sarada Krishnan (2017). Sustainable Coffee Production. Oxford Research Encyclopedia of Environmental Science. 1-3 https://doi.org/10.1093/acrefore/9780199389414.013.224

Selvakumar, M. and Sreenivasan, M.S., 1989. Studies on morphology and quality of Ethiopian arabica coffee. Journal of Plantation Crops, 16, pp.321-324.

Shannon, C. E., & Weaver, W. (1949). The Mathematical Theory of Communication. Urbana, IL: The University of Illinois Press, 1-117.

Singh, B.D., (2001). Plant breeding: Principles and Methods. Kalyani Publishers, New Delhi. 896p.

Tatari, M., & Abdollahi, H. (2021). Evaluation of Vegetative and Reproductive Characteristics of Some Quince (Cydonia Oblonga Mill.) Genotypes from Central Regions of Iran. International Journal of Fruit Science, 21(1), 945–954. https://doi.org/10.1080/15538362.2021.1948377

Temesgen, B. (2021). Role and economic importance of crop genetic diversity in food security. International Journal of Agricultural Science and Food Technology, 7, 164–169. https://doi.org/10.17352/2455-815x.000104

Tesfa, M. (2022). Characterization and Estimation of Diversity of Sugarcane (Saccharum officinarum) Genotypes Based on Qualitative Morphological Traits . 1–17.

Tsehaye Y., Kebebew F., (2002) Morphological diversity and geographic distribution of adaptive traits in finger millet (*Eleusine coracana* (L.) Gaertn. Subsp. coracana [Poaceae]) populations from Ethiopia. Addis Ababa, Ethiopia.

USDA (The United States Department of Agriculture), (2023). Ethiopia Coffee Annual Report, GAIN Report Number: ET2023-0014, 7p. https://apps.fas.usda.gov/newgainapi/api/Report/DownloadReportByFileName?fileName=Coffee%20Annual_Addis%20Ababa_Ethiopia_ET2023-0014.pdf

Vavilov, N.I., 1951. The origin, variation, immunity and breeding of cultivated plants. (Translated by S. K. Chestitee). Chronica Botonica, 13, 1-366

Weldemichael, G. (2020). Phenotypic Diversity of Ethiopian Coffee (Coffea arabica L .) Accessions Collected from Gomma District in Jimma Zone for Qualitative Traits. nternational Journal of Research Studies in Science, Engineering and Technology, 7(8), 8–14.

Weldemichael, G. (2021). Phenotypic Diversity of Ethiopian Coffee (*Coffea arabica* L .) Accessions Collected from Gomma District in Jimma Zone for Qualitative Traits. *International Journal of Research Studies in Science, Engineering and Technology 7(8): 08-14.* https://www.researchgate.net/publication/349916670

Yigzaw Dessalegn (2005). Assessment of cup quality, morphological, biochemical and molecular diversity of Coffea arabica L. genotypes of Ethiopia. PhD. Thesis, University Free State. 197 pp.

Yirga, M. (2021). Phenotypic Characterization of Coffee (Coffea Arabica L.) Germplasm, in Ethiopia. American Journal of BioScience, 9(2), 34. https://doi.org/10.11648/j.ajbio.20210902.11

Zhu, Y., Liang, D., Song, Z., Tan, Y., Guo, X., & Wang, D. (2022). Genetic Diversity Analysis and Core Germplasm Collection Construction of Camellia oleifera Based on Fruit Phenotype and SSR Data. Genes, 13(12). https://doi.org/10.3390/genes13122351

3. Genetic Diversity of Arabica Coffee Genotypes in South Ethiopia Using Quantitative Agro-morphological Traits

Abstract

The investigation was conducted to assess the extent of genetic diversity of South Ethiopian coffee genotypes for agro-morphological characters. Seventeen Arabica coffee genotypes were evaluated at Awada, Shebedino, and Wonago in south Ethiopia. The analysis of variance revealed significant variation among genotypes, environments, and GEI for number of primary-branches, fruit length, fruit width, fruit thickness, empty fruit rate, fruit filling coefficient, seed length, seed width, seed thickness, and yield characters. Mean performance analysis confirmed the presence of genotypes superior for yield. Moderately high-to-high broad-sense heritability along with moderate-to-high genetic advance values were found for 15 traits, indicating the possibilities of improvement through selection. Most agro-morphological traits were positively associated among themselves and with yield. Path coefficient analysis showed a positive direct effect of 11 characters (number of primary branches, number of secondary branches, leaf length, length of longest primary branch, leaf width, inter node length on longest primary branch, canopy diameter, seed length, single berry weight, stem girth, and stamen stalk length) on yield; indicating the significance of those traits for direct selection to increase coffee yield. Cluster analysis grouped genotypes into two main clusters and four sub-clusters. The first five principal components explained 74.05% of the total variation. Yield, inter node length on main stem, number of primary branches, number of secondary branches, length of longest primary branch, inter node length on longest primary branch, leaf length, leaf width, fruit length, fruit width, single berry weight, and seed thickness were the most important traits contributing more to variation, and these traits could be considered for effective parent selection in Arabica coffee improvement programs. The highest over laction and season mean yield of 1.37 ton per hectare was recorded for genotypes AW7705 and AW105, which is 0.4 ton ha-1 times the best check variety. Therefore, AW7705 and AW105 could be promoted as a promising candidate for the release of a new variety for Sidama and Gedeo coffee growing regions in South Ethiopia and other similar agro-ecologies. Hence, genotype, environment, and GEI have a significant effect on yield performance; the coffee yield improvement program should pay careful attention to a multi-location and season testing strategy to develop high-yielding varieties. Investigation of management options after the high-yielding season is also advised to minimize high-seasonal yield oscillation.

Keywords: Arabica coffee; Correlation coefficient, Genetic diversity; Quantitative traits; Path coefficient; Principal component analysis.

3.1 Introduction

Coffee has a tremendous economic, social, and spiritual impact on many people of different geographical locations, cultural backgrounds, ethnic groups, and psychological makeup (Yilma, 2017). It is the most beloved international non-alcoholic beverage. Arabica coffee (*Coffea arabica* L.) is a vital crop in the national economy of Ethiopia. The country has earned 1.4 billion USD by exporting over 300,000 tons of coffee for the global market in the 2022 fiscal year (ECTA, 2023; ENA, 2023). This has an increment of more than 20.54% and 53.8% in volume and revenue, respectively, as compared to the preceding fiscal year (ENA, 2023).

Ethiopia is the origin and center of genetic diversity for the Arabica coffee species (Vavilov, 1951). Different researchers witnessed the existence of abundant genetic diversity among Ethiopian Arabica coffee genotypes (Abdulfeta, 2018; Argaw & Taye, 2018; Beksisa *et al*., 2018; Dawit, 2018; Desalegn, 2018; Beksisa, 2021; Merga *et al*., 2021; Asefa, 2022; Legesse *et al*., 2022). As a result, the Ethiopian Arabica coffee gene pool is the most important and the basis for future coffee breeding programs (Bakala *et al*., 2022).

Despite the importance of coffee in Ethiopia's economy and the presence of huge genetic resources, a favorable climate, and suitable soil types for coffee production and productivity, the crop yield remains low (0.683 ton ha^{-1}) as compared to yields recorded in large producing countries and the world average (Motebayenore, 2022). Among others, the major contributing factor for the low production and productivity is the lack of yield competitive varieties that exhibit best performance across wide ranges of environments (Motebayenore, 2022).

Different genetic diversity assessment methods, viz., molecular, biochemical, and morphological markers, are available. Of these, the morphological method is direct, inexpensive, simple to score, and easy to apply, and its estimations were perceived as the determinant of the plant's agronomic value and taxonomic classification (Bhandari *et al*., 2017). Plant breeders can differentiate and identify the promising genotypes for variety development by assessing morphological characters (Akpertey *et al*., 2019). In Ethiopia, until today, coffee genetic diversity assessment through morphological traits has been the main tool that has been successfully used to analyze genetic diversity for improved variety development (Benti, 2017).

For efficient and effective crop breeding activity, investigation and a good understanding of the genetic diversity present in a crop population are essential. Observed variability in a population is due to genetic and environmental origins, but the only form that is heritable from generation to generation is genetic variability (Ahsan *et al*., 2015). Thus, the degree of crop improvement is dependent on the extent of the available beneficial genetic variability (Bello *et al*., 2014). In practice, the true variance components are unknown but are estimated from the data (Gebreselassie, 2023). The degree to which a character may flow from parent to offspring is typically assessed using heritability, which provides information on the extent to which a particular character can be transmitted from the parent to the progeny (Allard, 1960; Poehlman & Sleper, 1995). Similarly, genetic advance is important because it demonstrates the level of improvement in a character that resulted from one cycle of selection, and high genetic advance combined with high heritability estimates gives the optimal scenario for determining selection criteria (Syukur *et al*., 2012). Therefore, estimating the genetic coefficient of variation, genetic advance, and broad-sense heritability (h^2) would be useful for plant breeders to execute selection in breeding programs (Johnson *et al*., 1955).

Coffee bean yield has a complex character and is an economically important trait, but it is associated with many interrelated components. Accordingly, understanding the association between plant characteristics and yield is very crucial for successful breeding (Begna, 2021). The correlation is very important for measuring the degree and direction of linkage between different parameters that affect the yield either positively or negatively (Kasu-bandi *et al*., 2019). Path analysis divides the association among yield and its component traits into direct and indirect effects, and it has been successfully used to identify useful traits as selection criteria to improve yield (Getachew, 2019). Hence, estimates of correlation coefficients in combination with path coefficient analysis help to explain the direct and indirect contribution of one character upon another.

Cluster and principal component analysis (PCA) are widely used and useful tools for the determination of genetic relationships among genotypes in crop improvement. The PCA has been used to partition observed trait variations in the genotypes of many crops. Clustering is a classification technique that is used to arrange a set of cases into clusters. The aim of setting cases within a cluster is to make them more similar to each other and help the researchers give summary information on the data (Galbraith *et al*., 2010).

South Ethiopia is among the largest coffee-growing agro-ecological areas of the country, which contributes to more than 36 percent of the national market for coffee production (CSA, 2019). Its production potential is so high due to the suitable altitude, adequate rainfall, ideal temperature, and fertile soils (Yilma, 2017). The coffee-growing zones of south Ethiopia known for their high production potentials are Sidama, Gedeo, Gujji, Amaro, Gamo Goffa, Jinka, Wolayta, and Kembata-Tembaro. Despite its high potential for Arabica coffee growing and the existence of abundant genetic diversity, only six Arabica coffee varieties (four pure lines and two hybrids) had been released from this area's coffee gene pool. Although there is high demand for Yirgachaffe, Sidama, Amaro, and Gujji specialty coffees on the international market, the yield potential in the ecology is low. Therefore, the development of high-yielding, disease-resistant, and best-quality coffee varieties with outstanding processing and pest management technologies is an essential task.

One of the strategies the Ethiopian national coffee research program follows is the landrace variety development program. As a result, indigenous coffee types have been collected, characterized, and evaluated in the major coffee-growing areas of southern Ethiopian agroecology. Through these efforts, over 700 accessions of coffee were collected from farmers' fields and forests in south Ethiopian coffee-growing areas, viz., Sidama, Gedeo, Jinka, Amaro, Gamo Gofa, and Gujji, and maintained in the Awada field bank. Evaluation and characterization of these accessions are underway, and 14 promising selections were promoted from different collection batches for variety development. Although these selections were promoted for their high yield potential, coffee berry disease resistance, and coffee leaf rust resistance at Awada, they were not yet systematically characterized for quantitative traits and not evaluated in different environments to test wider adaptation. Therefore, the current study was conducted to estimate the extent of genetic variability, heritability, and genetic advance; examine the association among agro-morphological traits; assess the effect of environment on agro-morphological traits; and identify high-yielding coffee genotypes for the southern Ethiopian agro-ecological growing environment.

3.2 Materials and Methods

3.2.1 Study Area

The experiment was established at the Awada, Shebedino, and Wonago substations in August 2015. Awada is located 315 kilometers from Addis Ababa and 40 kilometers from Hawassa city, near Yirgalem town. Geographically, the site is located at latitude of 6^{0}45'46"N, longitude of 38^{0}22'36"E, and elevation 1740 m.a.s.l. With an average annual rainfall of

1269mm, the study area has a semi-bimodal rainfall pattern that is characterized by double wet and dry seasons (AARSC, 2022).

The Wonago sub-station is located at 6^{0}17'54"N latitude, and 38^{0}13'05"E, longitudes and at an altitude of 1850m.a.s.l. while Shebedino trial site is located at 6^{0}50'48"N latitude, 38^{0}27'6"E longitude and an altitude of 1845masl. Awada and Shebedino growing environments are found in Sidama regional state whereas Wonago is found at Gedeo Zone in south Ethiopian regional state; all situated in the south Ethiopian growing agro-ecological areas. All three experimental sites have eutric-nitosol and chromotic-cambisol soil types, which are ideal for coffee production (Kebede & Bellachew, 2008).

3.2.2 Planting materials

A total of 17 genotypes, comprising fourteen promising selections and three standard check varieties, were used for this experiment. The promising selections were promoted from different south Ethiopian coffee collection batches for their high potential for coffee berry disease resistance, coffee leaf rust resistance, and high yield performance through a preliminary assessment carried out at Awada (Table 3.1).

Table 3. 1 Description of experimental materials

No.	Genotypes	Some Distinct Characteristics of Genotypes	Source
1	AW1777	High yielder, Vigorous, medium resistance for CLR, 100% survival	AARSC
2	AW1995	High yielder, Vigorous, low CLR, 100% survival, good bearing area	AARSC
3	AW9644	High yielder, Vigorous, low CLR, 100% survival, bronze tipped	AARSC
4	AW7494	High yield, low CBD, low CLR, 100% survival, green tipped, bold fruit	AARSC
5	**Angafa**	standard check variety (commercially released cultivar)	AARSC
6	AW9662	High yielder, Vigorous, 100% survival, green tipped	AARSC
7	AW105	Reasonable resistance for CBD, good yield, 80% survival, bronze tipped	AARSC
8	AW9622	High yielder, 100% survival, bronze tipped	AARSC
9	AW9628	Vigorous, 80% survival, bronze tipped	AARSC
10	**74112**	standard check variety (commercially released cultivar)	JARC
11	AW4994	High yielder, 100% survival, green tipped, bold fruit	AARSC
12	AW5994	High yielder, Vigorous, low CLR, 57% survival, bronze tipped	AARSC
13	AW9641	High yielder, 100% survival, bronze tipped	AARSC
14	AW3106	High yielder, good resistance to CBD, survival 100%, bronze tipped	AARSC
15	**Feyate**	standard check variety (commercially released cultivar)	AARSC
16	AW7705	Reasonable yield, resistance for CBD, 90% survival, bronze tipped	AARSC
17	AW9623	High yielder, 100% survival, light bronze tipped	AARSC

AARSC= Awada Agricultural Research Sub-center; JARC=Jima Agricultural Research Center

3.2.3 Experimental design

The experiment was established in August 2015 using a randomized complete block design with three replications (Gomez and Gomez 1984). The experimental plots consisted of eight coffee trees planted in a single row for each genotype, with a spacing of 2 meters between plants and rows and 4 meters between replications at each location.

3.2.4 Experimental Procedures

Seed preparation, seedling media preparation, seed sowing, seedling management, land preparation, planting, and tree management (weeding, nutrient management, permanent and temporary shedding, soil conservation, and sucker management) were carried out as per recommendation (Endale *et al*., 2008).

3.2.5.Data collection

Agro-morphological trait data were recorded following the descriptor lists given for Arabica coffee by IPGRI (IPGRI, 1996). Yield data was collected from 2018 to 2021 for four consecutive harvesting seasons. All other quantitative data were collected at the optimum stage from all central row plants (6 plants per each replication) of each plot in 2022. A list of agro-morphological traits with their assessment methods is described below in Table 3.2.

Table 3. 2 List of 26 agro-morphological traits and method of data collection

No.	Character	Unit	Methods of assessment and scales used
1	Height up-to-first-primary branches	cm	measured from the ground to the main-stem's first primary branch
2	Plant height	cm	measured from ground level to the top of the trunk
3	Stem diameter	cm	Main-stem diameter mmeasured from five cm above-ground
4	Canopy diameter	cm	The tree canopy's average length measured from the widest part of the tree canopy east-west and north-south
5	Length of longest-primary branches	cm	measured from the tip of the longest primary to the stem
6	Leaf length	mm	Average of five mature leaves, measured from petiole end to apex.
7	Leaf width	mm	Average of five mature leaves, measured at their broadest part.
8	Leaf petiole length	mm	Average of five mature leaves, measured from the base to the insertion with the blade
9	Average internode length of main-stem	cm	Computing per tree by the formula (TH–HFPB)/TNN-1, where TH=plant height; TNN=total number of main-stem nodes; and HFPB=height up-to-first primary branch
10	Number of Nodes on longest primary branches	number	Recorded by counting total number of nodes per longest primary.
11	Number of primary-branches	mm	Recorded by counting over all primary branches per tree
12	Number of secondary-branches	number	Recorded by counting per tree
13	Inflorescence stalk Length	number	Average of five randomly selected inflorescences length
14	Stamen stalk Length	number	Average of five randomly selected stamen stalk length
15	Number of stamens per flower	number	Recorded by counting average of 10 randomly selected flowers from different nodes
16	Fruit length	mm	Average of five normal, mature green fruits, measured at the longest part
17	Fruit thickness	mm	Average of five normal, mature green fruits, measured at the thickest part
18	Fruit width	mm	Average of five normal, mature green fruits, measured at the thickest part
19	Single berry weight	gm	Average of 200 mature fruits weight
20	Empty fruit rate (%)	%	Scored by floating fruits
21	Fruit filling coefficient	ratio	Computed by ratio of bean numbers over cherry numbers. The fruit filling coefficients range from 0 to 2, representing sterility and full fertility, respectively.
22	Yield	kg	Fresh cherry weight per tree recorded in kg and then converted to clean coffee in ton per hectare.
23	Seed length	mm	Average of five normal, mature seeds, measured at the longest part
24	Seed width	mm	Average of five mature seeds, measured at their widest part
25	Seed thickness	mm	Average of five mature seeds, measured at at their thickest part
26	Yield	ton	Bean yields were weighted on a tree basis and converted to clean coffee in ton ha^{-1}

3.2.6 Data Analysis

A separate analysis of variance (ANOVA) was conducted using the Statistical Analysis System (SAS 9.4 version statistical package) to examine the presence of genetic variability among genotypes in their performance for agro-morphological characters. A combined analysis of variance was done to obtain an estimate of the environment-genotype interaction effect after the homogeneity of variances test between environments using Levene's test. The mean comparison was made using the Turkey test. Genotypic and phenotypic correlation analyses were conducted to determine the association among the traits using SAS. Path coefficient analysis was conducted to study independent traits direct and indirect effects. For path coefficient analysis, coffee yield ton ha^{-1} was taken as a dependent variable, while the rest of the variables were considered contributory factors. The direct and indirect effects of the independent characters on coffee yield were studied using the formula suggested by Dewey and Lu (1959). Principal component analysis was done using SAS software to determine the relative importance of the traits responsible for variation among the coffee genotypes. Coffee genotypes were clustered by R software using the variability package with the Ward method of clustering strategy, which groups and sorts genotypes into clusters to form a dendogram (Popat *et al*., 2020). The numbers of clusters were determined by following the approach suggested by Copper and Miligan (1988) by looking at three statistics, namely Pseudo F, Pseudo t^2 and cubic clustering criteria.

The genotypic, phenotypic, and environmental coefficients of variance were estimated using the formula suggested by Allard (1960) and Singh & Chaudhary (1985) as follows:

$$G = \frac{MSg - MSe}{r}$$

$$P = G + E$$

$$E = \frac{MSe}{r}$$

where σ^2_G = genotypic variance, σ^2_P = phenotypic variance, σ^2_E = Environmental variance, MSg = mean square of genotypes, MSe = mean square of error, and r = number of replications

$$PCV = \frac{(\sigma^2 p)^{1/2}}{\bar{X}} \times 100$$

$$GCV = \frac{(\sigma^2 G)^{1/2}}{\bar{X}} \times 100$$

Where: GCV=Genotypic coefficient of variation, PCV=Phenotypic coefficient of variation, and $\bar{X}$ = grand mean of a character.

Heritability in the broad sense (h^2**)** of the characters was computed according to the formula as described by Allard (Allard, 1960) as follows:

$$H(h^2 b) = \frac{\sigma^2 G}{\sigma^2 P} \times 100$$

Where: $H(h^2_b)$ = Heritability in broad sense, σ^2G= Genotypic variance, σ^2P= Phenotypic variance

Genetic advance (GA) was determined as described by Johnson *et al.* (1955)

$$GA = K \times \sigma P \times h^2$$

Where: K = constant (which varies depending upon the selection intensity and, 2.06 at 5% selection intensity), σp = Phenotypic standard deviation calculated as square root of phenotypic variance, h^2= Heritability in broad sense, GA = Genetic advance.

Johnson et al. (1955) described how to quantify **genetic advance as a percentage of the mean (GAM)** as follows:

$$GAM(\%) = \frac{(GA)}{\bar{X}} \times 100$$

Where: G=genetic advance, and $\bar{X}$ =grand mean of a character.

3.3 Results and Discussion

3.3.1 Analysis of variance

A separate analysis of variance showed significant variation ($P < 0.01$ or $P < 0.05$) among seventeen genotypes at each location for all the studied quantitative morphological characters except the number of flowers per axil (Table 3.3). The existence of statistically significant variation among Arabica coffee genotypes for quantitative agro-morphological traits has been reported by many authors (Abdulfeta, 2018; Dawit, 2018; Desalegn, 2018; Adem *et al*., 2020; Merga *et al*., 2021; and Ndikumana, 2022). The significant variation for the traits indicated the presence of a considerable amount of variation among the studied Arabica coffee genotypes, and this variation could be exploited to improve coffee yield through selection and hybridization.

Combined analyses of variance showed a significant difference ($P < 0.01$ or $P < 0.05$) among genotypes and locations for all the traits studied except for the number of flowers per axil. In addition to that, significant genotype by environment interaction (GEI) was also observed for the traits viz. number of primary-branches (No.), fruit length (cm), fruit width (cm), fruit thickness (mm), empty fruit rate (%), fruit filling coefficient (%), seed length (mm), seed width (mm), seed thickness (mm), and yield (ton ha^{-1}) (Table 3.3). The existence of significant GEI variation for the aforementioned traits indicated the inconsistency in performance of the genotypes for these traits across environments. This suggests that the phenotypic expression of one genotype might be superior to another genotype in one environment but inferior in a different environment. Different researchers reported differential performance of genotypes when grown in different environments for most morphological traits (Marie *et al*., 2020; Beksisa, 2021; Cheserek *et al*., 2021; Merga *et al.*, 2021; Ndikumana, 2022).

Table 3. 3 Separate and combined analysis of variance for the 26 agro-morphological characters of 17 genotypes grown at Awada, Shebedino and Wonago

Traits	Awada				Shebedino				Wonago				Combined					
	Rep (df=2)	Gen (df=16)	Error (df=32)	CV	Rep (df=2)	Gen (df=16)	Error (df=32)	CV	Rep (df=2)	Gen (df=16)	Error (df=32)	CV	Rep (df=2)	Loc (df=2)	Gen (df=16)	Gen*Loc (df=32)	Error (df=100)	CV
HPFP	20.59^{ns}	52.36**	10.69	15.28	40.89 ^{ns}	146.15*	36.52	33.16	15.40^{ns}	30.37*	14.57	19.59	42.65^{ns}	130.21**	92.27**	18.31^{ns}	20.45	22.95
SG	0.04^{ns}	0.39**	0.05	6.55	0.05 ^{ns}	0.34**	0.05	4.75	0.44^{ns}	0.35**	0.08	6.94	0.13^{ns}	22.22**	0.74**	0.17^{ns}	0.07	6.31
PH	123.63^{ns}	1220.55**	354.52	7.33	459.28^{ns}	2345.99*	674.13	26.96	6232.89^{ns}	1427.62*	590.00	9.60	1880.2^{ns}	42250.83**	5479.14*	2257.51^{ns}	2723.8	19.23
INLMS	1.24^{ns}	3.58*	1.00	12.90	11.03 ^{ns}	29.85*	7.41	27.53	6.95^{ns}	3.37*	1.45	13.76	2.37^{ns}	58.75**	5.68*	4.39^{ns}	3.54	21.39
CD	695.56^{ns}	624.75**	130.26	5.40	393.77^{ns}	719.23*	309.92	25.66	954.35^{ns}	828.66**	165.70	6.45	69.48^{ns}	3208.8*	3092.25*	1235.19^{ns}	1177.71	16.45
NPB	1.65^{ns}	84.55**	6.27	3.23	2.63^{ns}	22.85**	2.78	2.16	17.51^{ns}	38.02*	15.64	5.97	9.07^{ns}	2061.59**	70.27**	37.57**	8.14	3..87
NSB	508.45^{ns}	4352.82**	292.95	17.12	19.56^{ns}	547.06**	72.33	3.18	41.51^{ns}	2811.06**	160.57	6.74	248.82^{ns}	169293.86**	4368.52**	1671.21^{ns}	174.67	5.25
LLP	28.37^{ns}	247.64**	37.80	5.42	843.27^{ns}	1579.78*	972.98	25.73	181.82^{ns}	207.97**	29.47	5.41	71.64^{ns}	1772..06**	512.27**	19.53^{ns}	36.18	5.41
NNLP	3.20^{ns}	14.30*	7.42	10.46	64.79^{ns}	149.42*	52.22	27.44	23.15^{ns}	5.67*	3.16	7.53	4.84^{ns}	66.93*	9.17**	7.92^{ns}	4.46	8.42
INLLP	0.19^{ns}	1.20**	0.40	12.08	1.78^{ns}	3.65^{ns}	2.44	24.45	0.41^{ns}	1.20*	0.24	10.17	0.32^{ns}	32.88**	1.81*	1.72^{ns}	1.04	18.6
LL	8.05^{ns}	107.76*	34.50	4.05	3.57^{ns}	184.28**	50.48	4.48	108.40^{ns}	156.41*	43.20	4.55	44.57^{ns}	3368.11**	366.57**	40.9^{4ns}	42.53	4.37
LW	27.70^{ns}	47.48*	15.02	5.84	8.96^{ns}	65.93*	18.66	5.73	18.94^{ns}	48.92*	14.41	5.33	47.13^{ns}	1022.63**	126.02**	18.16^{ns}	17.56	5.9
LPL	0.48^{ns}	1.42*	0.50	8.15	4.13^{ns}	1.30*	0.36	7.19	0.36^{ns}	1.01**	0.19	5.13	0.83*	0.96*	3.01*	0.36^{ns}	0.36	7.04
ISL	1.76^{ns}	1.41*	0.61	10.04	0.07^{ns}	2.84*	0.48	8.43	0.26^{ns}	0.89^{ns}	0.62	10.01	1.38^{ns}	2.75*	2.21**	0.47^{ns}	0.68	8.73
NFPA	6.05^{ns}	2.27^{ns}	3.11	16.68	4.98^{ns}	3.83^{ns}	7.22	18.92	2.85^{ns}	4.78^{ns}	5.55	23.77	4.1^{ns}	7.34^{ns}	4.22^{ns}	2.37^{ns}	3.01	15.51
SSL	0.19^{ns}	1.62**	0.23	4.60	0.60^{ns}	7.26*	4.75	18.33	3.11^{ns}	1.70^{ns}	2.60	15.98	0.35^{ns}	6.22*	3.46*	0.47^{ns}	1.29	10.64
FL	0.07^{ns}	1.87**	0.54	4.52	0.38^{ns}	0.61*	0.31	3.60	1.00^{ns}	0.88*	0.28	3.38	0.09^{ns}	6.61**	1.82**	0.78*	0.39	3.95
FW	0.00^{ns}	0.91**	0.14	2.65	0.07^{ns}	0.40*	0.21	3.29	0.41^{ns}	0.50*	0.20	3.25	0.22^{ns}	5.78**	4.98**	1.38*	0.35	5.94
FT	0.05^{ns}	0.49**	0.12	2.75	0.01^{ns}	0.52*	0.20	3.69	0.25^{ns}	0.35*	0.16	3.48	0.19^{ns}	36.14**	0.88**	0.32*	0.18	3.31
SBW	113.85^{ns}	393.38**	126.87	6.04	76.66^{ns}	310.30*	150.23	6.61	39.27^{ns}	262.05*	131.27	6.23	126.8^{ns}	517083**	468.56**	117.73^{ns}	90.01	7.43
EFR	6.07^{ns}	31.17*	14.40	4.19	6.07^{ns}	31.17*	14.40	4.19	41.37^{ns}	100.83*	37.40	6.66	30.33^{ns}	147682.24**	105.56*	109.41*	51.65	5.91
FFC	0.01^{ns}	0.05**	0.00	2.63	0.00^{ns}	0.702*	0.01	3.28	0.00^{ns}	0.01*	0.00	3.17	13.88^{ns}	137899.76**	18.91**	19.99**	12.52	11.12
SL	0.22^{ns}	0.70**	0.17	3.94	0.61^{ns}	0.74*	0.21	4.37	0.10^{ns}	0.99**	0.12	3.49	0.19^{ns}	1251.47**	0.63**	0.41**	0.13	4.73
SW	0.32^{ns}	0.21*	0.09	4.08	0.14^{ns}	0.34**	0.08	3.85	0.07^{ns}	0.40**	0.10	4.66	0.30^{ns}	144.78**	0.89**	0.32*	0.1	3.8
ST	0.01^{ns}	1.04*	0.05	5.19	0.03^{ns}	0.17**	0.04	4.73	0.01^{ns}	0.13*	0.05	5.27	0.06^{ns}	110.19**	0.22**	0.19**	0.06	4.85
YI	2.78^{ns}	16.53**	1.70	8.53	5.98^{ns}	6.22*	2.56	13.74	0.37^{ns}	14.96**	1.69	15.14	1.85^{ns}	568.74**	18.58**	9.56**	2.05	12.09

Where; *& ** = Significant at P<0.05 and P<0.01 respectively; Rep= Replication; Loc=Location; Gen=Genotype; Gen*Loc =genotype by environment interaction; Numbers in parenthesis stands for the degree of freedom; CV= coefficient of variation; HPFP=Height up-to-first primary (cm); SG=stem-girth (cm); PH=Plant Height (cm); INLMS= Inter Node length on Main-stem (cm); CD=Canopy diameter (cm); NPB=Number of Primary Branch (No.); NSB=Number of Secondary Branch (No.); LLP=Length of longest-primary (cm); NNLP=Number of nodes on longest primary (No.); INLLP=Inter-node-length on longest primary (cm); LL=Leaf length (mm); LW=Leaf width (mm); LPL=Leaf petiole length (mm); ISL=Inflorescence Stalk Length (mm); SSL=Stamen stalk Length (mm); FL=Fruit length (cm); FW=Fruit width (mm);

FT=Fruit thickness (mm); SBW=Single berry weight (gm); EFR=Empty fruit rate (%); FFC=Fruit filling coefficient; SL=Seed length (mm); SW=Seed width (mm); ST=Seed thickness (mm); YI=Yield (ton ha-1)

3.3.2 Mean Performance

The mean, range, and standard deviation of the mean value for 26 traits are shown in Table 3.4. Wider ranges between the minimum and maximum values were observed for the majority of the studied traits (Table 3.4), reflecting the existence of ample amounts of variation among the tested genotypes for agro-morphological traits. This study result also revealed high performance of promising selections at each of the three locations over the standard check varieties for the majority of the agro-morphological traits. Therefore, any improvement through selection and/or hybridization could be advised to exploit the abundant genetic resources of the country.

The mean performance for yield revealed highly significant ($p<0.01$) variation among main effects (genotypes and locations) as well as interaction effects (Table 3.3). In line with the present results, many authors also reported significant variation among genotypes, environment, and GEI for Arabica coffee bean yield (Argaw & Taye, 2018; Beksisa *et al*, 2018; Marie *et al*., 2020; Beksisa, 2021; Cheserek *et al*., 2021; Merga, 2021; Legesse *et al*., 2022; Merga, 2022).

When we consider the four-year harvesting season mean yield (combined over season) in tons per hectare, the highest yield at Awada was recorded for genotype AW5994 (1.86 ton ha^{-1}), followed by AW3106 (1.83 ton ha^{-1}), Angafa (1.8 ton ha^{-1}), and AW105 (1.76 ton ha^{-1}). The highest yield at Shebedino was recorded for AW7705 (1.52 ton ha^{-1}) followed by AW9641 (1.34 ton ha^{-1}), AW9622 (1.29 ton ha^{-1}), Angafa (1.27 ton ha^{-1}), and AW5994 (1.20 ton ha^{-1}). The highest yield at Wonago was recorded for AW105 (1.32 tons/ha) followed by AW7705 (1.22 ton/ha), AW1777 (1.09 ton ha^{-1}), AW4994 (1.05 ton ha^{-1}), Angafa (0.98 ton ha^{-1}), and AW7494 (0.96 ton ha^{-1}) (Table 3.4). In this study, the coffee bean mean yield of the 14 selections was higher than the mean of the three check varieties in three locations and four harvesting years except in 2019 at Awada, 2020 at Awada & Wonago, and 2021 at Awada & Shebedino (Table 3.4).

Yield performance variation was also observed between growing locations. The highest yield was recorded at Awada (1.53 ton ha-1) followed by Shebedino (1.16 ton ha-1) and the lowest at Wonago (0.86 ton ha-1) (Table 3.4).

In addition to genotypic and location effects on yield performance, the coffee yield was highly oscillating in season. The highest yield was recorded in the 2018 and 2020 harvesting seasons at three locations (2.09 ton ha^{-1} and 2.37 ton ha^{-1} at Awada; 1.59 ton ha^{-1} and 1.874 ton ha^{-1} at Shebedino; and 1.48 ton ha^{-1} at Wonago). The lowest was recorded in 2019 and 2021 at three locations (0.65 & 0.99 ton ha-1 at Awada, 0.31 & 0.89 ton ha^{-1} at Shebedino and 0.62 & 0.39 ton ha^{-1} at Wonago) showing the yearly variation of yield. In line to this study, Antonio *et al*., 2010 and Atinafu *et al*., 2017, reported yield variation by year (seasonal oscillation) for Arabica coffee. Many authors reported seasonal yield variation due to the biennial nature of the plant or seasonal environmental variation (Camargo *et al*., 2010; Jaramillo-Botero *et al*., 2010; Bernardes *et al*., 2012; Carvalho, 2019; Merga et al., 2023). Hence, season has a great impact on yield variation; it is difficult to identify a superior variety in a single harvesting year. Therefore, considering seasonal effect and repeating the experiment over seasons is advised to identify best performing and stable genotypes for variety development. In addition, examining different management option after high yielding season is also recommended.

When we consider over three locations and four harvesting seasons mean yield, the highest mean bean yield value was recorded for selections AW105 (1.37 ton ha^{-1}) and AW7705 (1.37 ton ha^{-1}) followed by Angafa (1.35 ton ha^{-1}), AW5994 (1.28 ton ha^{-1}), AW4994 (1.26 ton ha^{-1}), AW3106 (1.25 ha^{-1}), and AW9641 (1.22 ha^{-1}) (Table 3.4). These promising selections outperformed the released varieties and could be used for future improved variety development through selection and hybridization.

When we compare the performance of check variety, Angafa was the highest yielding and best check at each location for 4 harvesting years (2018–2021) than Feyate and 74112, except in the 2019 harvesting season. In the 2019 harvesting season, variety Feyate was the highest yielding check at Awada and Wonago, whereas variety 74112 was the highest yielder at Shebedino. On the other side, the check variety 74112 was the lowest yielder and poorest performer in all seasons and locations except for 2019 at Shebedino (Table 3.4). As a result, coffee growers in south Ethiopia should search for other high-yielding varieties to cultivate in place of 74112, and coffee breeders need to use other varieties as a standard check for comparison.

Table 3. 4 Mean, standard deviation of the mean, maximum value, minimum value, and minimum significant difference of 26 characteristics of 17 genotypes evaluated at Awada, Shebedino and Wonago

	Awada					Leku					Wonago					Combined				
Trait	Mean	Max	Min	STD	MSD	Mean	Max	Min	STD	MSD	Mean	Max	Min	STD	DMR	Mean	Max	Min	STD	MSD
HPFP	21.40	30.56	12.78	4.18	10.01	18.22	29.00	10.33	3.92	10.05	19.49	24.33	13.44	2.94	11.68	19.70	25.74	12.18	3.20	7.57
SG	3.42	3.94	2.64	0.36	0.69	4.73	5.33	3.86	0.34	0.69	4.20	4.78	3.44	0.35	0.89	4.11	4.60	3.32	0.29	0.43
PH	256.71	288.22	215.44	20.17	31.32	304.58	485.56	256.67	49.48	60.55	252.95	287.00	222.78	21.05	40.40	271.00	340.52	231.63	24.65	48.81
INLMS	7.75	8.67	6.28	0.64	1.66	9.89	16.33	7.22	1.81	4.53	8.74	10.56	6.33	1.05	3.68	8.79	10.63	6.61	0.79	3.15
CD	211.24	230.89	166.50	14.43	18.98	214.90	349.44	171.67	37.01	91.70	199.70	222.78	159.17	15.24	21.41	209.00	258.15	165.78	18.58	32.10
NPB	77.62	89.41	67.89	5.31	7.66	76.96	81.44	71.44	2.76	5.10	66.29	72.52	59.11	3.65	6.58	73.62	77.83	68.39	2.78	4.77
NSB	300.05	362.15	233.30	38.09	28.47	267.27	296.11	250.22	13.50	14.15	187.99	275.00	141.00	29.04	21.08	251.77	289.94	224.12	22.08	12.36
LLP	113.42	121.78	82.11	9.09	10.23	121.24	192.33	95.00	19.83	51.88	100.26	108.33	80.89	7.60	9.03	111.18	120.96	86.00	7.55	31.41
NNLP	26.03	29.00	19.78	2.18	4.53	26.33	43.56	23.11	4.65	12.02	23.59	26.33	21.44	1.38	2.95	25.08	27.56	24.00	1.01	4.34
INLLP	5.26	6.11	3.74	0.63	1.94	6.39	10.00	4.78	1.10	2.60	4.83	5.33	4.00	0.36	0.82	5.49	6.37	4.32	0.45	1.71
LL	144.92	153.67	130.00	5.99	9.77	158.69	170.39	135.89	7.84	11.82	144.32	157.44	127.61	7.42	20.11	149.00	157.19	131.17	6.34	10.91
LW	66.41	71.50	53.28	3.98	11.86	75.36	83.67	61.28	4.69	7.18	71.23	77.11	60.50	4.17	11.61	71.00	74.50	58.35	3.80	7.01
LPL	8.64	9.89	7.22	0.69	2.15	8.37	9.56	7.11	0.66	1.84	8.47	9.33	7.00	0.60	1.33	8.50	9.59	7.11	0.57	1.00
ISL	7.75	8.73	6.50	0.69	1.30	8.18	8.87	7.27	0.53	2.11	7.88	8.93	7.00	0.51	1.31	7.98	8.60	7.16	0.49	1.26
NFPA	10.58	13.13	9.53	0.87	ns	14.20	16.27	12.60	1.13	ns	9.91	12.27	8.27	1.25	ns	11.19	12.53	10.31	0.56	ns
SSL	10.47	11.27	9.00	0.73	1.47	11.89	14.33	9.20	1.44	3.62	10.08	11.60	9.00	0.74	2.68	10.70	11.36	9.27	0.62	1.47
FL	16.18	17.95	14.93	0.79	2.24	15.46	16.62	14.71	0.45	1.70	15.77	16.67	14.74	0.55	1.63	15.80	16.76	15.08	0.45	1.04
FW	14.27	15.46	13.48	0.55	1.16	13.74	14.52	13.24	0.36	0.75	13.64	14.61	13.01	0.42	1.36	14.30	15.93	13.49	0.34	0.72
FT	12.45	13.30	11.79	0.41	1.05	12.02	12.53	11.29	0.42	0.74	11.50	12.26	10.86	0.35	1.23	12.10	13.69	11.46	0.31	0.68
SBW	186.34	208.70	161.44	11.45	18.73	185.47	205.00	162.66	10.17	20.39	183.78	199.17	162.27	9.36	19.06	185.00	207.40	159.42	10.14	10.71
EFR	90.57	94.50	83.99	3.22	6.31	90.57	94.50	83.99	3.22	6.31	91.87	98.44	79.65	5.72	18.71	87.80	91.59	81.84	3.98	7.77
FFC	1.75	1.85	1.44	0.12	0.14	1.87	1.96	1.68	0.08	0.10	1.90	1.98	1.80	0.06	0.10	1.81	1.91	1.60	0.08	0.09
SL	10.36	11.14	9.57	0.48	1.25	10.59	11.31	9.34	0.50	1.42	10.12	11.01	8.78	0.58	1.08	10.60	12.38	9.34	0.39	0.69
SW	7.21	7.68	6.72	0.27	0.90	7.19	7.94	6.62	0.34	0.85	6.85	7.50	6.17	0.38	0.98	7.25	9.26	6.61	0.27	0.49
ST	4.26	4.41	4.01	0.12	0.37	4.34	5.05	3.95	0.24	0.63	4.10	4.57	3.79	0.21	0.66	4.42	6.02	4.02	0.15	0.35
YI	1.53	1.86	1.07	2.35	0.40	1.16	1.52	0.85	0.14	0.35	0.86	1.32	0.52	0.23	0.40	1.19	1.37	0.82	1.43	0.24

Where: Max=maximum; min=minimum; StD=Standard deviation; MSD=minimum significant difference (Tukey test at 5%); ns=non-significant; HPFP=Height Up-to-first-primary (cm); SG=stem-girth (cm); PH=Plant Height (cm); INLMS= Inter Node length on Main-stem (cm); CD=Canopy diameter (cm); NPB=Number of Primary Branch (No.); NSB=Number of Secondary Branch (No.); LLP=length of longest-primary (cm); NNLP=Number of nodes on longest primary (No.); INLLP=Inter-node length on longest primary (cm); LL=Leaf length (mm); LW=Leaf width (mm); LPL=Leaf petiole length (mm); ISL=Inflorescence Stalk Length (mm); SSL=Stamen stalk Length (mm); FL=Fruit length (cm); FW=Fruit width (mm); FT=Fruit thickness (mm); SBW=Single berry weight (gm); EFR=Empty fruit rate (%); FFC=Fruit filling coefficient; SL=Seed length (mm); SW=Seed width (mm); ST=Seed thickness (mm); YI=Yield (ton ha-1)

Table 3. 5 The four consecutive harvesting season mean yield of 17 genotypes grown at Awada, Shebedino and Wonago

Genotype	2018/19 harvesting year			2019/20 harvesting year			202021 harvesting year			2021/22 harvesting year			Over season Combined			Pooled (Over all season & location)
	Awada	Shebedino	Wonago	Awada	Shebedino	Wonago	Awada	Shebedino	Wonago	Awada	Shebedino	Wonago	Awada	Shebedino	Wonago	
AW1777	1.3	0.9	2.02	0.54	0.4	0.6	2.61	2.04	1.49	0.83	1.22	0.27	1.32	1.14	1.09	1.19
AW105	2.06	1.64	1.11	0.37	0.46	0.9	2.97	1.35	2.7	1.21	0.66	0.57	1.76	1.03	1.32	1.37
AW1995	2.45	1.39	1.12	0.23	0.36	0.65	2.99	1.78	1.47	0.39	0.94	0.25	1.51	1.12	0.87	1.17
AW3106	2.57	1.9	0.97	0.91	0.28	0.51	2.96	1.89	1.07	0.88	0.66	0.47	1.83	1.18	0.76	1.26
AW4994	1.92	1.49	0.81	1.01	0.4	0.92	2.02	1.77	1.67	1.62	0.67	0.79	1.64	1.08	1.05	1.26
AW5994	2.68	1.97	1.02	1.05	0.33	0.52	2.33	2	1.34	1.38	0.52	0.25	1.86	1.2	0.78	1.28
AW7494	2.14	2.08	0.97	0.36	0.29	0.57	2.59	1.41	1.93	0.24	0.76	0.36	1.33	1.13	0.96	1.14
AW7705	1.94	2.2	1.57	0.79	0.24	0.65	1.69	2.39	2.09	1.06	1.25	0.55	1.37	1.52	1.22	1.37
AW9622	2.45	2.37	0.66	0.47	0.2	0.58	2.24	1.93	1.2	0.69	0.67	0.24	1.47	1.29	0.67	1.14
AW9623	2.62	1.59	0.87	0.32	0.19	0.47	2.32	2.09	1.34	1.31	0.68	0.49	1.64	1.14	0.79	1.19
AW9628	1.73	1.12	0.64	0.58	0.29	0.53	1.6	1.78	0.63	0.64	1.06	0.27	1.14	1.06	0.52	0.91
AW9641	2.35	1.37	0.81	0.93	0.35	0.52	1.56	2.47	0.93	1.8	1.18	0.36	1.66	1.34	0.66	1.22
AW9644	1.84	1.54	0.58	0.42	0.53	0.56	2.74	1.57	1.21	0.37	1.13	0.4	1.34	1.19	0.69	1.07
AW9662	2.38	1.43	0.97	0.99	0.33	0.85	2.26	1.92	1.1	1.09	0.85	0.4	1.68	1.13	0.83	1.21
74112	0.84	0.77	0.39	0.75	0.23	0.32	2.36	1.67	1.21	0.33	0.75	0.25	1.07	0.85	0.54	0.82
Angafa	2.38	1.9	0.88	0.37	0.14	0.63	2.76	1.92	1.98	1.68	1.12	0.42	1.8	1.27	0.98	1.35
Feyate	1.89	1.37	0.64	1.06	0.19	0.7	2.3	1.89	1.87	1.36	0.94	0.39	1.65	1.1	0.9	1.22
Mean	**2.09**	**1.59**	**0.94**	**0.65**	**0.31**	**0.62**	**2.37**	**1.87**	**1.48**	**0.99**	**0.89**	**0.39**	**1.53**	**1.16**	**0.86**	**1.19**
CV	16.53	24.12	14.28	9.00	20.13	28.88	18.30	21.04	28.75	24.15	17.44	15.14	9.00	13.74	15.14	12.10
MSD	**0.76**	**0.48**	**0.94**	**0.76**	**0.98**	**0.27**	**0.85**	**0.57**	**0.83**	**0.45**	**0.79**	**0.36**	**0.42**	**0.49**	**0.40**	**0.24**
P-value	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**

Where: CV (%) =coefficient of variation in percent; MSD= Minimu significant difference (Tukey Test at 5%); and ** = highly significant

3.3.3 Variance Components

The estimation of genotypic, phenotypic, and environmental coefficients of variance for 25 agro-morphological traits are given in Table 3.6. Genotypic variance values ranged from 0.05 for seed thickness to 1397.5 for number of secondary-branches, while phenotypic variance values ranged from 0.07 to 1826.38 for seed thickness and plant height, respectively. The magnitudes of genetic variances were higher for plant height (918.45), canopy diameter (638.18), number of secondary-branches (1397.95), length of longest-primary (158.7), single berry weight (126.18), leaf length (108.01), and leaf width (36.15) (Table 3.5). The level of variation was shown to be different among different quantitative characters. In current study, the phenotypic variance was higher than the genotypic variance for the characters studied (Table 3.6). Thus, it suggests the substantial influence of the environment besides the genetic variation for the expression of these traits.

The genotypic coefficient of variation (GCV) values ranged from 3.75% for fruit width to 80.64% for fruit filling coefficient; whereas, the phenotypic coefficient of variation (PCV) values ranged from 4.13% for fruit width to 138.76% for fruit filling coefficient (Table 3.6). According to Terfa and Gurmu (2020), PCV and GCV values greater than 20% are considered high; values between 10% and 20% are medium; and less than 10% are considered low. In this study, the high (>20%) PCV and GCV values were noted for the fruit filling coefficient (80.65%, 138.72%), height up to the first primary (24.84%, 28.15%), and bean yield (20.16%, 21.38%), respectively. In addition, moderate GCV and PCV were calculated for plant height (11.17%, 15.75%), canopy diameter (12.11%, 15.39%), number of secondary-branches (14.85%, 15.16%), length of longest-primary (11.33, 11.75), and leaf petiole length (11.05, 11.78), respectively.

According to Sing (1990), traits performing high estimates of GCV and PCV have a high potential for effective selection, but traits with low estimates for both variability components are difficult or impractical for selection due to the masking effect of environment on the genotypic effect. As the present study result reveals, fruit filling coefficient, height up to the first primary, bean yield, plant height, canopy diameter, number of secondary-branches, length of longest primary, and leaf petiole length traits had high to moderate GCV and PCV values and thus, can provide a high chance for effective selection. Moderate to high PCV and GCV values for the plant vigor and yield components were also reported by many authors (Kebede & Belachew 2008; Kitila *et al.* 2011; Tessema *et al.* 2011; Atinafu *et al.*, 2017;

Malau & Pandiangan, 2018; Ndikumana, 2022). Low and moderate genotypic variation in most of the parameters might indicate the self-fertilized nature of Arabica coffee plants. Broad genetic variability may be obtained through hybridization.

In this study, the values of PCV were relatively higher than GCV for all traits; however, there was a slight variation between the PCV and GCV values for traits of leaf width, leaf length, leaf petiole length, fruit length, fruit width, fruit thickness, single berry weight, empty fruit rate, seed width, seed length, and seed thickness. This indicated that the environment influenced to a lesser extent these characters and that there was a high contribution of genotypic effect for the phenotypic expression of these characters. Therefore, selection based on these characters' phenotypic performance would be reliable.

3.3.4 Estimation of broad sense heritability and genetic advance

The estimation of broad sense heritability (Hb%), Genetic advance (GA), and genetic advance as a percentage of the mean (GAM) for 25 agro-morphological traits were computed using combined mean values and presented in Table 3.6. The GCV shows how much of the observed phenotypic variability is due to genetic factors, but it does not show how much of the variation is heritable or not. Therefore, Hb% estimate is required to forecast the expected gain through the selection process. The Hb% estimates ranged from 96.0% for the number of secondary-branches to 33.8% for the fruit filling coefficient (Table 3.6). Singh (2001) asserts that, Hb% values above 80% are considered high, values between 60% and 79% are moderately high, values between 40% and 59% are moderate, and values below 40% are considered low. According to this benchmark, high and moderately high Hb% values (>80% & 60 to 79%) were noted for number of secondary-branch (96.0%); length of longest-primary (92.94%); stem-girth (90.91%); seed width (89.16%); yield (88.95%); number of primary-branch (88.42%); leaf length (88.4%); leaf petiole length (88.09%); leaf width (86.07%); fruit width (82.11%); single berry weight (80.79%); seed length (79.55%); fruit thickness (79.91%); fruit length (78.59%); height up-to-first primary (77.83%); seed thickness (72%); inflorescence stalk length (69.23%); stamen stalk length (62.72%); and canopy diameter (61.91%) in descending order. According to Singh (2001), if a character's heritability is quite high, selecting for such characters may be simple due to the relatively little contribution of the environment on the phenotype; in contrast, the selection of low-heritability characters is rather difficult or practically impractical due to environmental masking effects. Therefore, the aforementioned characters are effective for selection to improve crop productivity.

The medium Hb% values (40 to 59%) were observed for empty fruit rate (51.07%); plant height (50.29%); number of nodes on longest primary (51.36%); and inter-node length on longest primary (42.34%). Whereas, low Hb% values (<40%) were observed for the inter-node length on the main-stem (37.77%) and fruit filling coefficient (33.8%). In line with this study result, the existence of high to moderate Hb% was reported for the traits of secondary branches, canopy diameter, fruit length, fruit width, bean length, bean width, bean weight, and plant height by different scholars (Yigzaw, 2005; Malau & Pandiangan, 2018; Kitila *et al.*, 2011; Olika *et al.*, 2001; Tessema *et al.*, 2011; Malau & Pandiangan, 2018; and Ndikumana, 2022). In contrast to this result, Bayetta (2001) observed high Hb% estimations ranging from 71.43 to 97.32% among the assessed characters, implying that the effect of environment on the phenotypic appearance of the characters was minor, and Ermias (2005) reported low Hb% for primary branches (13%). The present study results are generally in agreement with most of the findings of previous studies. Some discrepancies with some previous findings may be related to differences in the number and type of genotypes studied and the number of seasons and locations from which the data were collected.

The genetic advance as a percentage of the mean (GAM) ranged from 96.58% for the fruit filling coefficient to 6.99% for the fruit width. According to Jonhson *et al.* (1955), the value of GAM is categorized as high (> 20%), moderate (10–20%), and low (10%). Based on this category, high GAM were recorded for the fruit filling coefficient (96.58%); height up to the first primary (45.14); bean yield (3917%); number of secondary-branches (29.97%); stem-girth (22.65%); length of the longest primary (22.5%); and leaf petiole length (21.37%) in decreasing order. Medium GAM were recorded for canopy diameter (19.63%), plant height (16.31%), leaf width (16.18%), inflorescence stalk length (15.34%), seed width (13.81%), leaf length (13.48%), stamen stalk length (12.97%), inter-node length on longest primary (12.34%), inter-node length on main-stem (12.18%), number of primary-branches (11.97%), and single berry weight (11.25%), whereas low GAM was recorded for seed thickness (9.15%), fruit length (7.96%), number of nodes on longest primary (7.38%). Similar to this finding, high-heritability together with high-expected genetic advance as a percentage of the mean for Arabica coffee yield was reported by several researchers (Ermias, 2005; Yigzaw, 2005; Abdi, 2009; Olika *et al.*, 2011; Atinafu *et al.*, 2017). In contrast to this finding, low heritability coupled with low-expected GAM for Arabica coffee yield were reported by (Merga *et al.*, 2021).

The estimate of heritability alone does not indicate the expected gain in the next generation. Therefore, knowledge of heritability coupled with genetic advance will provide a clear base on the reliability of the particular traits for efficient selection. In predicting gain under selection, estimates of high heritability along with high genetic advances are usually more effective than estimates of heritability alone (Slam *et al*., 2015). Whereas, low heritability accompanied by genetic advance would offer less chance for selection due to non-additive gene effects and the influence of the environment. Moderately high-to-high heritability coupled with moderate to high GAM was observed for length of longest-primary branch, stem-girth, number of secondary branch, yield, canopy diameter, leaf length, number of primary branch, leaf petiole length, leaf width, single berry weight, height up to the first primary, inflorescence stalk length, stamen stalk length, and seed width. Therefore, selection in the next generation based on these characters would be effective.

Table 3.6 Estimates of variance components, broad-sense-heritability and genetic advance for combined 25 agro-morphological traits of 17 genotypes

Traits	σ2g	σ2p	σ2E	GCV(%)	PCV(%)	Hb (%)	GA	GAM
HPFP	23.94	30.76	6.82	24.84	28.15	77.83	8.89	45.14
SG	0.22	0.25	0.02	11.53	12.09	90.91	0.93	22.65
PH	918.45	1826.38	907.93	11.17	15.75	50.29	44.27	16.31
INLMS	0.72	1.89	1.18	9.62	15.66	37.77	1.07	12.18
CD	638.18	1030.75	392.57	12.11	15.39	61.91	40.95	19.63
NPB	20.71	23.42	2.71	6.18	6.57	88.42	8.82	11.97
NSB	1397.95	1456.17	58.22	14.85	15.16	96.00	75.47	29.97
LLP	158.70	170.76	12.06	11.33	11.75	92.94	25.02	22.50
NNLP	1.57	3.06	1.49	5.00	6.97	51.36	1.85	7.38
INLLP	0.26	0.60	0.35	9.21	14.15	42.34	0.68	12.34
LL	108.01	122.19	14.18	6.96	7.40	88.40	20.13	13.48
LW	36.15	42.01	5.85	8.47	9.13	86.07	11.49	16.18
LPL	0.88	1.00	0.12	11.05	11.78	88.09	1.82	21.37
ISL	0.51	0.74	0.23	8.95	10.76	69.23	1.22	15.34
SSL	0.72	1.15	0.43	7.95	10.04	62.72	1.39	12.97
FL	0.48	0.61	0.13	4.36	4.92	78.59	1.26	7.96
FW	0.29	0.35	0.06	3.75	4.13	82.11	1.00	6.99
FT	0.23	0.29	0.06	3.99	4.47	79.91	0.89	7.36
SBW	126.18	156.19	30.00	6.08	6.76	80.79	20.8	11.25

							0	
EFR	17.97	35.19	17.22	4.83	6.76	51.07	6.24	7.11
FFC	2.13	6.30	4.17	80.65	138.72	33.80	1.75	96.58
SL	0.17	0.21	0.04	3.88	4.35	79.55	0.75	7.13
SW	0.26	0.30	0.03	7.10	7.52	89.16	1.00	13.81
ST	0.05	0.07	0.02	5.23	6.17	72.00	0.40	9.15
YI	5.72	6.43	0.71	20.16	21.38	88.95	4.65	39.17

Where: σ^2g= Genotypic variance; σ^2p= Phenotypic variance; σ^2E= environmental variance; GCV(%)=Genotypic coefficient of variation; PCV(%)=Phenotypic coefficient of variation; Hb(%)=Heritability in broad sense; GA = Genetic advance ; GAM= genetic advance as a percentage of the mean

3.3.5 Correlations

To understand the magnitude of association of various quantitative traits and yields of Arabica coffee genotypes, genotypic and phenotypic correlation coefficients analysis were computed using combined mean values of 25 quantitative traits and presented in Table 3.7.

In this investigation, most plant vigor parameters were positively associated among themselves and with yield (Table 3.7). Yield ton ha^{-1} had significantly and positively associated with stem-girth (r_g = 0.59, r_p= 0.54), inter-node length on main-stem (r_g = 0.52, r_p= 0.41), canopy diameter (r_g = 0.53, r_p= 0.43), number of primary-branch (r_g = 0.35, r_p= 0.34), number of secondary-branch (r_g = 0.49, r_p= 0.45), length of longest-primary branch (r_g = 0.54, r_p= 0.59), inter node length on longest primary (r_g = 0.55, r_p= 0.41), leaf length (r_g = 0.72, r_p= 0.56), leaf width (r_g=0.54, r_p= 0.37), stamen stalk length (r_g = 0.59, r_p= 0.35), fruit length (r_g = 0.49, r_p= 0.39), single berry weight (r_g = 0.67, r_p = 0.32), and seed length (r_g = 0.47, r_p= 0.55). Therefore, those traits that are significantly and positively associated with yield could be used for indirect selection in the yield improvement program. Contrariwise, bean yield ton ha^{-1} showed a negative but statistically non-significant association with empty fruit rate (r_g = -0.07, r_p= 0.03) (Table 3.7). According to this result, empty fruit rate traits require careful selection because improving them may result in a decrease in yield. In line with the present study, many researchers also reported a significant and positive correlation between several vigour parameters (stem diameter, plan height, number of primary-branches, length of the longest primary branch, leaf width, and leaf length) among themselves and with yield per plant (Yigzaw, 2005; Kitila *et al*., 2011; Malau & Pandiangan, 2018; Yirga *et al*., 2021; Ndikumana, 2022).

Table 3. 7 Genotypic and phenotypic correlation coefficients among 25 agro-morphological characters of Arabica coffee

	YI	HPFP	SG	PH	INLMS	CD	NPB	NSB	LLP	NNLP	INLLP	LL	LW	LPL	ISL	SSL	FL	FW	FT	SBW	EFR	FFC	SL	SW	ST
YI	1	0.21^{ns}	0.54*	0.32*	0.41*	0.48*	0.33^{ns}	0.45*	0.59**	0.30^{ns}	0.41*	0.56**	0.37*	0.34*	0.12^{ns}	0.35*	0.39*	0.27^{ns}	0.2^{ns}	0.32*	-0.03^{ns}	0.11^{ns}	0.55*	0.22^{ns}	0.24^{ns}
HPFP	0.25^{ns}	1	0.25^{ns}	0.47*	0.62**	0.6**	-0.13^{ns}	0.42*	0.5*	0.16^{ns}	0.44*	0.24^{ns}	0.48*	0.45*	0.05^{ns}	0.37*	0.0^{ns}	-0.01^{ns}	0.01^{ns}	0.03^{ns}	0.16^{ns}	-0.34*	0.31*	0.12^{ns}	0.3*
SG	0.59**	0.2^{2ns}	1	0.25	0.31*	0.35*	0.06ns	0.39*	0.51*	0.08ns	0.35*	0.58**	0.5*	0.28*	0.09ns	0.19ns	0.34*	0.37*	0.34*	0.22ns	-0.22^{ns}	0.02^{ns}	0.37*	0.38*	0.05^{ns}
PH	0.31^{ns}	0.51*	0.2	1	0.84	0.9**	0.24^{ns}	0.15^{ns}	0.37*	0.13^{ns}	0.74**	0.22^{ns}	0.08^{ns}	0.19^{ns}	0.01^{ns}	0.11^{ns}	-0.02^{ns}	0.06^{ns}	0.11^{ns}	0.06^{ns}	-0.05^{ns}	-0.38*	0.14^{ns}	-0.02^{ns}	0.31*
INLMS	0.52*	0.61*	0.41*	0.82**	1	0.9	0.01^{ns}	0.11^{ns}	0.52*	0.16^{ns}	0.81**	0.41*	0.35*	0.41*	0.01^{ns}	0.29*	0.14^{ns}	0.15^{ns}	0.08^{ns}	0.12^{ns}	-0.05^{ns}	-0.27*	0.38*	0.17^{ns}	0.31*
CD	0.53*	0.66**	0.37*	0.86**	0.92**	1	0.1^{ns}	0.28*	0.49*	0.27*	0.79**	0.36*	0.27*	0.48*	-0.03^{ns}	0.24^{ns}	0.06^{ns}	0.06^{ns}	0.06^{ns}	0.03^{ns}	0.09^{ns}	-0.4*	0.34*	0.15^{ns}	0.37*
NPB	0.35*	-0.12ns	0.04^{ns}	0.32*	0.03*	0.12*	1	0.42*	0.01^{ns}	0.08^{ns}	-0.07^{ns}	0.01^{ns}	-0.13^{ns}	-0.04^{ns}	0.01^{ns}	-0.2^{ns}	-0.14^{ns}	-0.05^{ns}	0.01^{ns}	-0.2^{ns}	0.06^{ns}	0.01^{ns}	-0.17^{ns}	-0.13^{ns}	-0.26^{ns}
NSB	0.49*	0.48*	0.41*	0.16*	0.12^{ns}	0.34*	0.45*	1	0.37*	0.32*	0.13^{ns}	0.23^{ns}	0.31^{ns}	0.26^{ns}	-0.03^{ns}	-0.04^{ns}	-0.04^{ns}	0.01^{ns}	0.09^{ns}	-0.12^{ns}	0.19^{ns}	-0.15^{ns}	0.04*	0.11^{ns}	-0.06^{ns}
LLP	0.54*	0.7**	0.31*	0.84**	0.95**	0.96**	0.17^{ns}	0.30*	1	0.5*	0.43*	0.6**	0.68**	0.4*	0.01^{ns}	0.37*	0.24^{ns}	0.28^{ns}	0.25^{ns}	0.25^{ns}	-0.13^{ns}	-0.22^{ns}	0.38*	0.19^{ns}	-0.03^{ns}
NNLP	0.34^{ns}	0.46^{ns}	0.05^{ns}	0.65**	0.67**	0.79**	-0.18^{ns}	0.15^{ns}	0.81**	1	0.16^{ns}	0.04^{ns}	0.11^{ns}	0.18^{ns}	0.20^{ns}	0.05^{ns}	0.09^{ns}	0.09^{ns}	0.06^{ns}	0.25^{ns}	-0.06^{ns}	-0.03^{ns}	0.17^{ns}	0.08^{ns}	0.07^{ns}
INLLP	0.55*	0.4^{ns}	0.46*	0.67**	0.81**	0.77**	0.02^{ns}	0.12^{ns}	0.77**	0.01 ^{ns}	1	0.33*	0.25^{ns}	0.42*	-0.15^{ns}	0.17^{ns}	0.17^{ns}	0.2^{ns}	0.18^{ns}	0.16^{ns}	-0.2^{ns}	-0.18^{ns}	0.32*	0.21^{ns}	0.31*
LL	0.72**	0.42^{ns}	0.64**	0.37 ^{ns}	0.65*	0.53*	-0.06^{ns}	0.26*	0.55*	0.56*	0.52*	1	0.66**	0.49*	0.2^{ns}	0.39*	0.54*	0.43*	0.16^{ns}	0.23^{ns}	0.06^{ns}	0.01^{ns}	0.61**	0.44*	0.11^{ns}
LW	0.54*	0.64*	0.66**	0.29 ^{ns}	0.6*	0.45^{ns}	-0.11^{ns}	0.37*	0.53*	0.72**	0.45^{ns}	0.7**	1	0.42*	-0.11^{ns}	0.36*	0.29^{ns}	0.27^{ns}	0.23^{ns}	0.17^{ns}	-0.08^{ns}	0.02^{ns}	0.43*	0.43*	0.09^{ns}
LPL	0.14ns	0.53*	0.32*	0.26^{ns}	0.58*	0.62*	-0.02^{ns}	0.26*	0.61*	0.1 ^{ns}	0.51*	0.53*	0.41^{ns}	1	-0.13^{ns}	0.25^{ns}	0.11^{ns}	-0.03^{ns}	-0.15^{ns}	-0.03^{ns}	-0.01^{ns}	-0.28^{ns}	0.59**	0.3*	-0.09^{ns}
ISL	0.21^{ns}	0.24^{ns}	0.38*	0.41 ^{ns}	0.59*	0.48*	0.04^{ns}	0.15^{ns}	0.48*	0.37 ^{ns}	0.4 ^{ns}	0.73**	0.35 ^{ns}	0.37 ^{ns}	1	0.44*	0.14^{ns}	0.23^{ns}	0.27^{ns}	0.19^{ns}	0.37*	0.09^{ns}	-0.1^{ns}	-0.11^{ns}	-0.05^{ns}
SSL	0.59*	0.29^{ns}	0.39*	0.27 ^{ns}	0.59*	0.43 ^{ns}	-0.11^{ns}	0.07^{ns}	0.47 ^{ns}	0.34 ^{ns}	0.44 ^{ns}	0.67**	0.46 ^{ns}	0.36 ^{ns}	0.82**	1	0.37*	0.3^{ns}	0.17^{ns}	0.12^{ns}	0.04^{ns}	-0.02^{ns}	0.33*	0.17^{ns}	0.19^{ns}
FL	0.49*	0.07^{ns}	0.34*	0.10^{ns}	0.32^{ns}	0.18^{ns}	-0.13^{ns}	-0.07^{ns}	0.21^{ns}	0.74**	0.26^{ns}	0.76**	0.37^{ns}	0.2^{ns}	0.56*	0.53*	1	0.86**	0.47*	0.37*	-0.05^{ns}	0.27^{ns}	0.51*	0.47*	0.16^{ns}
FW	0.22^{ns}	-0.17^{ns}	0.39*	0.01^{ns}	0.03^{ns}	-0.01^{ns}	0.02^{ns}	-0.05*	-0.06^{ns}	0.82**	0.29 ^{ns}	0.34^{ns}	-0.02^{ns}	0.04^{ns}	0.46^{ns}	0.38^{ns}	0.59*	1	0.81**	0.42*	-0.04^{ns}	0.22^{ns}	0.32*	0.5*	0.22^{ns}
FT	0.25^{ns}	0.18^{ns}	0.38*	0.13^{ns}	0.28^{ns}	0.09^{ns}	-0.04^{ns}	-0.09^{ns}	0.15 ^{ns}	0.87**	0.06 ^{ns}	0.46 ^{ns}	0.47^{ns}	0.06^{ns}	0.54*	0.47 ^{ns}	0.55*	0.45 ^{ns}	1	0.42*	-0.07^{ns}	0.1^{ns}	-0.03^{ns}	0.38*	0.18^{ns}
SBW	0.67**	0.01^{ns}	0.35*	0.17 ^{ns}	0.41 ^{ns}	0.24 ^{ns}	-0.02^{ns}	-0.03^{ns}	0.32 ^{ns}	0.35 ^{ns}	0.42 ^{ns}	0.63*	0.39 ^{ns}	0.1 ^{ns}	0.61*	0.62*	0.73**	0.4 ^{ns}	0.56*	1	-0.12^{ns}	0.29^{ns}	0.25^{ns}	0.27^{ns}	0.18^{ns}
EFR	-0.07^{ns}	0.11^{ns}	-0.31*	-0.16 ^{ns}	-0.07 ^{ns}	-0.01^{ns}	-0.13^{ns}	0.25*	-0.03 ^{ns}	0.75**	-0.35 ^{ns}	-0.02 ^{ns}	-0.16 ^{ns}	0.05 ^{ns}	0.36 ^{ns}	0.23 ^{ns}	-0.01 ^{ns}	-0.17 ^{ns}	-0.08 ^{ns}	-0.04 ^{ns}	1	-0.17^{ns}	0.06^{ns}	0.01^{ns}	0.09^{ns}
FFC	0.1^{ns}	-0.5*	0.06^{ns}	-0.52*	-0.45^{ns}	-0.53*	0.09**	-0.24*	-0.47 ^{ns}	0.28 ^{ns}	-0.26 ^{ns}	-0.18 ^{ns}	-0.15 ^{ns}	-0.32 ^{ns}	-0.3 ^{ns}	-0.1^{ns}	0.22^{ns}	0.2 ^{ns}	0.25 ^{ns}	0.4 ^{ns}	-0.33 ^{ns}	1	0.09^{ns}	0.26^{ns}	0.03^{ns}
SL	0.47*	0.33^{ns}	0.186	0.05^{ns}	0.19^{ns}	0.19^{ns}	-0.25^{ns}	0.11^{ns}	0.14 ^{ns}	0.96**	0.14 ^{ns}	0.48*	0.37 ^{ns}	0.18 ^{ns}	0.09 ^{ns}	0.37 ^{ns}	0.55*	0.11 ^{ns}	0.12 ^{ns}	0.27 ^{ns}	-0.01 ^{ns}	0.15 ^{ns}	1	0.56**	0.39*
SW	0.23^{ns}	0.22^{ns}	0.11^{ns}	0.01^{ns}	0.05 ^{ns}	0.06 ^{ns}	-0.24*	0.06^{ns}	0.01 ^{ns}	0.71**	0.03 ^{ns}	0.3 ^{ns}	0.31 ^{ns}	-0.1 ^{ns}	0.01 ^{ns}	0.24 ^{ns}	0.45 ^{ns}	0.15 ^{ns}	0.21 ^{ns}	0.21 ^{ns}	-0.02 ^{ns}	0.2 ^{ns}	0.9**	1	0.51
ST	0.22^{ns}	0.22^{ns}	0.03^{ns}	0.03 ^{ns}	0.01 ^{ns}	0.06 ^{ns}	-0.26*	0.05^{ns}	-0.01 ^{ns}	0.09^{ns}	-0.01 ^{ns}	0.17 ^{ns}	0.16 ^{ns}	-0.18 ^{ns}	-0.1 ^{ns}	0.19 ^{ns}	0.31 ^{ns}	0.03 ^{ns}	0.03 ^{ns}	0.11 ^{ns}	0.03 ^{ns}	0.16 ^{ns}	0.89**	0.96*	1

Where; YI=Yield (ton ha-1); HPFP=Height up-to first primary (cm); SG=stem-girth (cm); PH=Plant Height (cm); INLMS= Inter Node length on Main-stem (cm); CD=Canopy diameter (cm); NPB=Number of Primary Branch (No.); NSB=Number of Secondary Branch (No.); LLP=length of longest-primary (cm); NNLP=Number of nodes on longest primary (No.); INLLP=Inter-node length on longest primary (cm); LL=Leaf length (mm); LW=Leaf width (mm); LPL=Leaf petiole length (mm); ISL=Inflorescence Stalk Length (mm); SSL=Stamen stalk Length (mm); FL=Fruit length (cm); FW=Fruit width (mm); FT=Fruit thickness (mm); SBW=Single berry weight (gm); EFR=Empty fruit rate (%); FFC=Fruit filling coefficient; SL=Seed length (mm); SW=Seed width (mm); ST=Seed thickness (mm);

3.3.6 Path analysis

To assess the magnitude of direct and indirect contributions of various characters to Arabica coffee yield ton ha^{-1}, path coefficient analysis was performed using 13 significantly associated traits with yield in ton ha^{-1}. Among 13 significantly correlated characters with yield, eleven characters had a positive direct influence, whereas the remaining two characters had a negative direct effect on yield ton ha^{-1} at genotypic level (Table 3.8). The number of primary-branches (0.451) exerted the highest positive direct effect on yield ton ha^{-1} (Table 3.8). The indirect effect of the number of primary-branches on yield showed a positive indirect effect through all traits except internode length, main-stem leaf width, and single berry weight. Therefore, the number of primary-branches is found to be an important component, and direct selection for this trait may be rewarding for the improvement of Arabica coffee yield.

Number of secondary-branches (0.433), leaf length (0.42), length of longest-primary branch (0.413), leaf width (0.383), inter-node length on longest primary branch (0.378), canopy diameter (0.270), seed length (0.21), single berry weight (0.142), stem-girth (0.101), and stamen stalk length (0.03) had a positive direct effect on yield ton ha^{-1} (Table, 3.8). Thus, considering genotypes with these traits would be effective in coffee yield improvement program.

On the contrary, internode length on the main-stem (-0.197) and fruit length (-0.282) had a negative direct effect on yield ton ha-1 (Table 3.8). The direct effect of fruit length on yield ton ha-1 was negative, and its indirect effect revealed a positive trend through stem girt, internode length on the longest primary branch, internode length on the main-stem, and leaf width.

In-line with this result, Yirga *et al*. (2021), stated positive direct effects of inter-node length of primary-branches, bean thickness, and average inter-node-length of the main-stem, number of primary-branches, stem diameter, and bean weight on yield per tree. Similarly, Getachew *et al.* (2013), also reported a positive direct effect of average inter-node-length of primary-branches, number of primary-branches, number of nodes on primary branches, fruit length and thickness, and stem diameter on yield per plant. In-contrast to this study, Yirga *et al.* (2021) reported negative direct effects of bean length, number of secondary-branches, average length of primary-branches, canopy diameter, and a positive direct effect of inter-node length of the main-stem on yield per tree.

Table 3. 8 Genotypic path coefficient analysis (bold and diagonal value indicating direct effect; above and below the diagonal value indicating indirect effect) of 13 agro-morphological traits on bean yield ton ha-1

	SG	INLMS	CD	NPB	NSB	LLP	INLLP	LL	LW	SSL	FL	SBW	SL	r_g
SG	**0.101**	-0.084	0.104	0.018	0.177	0.083	0.174	0.27	0.53	0.011	0.051	0.01	0.04	0.59*
INLMS	-0.07	**-0.197**	0.257	-0.002	-0.022	0.318	0.306	0.07	-0.058	0.008	0.028	0.007	0.04	0.52*
CD	-0.027	-0.15	**0.270**	0.02	0.095	0.382	0.291	0.03	-0.014	0.005	-0.015	0.004	0.03	0.53*
NPB	0.034	0.003	0.021	**0.451**	0.301	0.134	-0.29	0.04	-0.088	0.006	0.000	-0.006	0.02	0.35*
NSB	0.352	-0.084	0.095	0.329	**0.433**	0.096	0.045	0.11	0.297	0.005	-0.034	0.003	0.04	0.49*
LLP	-0.033	-0.152	0.182	0.036	0.145	**0.413**	0.238	0.06	-0.034	0.008	0.004	0.012	0.04	0.54*
INLLP	-0.046	-0.155	0.215	0.028	0.052	0.342	**0.378**	0.22	-0.128	-0.198	0.112	0.104	0.36	0.55*
LL	-0.063	-0.064	0.148	0.021	0.113	0.118	0.197	**0.420**	-0.19	-0.198	0.114	0.222	0.61	0.72**
LW	0.566	-0.052	0.129	-0.05	0.164	0.063	0.17	0.28	**0.383**	0.005	0.047	0.01	0.05	0.54*
SSL	-0.059	-0.05	0.117	0.023	0.113	0.113	0.197	0.22	-0.04	**0.03**	0.008	0.014	0.05	0.59*
FL	0.030	0.019	0.053	0.002	0.068	-0.006	0.197	0.22	0.037	-0.001	**-0.282**	0.035	0.05	0.49*
SBW	-0.011	-0.01	0.020	-0.005	-0.065	0.035	0.087	0.21	-0.016	0.003	-0.07	**0.142**	0.04	0.67**
SL	-0.032	-0.039	0.129	0.008	0.03	0.08	0.155	0.29	-0.053	0.008	-0.07	0.028	**0.21**	0.47*

Where: SG=stem-girth (cm), INLMS=Inter Node length on Main-stem (cm), CD=Canopy diameter (cm), NPB= Number of primary branch, NSB=Number of Secondary Branch (No.), LLP=length of longest-primary (cm), INLLP=Inter node length on longest primary (cm), LL=Leaf length (mm), LW=Leaf width (mm), LPL=Leaf petiole length (mm), SSL=Stamen stalk Length (mm), FL=Fruit length (cm), SBW=Single berry weight (gm), SL=Seed length (mm), SW=Seed width (mm), and r_g=genotypic correlation coefficient value with yield.

3.3.7 Principal component analysis

The principal component analysis (PCA) of seventeen Arabica coffee genotypes based on a combined mean value of 25 quantitative traits generated the twenty-five eigenvalues and eigenvectors. However, according to Kaiser (1960), as cited by Amare *et al*. (2018), factors to be retained should have more than 1 eigenvalue, at least 5% of the variance explained for each component, and/or more than 70% of the cumulative proportion of variance explained. Accordingly, the first five components that explained 74.05 percent of total variation were used for displaying characters (Table 3.9).

The first PCA explained 36.53% of the total variations (Table 3.9). According to Jeffers (1967), the relative weight given to the variables in each component is determined by the importance of the variables, which possess a high positive and negative weight. Based on this suggestion, the most important characters contributing more to the variation were length of the longest primary (0.28), inter node length on the main-stem (0.28), leaf length (0.28), yield (0.26), canopy diameter (0.26), inter-node length on the longest-primary (0.25), leaf width (0.25), seed width (0.24), height up to the first primary (0.21), stem-girth (0.21), leaf petiole length (0.2), stamen stalk length (0.21), fruit length (0.20), fruit width (0.20), and seed thickness (0.20). The sign indicates the direction of the relationship between the components and the characters (Tsehaye & Kebebew, 2002). Accordingly, in the first principal component, most traits were associated positively, whereas the fruit-filling coefficient was negatively associated with low values.

The second PCA explained 14.82% of the variation, and fruit length (0.32), fruit width (0.32), single berry weight (0.35), and fruit filling coefficient (0.38) mainly accredited this variation. The third PCA explained 8.33% of the total variation, and this variation is mainly contributed by seed length (-0.52), number of primary-branches (0.35), inflorescence stalk length (0.34), fruit thickness (0.33), and seed width (-0.27) in decreasing order.

The fourth and fifth principal components explained 7.69% and 6.68%, respectively. High variation in the fourth PC is attributed to stem-girth (-0.27), plant height (0.33), number of secondary-branches (-0.27), number of nodes on the longest-primary (-0.21), leaf width (-0.24), inflorescence stalk length (0.3), empty fruit rate (0.33), and fruit filling coefficient (-0.28), while high variation in the fifth PC is attributed to empty fruit rate (0.48), and inflorescence stalk length (0.42) (Table 3.9).

From this result, it may be concluded that important variables in Arabica coffee genotype with respect to agronomic traits were yield, number of primary-branches, internode length on the main-stem, number of secondary-branches, length of the longest primary, internode length on the longest primary, leaf length, leaf width, fruit length, fruit width, single berry weight, and seed thickness. The above-mentioned variables might be taken into consideration for effective parent selection.

Table 3. 9 Principal component analysis of 25 quantitative traits

Characters	Eigenvectors					
	PC1	PC2	PC3	PC4	PC5	PC6
YI	0.26	0.06	0.02	-0.11	0.24	0.11
HPFP	0.21	-0.24	-0.09	0.02	0.15	0.04
SG	0.21	0.04	0.17	-0.27	-0.1	-0.1
PH	0.19	-0.24	0.09	0.33	-0.26	0.03
INLMS	0.28	-0.17	-0.02	0.19	-0.1	-0.1
CD	0.26	-0.25	-0.01	0.17	-0.1	0.12
NPB	0.02	-0.19	0.35	-0.13	-0.12	0.26
NSB	0.11	-0.21	0.3	-0.27	0.23	0.3
LLP	0.28	-0.11	0.07	-0.13	0.06	-0.3
NNLP	0.15	-0.07	-0.03	-0.21	0.16	0.23
INLLP	0.25	-0.1	-0.01	0.09	-0.26	-0.1
LL	0.28	0.08	0.07	-0.07	0.1	-0.1
LW	0.25	-0.01	0.06	-0.24	-0.02	-0.2
LPL	0.2	-0.21	-0.16	-0.2	0.1	0.02
ISL	0.02	0.13	0.34	0.3	0.42	-0.2
SSL	0.21	0.03	-0.17	0.11	0.29	-0.3
FL	0.2	0.32	-0.03	0.01	0.01	0.09
FW	0.2	0.32	0.15	0.12	-0.12	0.1
FT	0.16	0.23	0.33	0.16	-0.25	0.04
SBW	0.15	0.35	0.08	0.12	0.12	-0.2
EFR	-0.01	-0.1	0.15	0.33	0.48	0.33
FFC	-0.05	0.38	0.03	-0.28	0.06	0.11
SL	0.04	0.1	-0.52	0.07	0.11	0.04
SW	0.24	0.08	-0.27	-0.15	0.05	0.15
ST	0.2	0.22	-0.03	-0.07	-0.18	0.36
EV	9.13	3.71	2.08	1.92	1.67	1.54
PVE	36.53	14.82	8.33	7.69	6.68	6.15
CPVE	36.53	51.35	59.68	67.37	74.05	80.2

Where; EV = Eigenvalue, PVE = Present variation explained. and CPVE = Cumulative present variation explained

3.3.8 Cluster analysis

To investigate the genetic relationship of 17 genotypes, cluster analysis was conducted and a dendrogram was generated using 25 agro-morphological traits data (Figure 3.1). Genotypes were grouped into two main clusters; the first main-cluster incorporated seven Arabica coffee genotypes, viz., 4112, AW9628, AW1777, AW1995, AW1962, AW7494, and AW9644, and bifurcated into two different sub-clusters. The first sub-cluster of the first main-cluster consisted of one genotype (4112), which is a standard check variety and characterized by low bean yield thon ha-1, height up to the first primary branch, stem-girth, plant height, internode length on the main-stem, canopy diameter, number of secondary-branches, length of the longest primary, number of nodes on the longest primary, leaf width, fruit length, fruit width, and seed length, whereas empty fruit rate and fruit filling coefficient were high.

The second sub-cluster of the first main-cluster consisted of six genotypes, which were further grouped into two sub-sub-sub-clusters. The first sub-sub-cluster of the second sub-cluster consisted of two genotypes, AW1777 and AW1995, that are characterized by average for bean yield ton ha-1, internode length on main-stem, internode length on longest primary, and fruit filling coefficient; high in seed length; and low in number of nodes on longest primary. The second sub-sub-cluster of the second sub-cluster consisted of three genotypes, namely, AW1962, AW7494, and AW9644, which are characterized by average height up to the first primary branch, stem-girth, plant height, length of the longest primary branch, empty fruit rate, and fruit filling coefficient; and low in fruit length, fruit width, fruit thickness, and seed thickness.

The second main-cluster contained ten genotypes (AW3106, AW105, AW9622, AW4994, AW9623, AW5994, AW9641, Feyate, AW1706, and Angafa) and bifurcated into two different sub-clusters. The first sub-cluster of the second main-cluster consists of one genotype, viz., AW3106, which is characterized by good yield, high canopy diameter, internode length on longest primary, inflorescence stalk length, low number of primary-branches, number of nodes on longest primary, fruit length, fruit width, fruit thickness, single berry weight, and empty fruit rate. The second sub-cluster of the second main-cluster consists of nine genotypes and is split into two different sub-sub-clusters. The first sub-sub-cluster of the second sub-cluster consisted of four genotypes, namely, AW105, AW4994, AW9622, and AW9623, whereas the second sub-sub-cluster of the second sub-cluster consisted of five genotypes, viz., AW5994, Feyate, AW9641, AW1706, and Angafa. Generally, the second sub-cluster of the second main-cluster was characterized by high yield (ton ha-1), stem-girth, plant height, inter-node length on the main-stem, canopy diameter, number of primary-branches, length of the longest primary, number of nodes on the longest primary, inter node length on the longest primary, number of secondary-branches, leaf width, leaf length, leaf petiole length, inflorescence stalk length, stamen stalk length, fruit length, fruit width, fruit thickness, single berry weight, empty fruit rate, fruit filling coefficient, seed width, and seed length (Figure 3.1). Genetic variation among coffee genotypes might contribute to the occurrence of different clusters.

In line with this study, clustering of Arabica coffee genotypes in diverse groups based on morphological traits has been reported by different researchers at different times (Yigzow, 2005; Kebede & Bellachew, 2008; Abdi, 2009; Olika *et al.* 2011; Atinafu *et al.*, 2017; Tounekti *et al.*, 2017; Ndikumana, 2022).

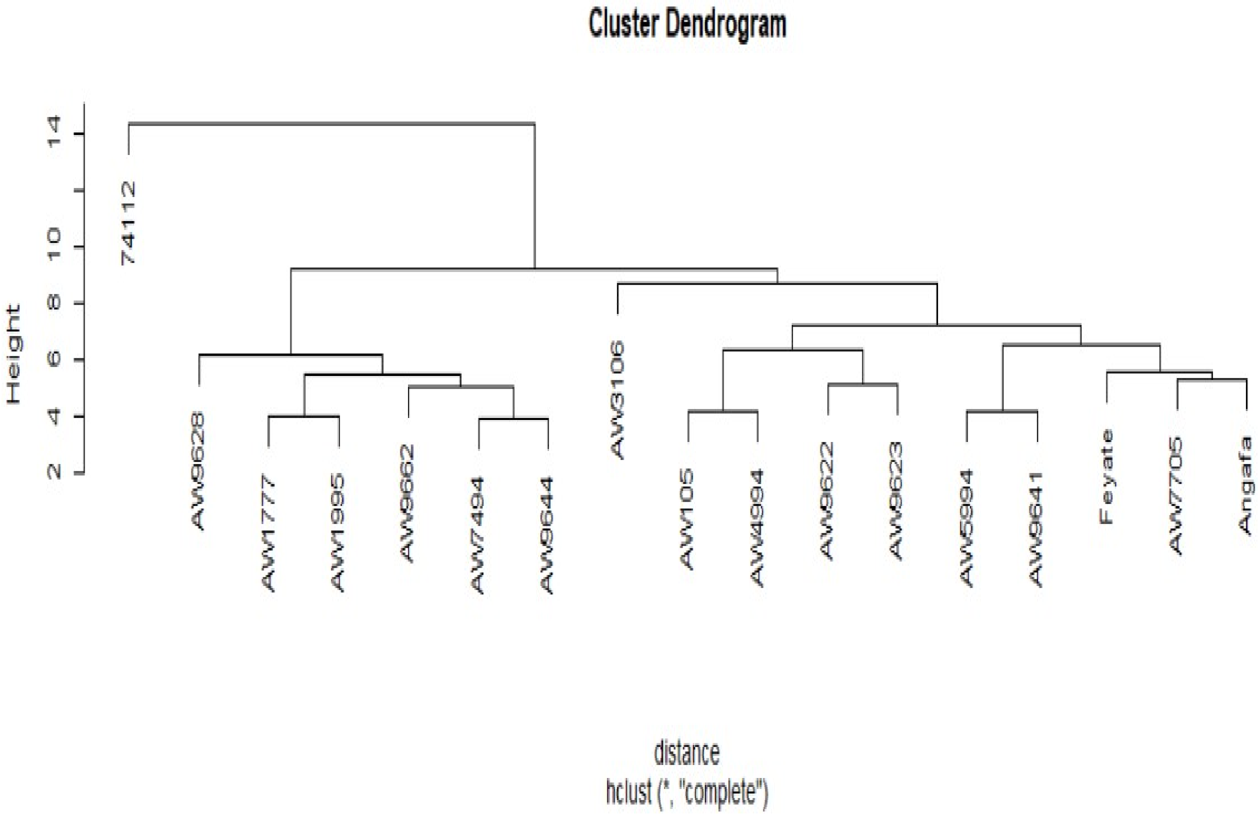

Figure 3. 1 Cluster denderogram describing variation among 17 genotypes grown at 3 location using 25 agromorphological trait data

3.4 Conclusion

The result of the present study confirmed the presence of diversity among south Ethiopian Arabica coffee genotypes for all studied agro-morphological characters except the number of flowers per axil. Mean performance analysis confirmed the presence of superior selection over the released check varieties bean yield. The presence of variability among the genotypes reveals that there is a good chance of improving south Ethiopian coffee through selection and hybridization.

The combined analysis of variance showed significant variation among genotypes and locations for most of the studied traits and GxE interaction for bean yield, fruit length, number of primary-branches, fruit thickness, fruit width, empty fruit rate, fruit filling coefficient, seed width, seed length, and seed thickness. The presence of significant GxE interaction indicated the inconsistency in performance of the genotypes for those traits across environments.

The estimation of genotypic, phenotypic, and environmental coefficients of variance showed a wider range of variation for the studied traits. The phenotypic variance was higher than the genotypic variance for the characters studied, suggesting a substantial influence of the environment besides the genetic variation on the expression of these traits. High-to-moderate GCV and PCV values were observed for the traits of fruit filling coefficient, height up to the first primary, bean yield, plant height, canopy diameter, number of secondary-branches, length of the longest primary, and leaf petiole length, and these traits can provide a high chance for effective selection.

Moderately high to very high heritability, along with moderate to high expected genetic advance as a percent of the mean, was observed for bean yield, length of longest primary branch, stem girth, number of secondary branches, canopy diameter, leaf length, number of primary branches, leaf petiole length, leaf width, single berry weight, height up to the first primary, inflorescence stalk length, stamen stalk length, and seed width. Therefore, selection in the next generation based on these characters would be effective.

The correlation coefficient analysis revealed positive associations for most of the studied agro-morphological characters among themselves and with yield in ton ha^{-1} showing the possibility of indirect selection in the yield improvement program. Genotypic path coefficient analysis showed a positive direct effect of 11 characters (number of secondary branches, number of primary branches, leaf length, length of longest primary branch, leaf width, inter node length on longest primary branch, canopy diameter, seed length, single berry weight, stem girth, and stamen stalk length) on yield, indicating the significance of these traits for direct selection to increase coffee yield ton ha^{-1}. The number of primary branches exerted the highest positive direct effect on bean yield and a positive indirect effect on all significantly associated traits except internode length, main-stem leaf width, and single berry weight. Therefore, direct selection using the number of primary branches would be advisable for the improvement of Arabica coffee yield.

Cluster analysis grouped genotypes into two main clusters and four sub-clusters. From the principal component analysis result, the most important traits contributing more to the variation were yield, number of primary branches, inter-node length on the main stem, number of secondary branches, length of the longest primary, inter-node length on the longest primary, leaf width, leaf length, fruit length, fruit width, single berry weight, and seed thickness. As a result, the aforementioned traits must be considered for effective parent selection in the Arabica coffee genotype.

Generally, the result confirmed the existence of sufficient diversity among quantitative agro-morphological traits in south Ethiopian Arabica coffee genotypes, which could make it possible to increase the yield potential of coffee genotypes under south Ethiopian specialty growing conditions. This study result revealed the presence of elite genotypes over the released standard check varieties, which could suggest the possibility of a direct selection and/or hybridization program. According to the four-year harvesting season mean yield, AW5994 and AW3106 at Awada; AW7705 and AW9641 at Shebedino; and AW105 and AW7705 at Wonago are the highest yielding promising genotypes over the standard check varieties. Therefore, these top-yielding genotypes could be used in breeding programs for yield improvement in their respective study areas. The highest overall three location and four harvesting season average bean yield in tons per hectare was recorded for genotypes AW7705 and AW105. Therefore, this genotype was found to be a promising candidate that could be included in the national testing program with a view to releasing a new variety for Arabica coffee growing areas in South Ethiopia and other similar agro-ecologies elsewhere. Hence, genotype, environment, and GEI have a significant effect on yield performance; identification of stable and high-yielding genotypes by conducting the experiment over locations and seasons is recommended. Investigation of different management options after the high-yielding season is also advised to minimize high-seasonal yield oscillation.

3.5 Reference

Abdulfeta, T. K. (2018). Characterization and Yield Performance Evaluation of Coffee (Coffea arabica L.) Germplasm Accessions from Tepi, Southwestern Ethiopia MSc. Thesis summated to Hawassa University College of Agriculture. 71p.

Abdi Adem (2009). Agro-morphological characterization of coffee (Coffea arabica L.) landrace collected from Mesela, West Harerge, Ethiopia. M.Sc. Thesis Submitted to Graduate Studies of Hawassa University, Hawassa, Ethiopia. 88 pp.

Abdulfeta T K (2018) Characterization and Yield Performance Evaluation of Coffee (Coffea arabica L.) Germplasm Accessions from Tepi, Southwestern Ethiopia MSc. Thesis summated to Hawassa University College of Agriculture. 71p.

Adem, A., Mohammed, H., & Ayana, A. (2020). Phenotypic Diversity in Arabica Coffee Genotypes from Eastern Ethiopia. 5(4), 42–47. https://doi.org/10.11648/j.ijee.20200504.11

Ahsan, M. Z., Majidano, M. S., Bhutto, H., Soomro, A. W., Panhwar, F. H., Channa, A. R., & Sial, K. B. (2015). Genetic Variability, Coefficient of Variance, Heritability and Genetic Advance of Some Gossypium hirsutum L. Accessions. Journal of Agricultural Science, 7(2). https://doi.org/10.5539/jas.v7n2p147

Akpertey, A., Anim-kwapong, E., & Ofori, A, (2019). Assessment of Genetic Diversity in Robusta Coffee Using Morphological Characters Assessment of Genetic Diversity in Robusta Coffee Using Morphological Characters. International Journal of Fruit Science, 19(3), 276–299. https://doi.org/10.1080/15538362.2018.1502723

Allard, R.W. 1960. Principles of Plant Breeding. John Wiley and Sons. Inc. New York, USA.

Antonio, C., Felipe, L., Juliana, C., Elias, B. &Gladyston, R (2010). Prediction of genetic gains from selection in arabica coffee progenies. Crop Breeding and Applied Biotechnology 11: 106-113.

Argaw, T., & Taye, G. (2018). Genotype by Environment Interaction Analysis of Arabica Coffee Bean Yield. 6(October), 509–519. https://doi.org/10.14662/ARJASR2018.090

Asefa, G., Mohammed, W., & Abebe, T. (2016). Genetic Variability Studies in Potato (Solanum Tuberosum L.) Genotypes in Bale Highlands , South Eastern Ethiopia. Journal of Biology, Agriculture and Healthcare, 6(3), 117–119.

Atinafu, G., Mohammed, H., & Kufa, T. (2017). Genetic Variability of Sidama Coffee (Coffea Arabica L .) Landrace for Agro-morphological Traits at Awada , Southern Ethiopia. 5(July), 263–275. https://doi.org/10.14662/ARJASR2017.025

Bakala, N., Bersisa, H., Tilehun, A., & Misgana, Z. (2022). Assessment on the Current Status of Coffee Arabica (Coffea arabica L) Landraces Diversity in Coffee Belt Areas of Western Oromia , Ethiopia. 10(1), 24–30. https://doi.org/10.11648/j.ijbmr.20221001.12

Bayetta, B. (2001) Arabica Coffee Breeding for Yield and Resistance to Coffee Berry Disease (Colletotichum kahawae sp.nor). Ph.D. Thesis, Imperial College of Wye, University of London.

Bayetta, B. (2001). Arabica coffee breeding for yield and resistance to coffee berry disease (Colletotrichum kahawae Sp.nov.). Unpublished PhD thesis University of London.

Begna T (2021) Role and economic importance of crop genetic diversity in food security. J Agric Sc Food Technol 7(1): 164-169. https://doi.org/10.17352/2455-815X.000104

Beksisa, L. (2021). GGE Biplot Analysis of Genotype x Environment Interaction and Bean Yield Stability of Arabica Coffee (Coffee arabica L .) Genotypes in Southwestern Ethiopia. 9(3), 110–115. https://doi.org/10.11648/j.ajbio.20210903.16

Beksisa, L., Alamerew, S., Ayano, A., & Daba, G. (2018). Genotype × environment interaction and yield stability of Arabica coffee (Coffea arabica L .) genotypes. 13(4), 210–219. https://doi.org/10.5897/AJAR2017.12788

Benti, T. (2017). Progress in Arabica Coffee Breeding in Ethiopia: Achievements, Challenges and Prospects. International Journal of Sciences: Basic and Applied Research, 33(2), 15–25. http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Bernardes, T., Moreira, M. A., Adami, M., Giarolla, A., & Rudorff, B. F. T. (2012). Monitoring biennial bearing effect on coffee yield using MODIS remote sensing imagery. Remote Sensing, 4(9), 2492–2509. https://doi.org/10.3390/rs4092492

Bhandari HR, Bhanu AN, Srivastava K, *et al* (2017). Assessment of genetic diversity in crop plants - an overview. Adv Plants Agric Res. 7(3):279-286. https://doi.org/10.15406/apar.2017.07.00255

Camargo, A.P.; Camargo, M.B.P. (2001). Definition and outline for the phenological phases of arabic coffee under brazilian tropical conditions. Bragantia 60(1):65-68

Carvalho, H. F. (2019). Genomic prediction in arabica coffee considering bienniality and threshold models. Thesis presented as requirement to obtain the title of Doctor in Tropical and Subtropical Agriculture, Area of Concentration in Genetics, Plant Breeding, and Biotechnology.

Cheserek, J. J., Ngugi, K., Muthomi, J. W., Omondi, C. O., & Ezekiel, K. N. (2021). Green bean biochemical attributes of Arabusta coffee hybrids from Kenya using HPLC and soxhlet extraction methods. Australian Journal of Crop Science, 15(2), 201–208. https://doi.org/10.21475/ajcs.21.15.02.p2581

Copper M.C. and G.W. Milligan, (1988). The effect of error on determining the number of clusters. Proceedings of the International Workshop on Data Analysis, Decision Support and Expert Knowledge Representation in Marketing and Related Areas of Research, June 21-23, 1987, University of Karlsruhe, West Germany, pp: 319-328

CSA (Central Statistical Agency) (2019). Agricultural sample survey: report on area and production of major crops of private peasant holdings for meher season of 2018/19. 58. Addis Ababa, Ethiopia: Central statistical Agency.

Dawit, 2018. Genetic Diversity Analysis among Promising Wollega Coffee (Coffea arabica l.) Selections in Western Ethiopia. MSc. Thesis of Hawassa University. Thesis Submitted to Graduate Studies of in partial fulfillment of Hawassa University, Hawassa Ethiopia. P38.

Desalegn, A. (2018). Genetic Variability and Character Association of Amaro Coffee (Coffea arabica l.) Accessions at Awada, Southern Ethiopia. Thesis Submitted to Graduate Studies of Jimma University, Jimma, Ethiopia.

Deshmukh S.N.N., M.S. Basu, P.S. Reddy (1986). Genetic variability, character association and path coefficients of quantitative traits in Virginia bunch varieties of groundnut. Indian J. Agric. Sci., 56 (1986), pp. 816-821

Dewey, D.R. and K.H. Lu. (1959). A correlation and path coefficient analysis of components of crested wheat grass seed production. Agronomy Journal. 51: 515-518

ECTA (Ethiopian Coffee Tea and Spice Authority), (2023). available at https://borkena.com/2022/09/15/ethiopia-coffee-export-earned-1-4-billion/

ENA (Ethiopian News Agency), (2023). Ethiopia Generates More Than Half Billion USD From Coffee Export in Four Months of Fiscal Yr. https://www.ena.et/web/amh/ Retrieved on January, 2023.

Endale, T., T. Kufa, A. Nestre, T. Shimber, A. Yilma and T. Ayano, 2008. Research on coffee field management. Proceedings of the workshop on four decades of coffee research and development in Ethiopia: A National workshop, Addis Ababa, Ethiopia. pp.187-195.

Ermias, H. (2005). Evaluation of Wellega Coffee Germplasm for Yield, Yield Component and Resistant to Coffee Berry Disease at Early Bearing Stage. Ph.D. Dissertation, School of Graduate Studies of Alemaya University.

Fekadu, T., Sentayehu, A. and Dagne, W. 2016. Assessment of the Growth and Yield Characters of Some Promising Arabica Coffee Hybrids under Highland Environments in Southwestern Ethiopia. American-Eurasian J. Agric. & Environ. Sci., 16: 917-923. https://doi.org/10.5829/idosi.aejaes.2016.16.5.12889

Galbraith S., Daniel JA., Vissel B (2010). A study of clustered data and approaches to its analysis. J Neurosci. 30(32):10601-8. PMID: 20702692; PMCID: PMC6634702. https://doi.org/10.1523/JNEUROSCI.0362-10.2010.

Gebreselassie Habtamu. (2023). Estimation of Variability , Heritability , and Genetic Advance Among Released Varieties and Local Cultivar of Potato (Solanum tuberosum L .) in Eastern Ethiopia. 1(3), 38–44. https://doi.org/10.11648/j.wjast.20230103.11

Gebreselassie, H., Atinafu, G., Degefa, M., & Ayano, A. (2017). Advances in Crop Science and Technology Growth Characteristics and Yield Evaluation of Arabica Coffee (Coffea arabica L .) Promising Selections Under Sidama and Gedeo Growing. 5(6). https://doi.org/10.4172/2329-8863.1000317

Gessese, M. K., Bellachew, B., & Jarso, M. (2015). Advances in Crop Science and Technology Multivariate Analysis of Phenotypic Diversity in the South Ethiopian Coffee (Coffea arabica L .) for Quantitative Traits. https://doi.org/10.4172/2329-8863.S1-00

Getachew WeldeMichael (2019). Character Association and Path Coefficient Analysis for Yield and Its Related Traits in Ethiopian Coffee (Coffea Arabica L.) Accessions" International Journal of Research Studies in Agricultural Sciences (IJRSAS), 5(3), pp. 19-29, http://dx.doi.org/10.204.

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

IPGRI (International Plant Genetic Resources Board descriptor for coffee) (1996). Descriptions of coffea spp. and Psilanthus spp. International Plant Genetics Resource Institute, Rome. Johnson, H.W., Robinson, H.F. and Comstooc, R.E (1955). Estimates of genetic and environmental variability in soybeans.Agronomy Journal 47:314-318.

Islam, M., Raffi, S., Hossain, M., & Hasan, A. (2015). Analysis of genetic variability, heritability and genetic advance for yield and yield associated traits in some promising advanced lines of rice. Progressive Agriculture, 26(1), 26–31. https://doi.org/10.3329/pa.v26i1.24511

Jaramillo-Botero, C., Santos, R. H. S., Martinez, H. E. P., Cecon, P. R., & Fardin, M. P. (2010). Production and vegetative growth of coffee trees under fertilization and shade levels. Scientia Agricola, 67(6), 639–645. https://doi.org/10.1590/s0103-90162010000600004

Johnson H. W., Robinson H. F., Comstock R. E. (1955). Estimates of genetic and environmental variability in Soybeans. Agronomy Journal. 47(7):314–318. https://doi.org/10.2134/agronj1955.00021962004700070009x.

Kasu-bandi, B. T., Kidinda, L. K., Kasendue, G. N., Longanza, L. B., Emery, K. L., & Lubobo, A. K. (2019). Correlations between Growth and Yield Parameters of Soybean (Glycine max (L .) Merr .) under the Influence of Bradyrhizobium japonicum in Kipushi (The Democratic Republic of Congo). https://doi.org/10.3844/ajabssp.2019.86.94

Kebede, M. and Bellachew, B. (2008) Phenotyoic Diversity in the Hararge Coffee (Coffee arabica L) Germplasm for Quantitative Traits. East African Journal of Sciences, 2, 13-18. https://doi.org/10.4314/eajsci.v2i1.40358

Kitila, O., S. Alamerew, T. Kufa, W. Garedew. 2011. Variability of quantitative traits in limmu coffee (Coffea arabica L.) in Ethiopia. Int. J. Agr. Res. 6:482-493. https://doi.org/10.3923/ijar.2011.482.493.

Legesse, A., Alamrew, S., & Tesfaye, A. (2022). Stability Analysis of Coffee (Coffea arabica L .) Bean Yield Using GGE Biplot in South Western Ethiopia. 8(1), 36–42. https://doi.org/10.11648/j.ajbes.20220801.15

Malau, S., & Pandiangan, S. (2018). Morphological Variation in Arabica Coffee (Coffea arabica L .) Growing in North Sumatra Indonesia Variasi Morfologi Kopi Arabica (Coffea arabica L .) yang Tumbuh di Sumatera Utara Indonesia. 46(3), 314–321. https://doi.org/10.24831/jai.v46i3.19342

Marie, L., Abdallah, C., Campa, C., Courtel, P., Bordeaux, M., Navarini, L., Lonzarich, V., Bosselmann, A. S., Turreira-García, N., Alpizar, E., Georget, F., Breitler, J. C., Etienne, H., & Bertrand, B. (2020). G × E interactions on yield and quality in Coffea arabica: new F1 hybrids outperform American cultivars. Euphytica, 216(5). https://doi.org/10.1007/s10681-020-02608-8

Merga, D., Beksisa, L., Merga, W., & Alemayehu, D. (2023). Genetic Variability of Coffee (Coffea arabica L.) Germplasm in Biennial Bearing and Its Influence on Selection

Efficiency. International Journal of Agronomy, 2023. https://doi.org/10.1155/2023/9995531

Merga, D., Mohammed, H., & Ayano, A. (2021). Estimation of Genetic Variability , Heritability and Genetic Advance of Some Wollega Coffee (Coffea arabica L .) Landrace in Western Ethiopia Using Quantitative Traits. 9(4), 182–191. https://doi.org/10.11648/j.jps.20210904.18

Merga, W. (2021). An overview of genotype x environment interaction and yield stability analysis in applied plant breeding: great emphasis given to coffee (Coffea arabica L.). 11(2)(2224–0616), 117–123. https://www.banglajol.info/index.php/IJARIT

Merga, W. (2021). An overview of genotype x environment interaction and yield stability analysis in applied plant breeding: great emphasis given to coffee (Coffea arabica L.). 11(2)(2224–0616), 117–123. https://www.banglajol.info/index.php/IJARIT

Merga, W. (2022). Bean yield stability analysis of coffee (Coffea arabica L) genotypes in south western Ehiopia. Ternational Journal of Recent Scientific Research, 13(ISSN: 0976-3031), 1–8. https://doi.org/10.24327/IJRSR

Motebayenore, E. (2022). Review on Coffee Production Constraints and Opportunities in Ethiopia. Journal of Horticulture, 9(1), 1–7.

Ndikumana, J. (2022). Agro-morphological characterization of arabica coffee cultivars in burundi. Thesis Submitted to Graduate Studies of Jomo kenyatta university, Nirobi, Keniya.

Olika, K., Sentayehu, A., Taye, K. and Weyessa, G. (2011) Variability of Quantitative Traits in Limmu Coffee (Coffea arabica L.) in Ethiopia. International Journal of Agricultural Research, 6, 482-493. https://doi.org/10.3923/ijar.2011.482.493

Poehlman, J.M. and Sleeper, D.A. (1995). Breeding Field Crops. 4th Edition. Inc. Westport Connecticut, Ani.

Popat, R., Rumit, P., and Dinesh, P. (2020). Variability: Genetic variability analysis for plant breeding research. R Package version 0.1.0. Available at: https://cran.rproject.org/web/packages/variability/variability.pdf

Punitha B, Vindhiyavarman P, Manivannan N (2010) Genetic divergence study in sunflower (Helianthus annuus L.). Electron J Plant Breed 1(4): 426-430

SAS Institute. (2015). Base SAS® 9.4 procedures guide: Statistical Procedures (3rd, pp. 549). https://support.sas.com/documentation/onlinedoc/base/procstat93m1.pdf

Singh, B., A.K. Saxena, B.K. Chandan, D.K. Gupta, K.K. Bhutani and K.K. Anand. 2001. Adaptogenic activity of a novel, withanolide-free aqueous fraction from the root of Withania somnifera. Phytother Research 15:311-8.

Singh, R.K. and B.D. Chauhary. 1985. Biometrical methods in quantitative genetic analysis.Kalyani Publishers, New Delhi-Ludhiana.

Syukur, M., S. Sujiprihati and R. Yunianti, (2012). Teknik Pemuliaan Tanaman. Penebar Swadaya. Jakarta.

Terfa, G. N., & Gurmu, G. N. (2020). Genetic variability, heritability and genetic advance in linseed (Linum usitatissimum L) genotypes for seed yield and other agronomic traits. Oil Crop Science, 5(3), 156–160. https://doi.org/10.1016/j.ocsci.2020.08.002

Tessema, A., S. Alamerew, T. Kufa, W. Garedew. 2011.Variability and association of quality and biochemical attributes in some promising Coffea arabica germplasm collections in Southwestern Ethiopia. Internat. J. Plant Breeding Genet. 5:302-316. https://doi.org/10.3923/ijpbg.2011.302.316.

Tounekti, T., Mahdhi, M., Al-turki, T. A., & Khemira, H. (2017). Genetic Diversity Analysis of Coffee (Coffea arabica L .) Germplasm Accessions Growing in the Southwestern Saudi Arabia Using Quantitative Traits. 321–336. https://doi.org/10.4236/nr.2017.85020

Tsehaye Y. and F. Kebebew, (2002). Morphological diversity and geographic distribution of adaptive traits in finger millet (Eleusine coracana (L.) Gaertn. Subsp. coracana [Poaceae]) populations from Ethiopia. Addis Ababa, Ethiopia

Vavilov, N.I., 1951. The origin, variation, immunity and breeding of cultivated plants. (Translated by S. K. Chestitee). Chronica Botonica, 13, 1-366

WCR (2014) World coffee research annual report 2012–2013. http://worldcoffeeresearch.org

Yigzow, D. (2005) Assessment of Genetic Diversity of Ethiopian Arabica Coffee Genotypes Using Morphological, Biochemical and Molecular Markers. Ph.D. Dissertation, University of the Free State, South Africa.

Yilma Yemane Teferaa, (2017). A guide to coffee production in Ethiopia. Addis Ababa Ethiopia. P 1-280.

Yirga, M., Gebreselassie, W., & Tesfaye, A. (2021). Correlation and Path Coefficient Analysis in Coffee (Coffea arabica L .) Germplasm Accessions in Ethiopia. 9(2), 27–34. https://doi.org/10.11648/j.sr.20210902.12

Yonas, B., Bayetta, B., & Chemeda, F. (2014). Evaluation of bean qualities of indigenous Arabica coffee genotypes across different environments. Journal of Plant Breeding and Crop Science, 6(10), 135–143. https://doi.org/10.5897/jpbcs2014.0446

CHAPTER FOUR

4. Genotype by Environment Interaction and Stability Analysis for Yield of Arabica Coffee Genotypes in South Ethiopia

Abstract

Seventeen Arabica coffee genotypes were evaluated in twelve environments in south Ethiopia to analyze the extent of genotype by environment interaction (GEI) effect on Arabica coffee yield and to identify the highest yielding and stable genotypes. The experiment was established in 2015 using a randomized complete block design in a factorial arrangement with three replications at each location. The results revealed significant differences among genotypes, environments, and GEI, with an explained proportion of variation of 3.26%, 71.74%, and 17.52%, respectively. According to GGE-biplot and AMMI models, G7, G16, G5, G14, G15, G12, G11, and G13 were identified as high-yielding genotypes. Likewise, E7, E1, E5, E3, and E11 were classified as favorable environments. According to the AMMI and GGE biplot models and various stability indices (AMMI stability value, cultivar superiority index, and yield stability index), G16 (AW7705) was the highest bean yielder in tons per hectare and the most stable genotype. Therefore, AW7705 was discovered to be a promising candidate, which can be considered desirable genotypes in further tests, to releasing a new variety for Arabica coffee growing regions of southern Ethiopia and other similar agro-ecologies elsewhere. G7 (AW105) was the most yielding but an unstable genotype and can be considered in the specific target environments. Since the environment and GEI explained significant variations in coffee yield, testing genotypes in diverse environments prior to deciding on any variety to use under wider agro-ecology is recommended for coffee breeders to identify stable and high-yielding coffee varieties.

Key words: Adaptability, AMMI, Arabica Coffee, GEI, GGE-biplot, Stability

4.1 Introduction

Coffee is the most traded agricultural commodity and popular non-alcoholic beverage in the world (FAO, 2021). Arabica (*Coffea arabica* L.) and Robusta (*Coffea canephora* P.) coffee are the two important commercial coffee species, with about 60% and 40% of global coffee production, respectively (ICO, 2021).

Arabica coffee, which is treasured for its superior cup quality and aroma over Robusta coffee, is Ethiopia's main export crop and the backbone of the nation's economy. Until the 1990s, it was fetching about 60% of foreign exchange for the nation (Yilma, 2017). Science then, with gradual diversification of export commodities, its export share has declined; but, still, it plays a pivotal economic role by accounting on average, 32% of the total government's export revenue, 35% of agricultural export share, and its exports generated 1.1 billion USD in the 2020-2021 fiscal year for the country (USDA, 2023). Furthermore, the livelihood of 25 percent of the country’s population is directly or indirectly dependent on the coffee value chain (USD, 2023). In general, coffee plays a great role in the economic, socio-cultural, ritual, and spiritual life of Ethiopian societies.

Coffee grows in almost all areas of Ethiopia as forest, semi-forest, garden, and plantation, ranging from the semi-savannah Gambella’s plain climate to the Southwest’s mountain forest zones and moderately wet zones in the east and north, with altitudes ranging from 500 to 2600 m.a.s.l. (EIAR, 2017). However, it thrives and produces best between 1,300 m.a.s.l. and 1,800 m.a.s.l., with annual rainfall ranging from 1,500 mm to 2,500 mm and minimum and maximum air temperatures of 15 and 30° C, respectively (Tadesse *et al*., 2020). About 856,591.99 hectares of land are occupied by coffee, with an estimated annual production of 5,847,895.69 tons of clean coffee, with a national average productivity of 0.683 tonne per hectare in the 2020/21 cropping season (Motebayenore, 2022).

Despite the importance of coffee in Ethiopia’s economy and the presence of huge genetic resources, a favorable climate, and soil types for coffee production and productivity, the crop yield is by far very low as compared to the yield recorded in Vietnam (2.4 ton/ha), Brazil (1.4 ton/ha), Colombia (0.9 ton/ha), and the world average (0.8 ton/ha) (WEF, 2021). Many factors contributed to low level of production and productivity; including lack of high-yielding improved varieties with consistent performance in wide ranges of environments, biotic, abiotic (low soil fertility, poor agronomic management), and failure to use appropriate technology (improved variety, fertilizer) (Tadesse *et al*., 2020; Ayele *et al*., 2021). Therefore, developing coffee cultivars with high yield potential, resistance to disease, and adaptability and stability to wide agro-ecological zones is among the top breeding objectives of the country. Coffee is a perennial plant; once a grower plants a tree, it needs three years for the first harvest, and the growers may wish to keep it for a further twenty years or more. Therefore, coffee researchers have to pay serious attention to replanting costs and climate change risks during the development of new varieties.

Yield is a complex quantitative trait whose expression is determined by genotype, environment, and genotype by invirnomental interaction (GEI). Environmental conditions strongly influence agricultural production, leading to considerable variations in yield. Such influence is discriminated when yield experiments are performed in various locations and different years (Bacsi, 2019; Marie *et al*., 2020; Beksisa, 2021; Merga, 2021; Legesse *et al*., 2022; Ndikumana, 2022). Inconsistency in the performance of genotypes or a change in the magnitude of the difference between genotypes under diverse environments is called GEI (Cheng *et al*., 2018).

GEI is a significant obstacle to the successful selection of germplasm and the identification of superior genotypes in breeding programs. It threatens the repeatability of experimental results and reduces the selection efficiency of the cultivars (Enyew *et al*., 2021). GEI is important only when it is significant and causes a significant change in genotype ranks in different environments, i.e., different genotypes are superior in different environments (Luthra and Singh, 1974). GEI is frequently present in multi-environment-trials (MET). MET studies are essential since GEI causes a change in the performance ranking of genotypes over various environments, which complicates the evaluation of genotypes (Neisse & Kirch, 2018).

The presence of significant GEI has several implications for breeding strategies. Evaluating genotypes across a range of agro-ecological zones allows breeders to identify stable genotypes with consistent performance or those specifically adapted to certain environments (Annicchiarico, 2002). This helps in determining whether to pursue a broad adaptation strategy selecting genotypes that perform relatively well across all environments or a specific adaptation strategy, where genotypes are tailored for optimal performance in particular locations or agro-ecologies.

GEI has also implications for trait heritability and selection efficiency. In the presence of significant GEI, heritability estimates tend to decrease, leading to reduced efficiency of selection across environments. This necessitates the careful design of breeding trials, often requiring larger sample sizes and more replicates to accurately estimate genotype performance. It may also mean breeders must prioritize traits with high heritability and low GEI for early generation selection, and shift to multi-location trials for traits with strong GEI in later breeding stages (Falconer & Mackay, 1996).

Measuring and understanding the impacts of GEI and the stability of genotypes is an essential component in plant breeding programs for a decision-making process such as the identification of the relevant testing environments, the allocation of resources in a breeding program, as well as the choice of germplasm and breeding techniques (Lean et al., 2016). Once the crop breeders are well-equipped with the information related to the nature and extent of GEI, they may aim either at developing varieties for all environments of interest or specific varieties for a specific target environment (Egea-Gilabert et al., 2021).

Several multivariate statistical techniques such as analysis of variance (ANOVA), principal component analysis (PCA), joint linear regression, restricted maximum likelihood (REML), additive main-effects and multiplicative interaction (AMMI), and genotype plus genotype by environment (GGE) biplots analysis are used to explore GEI effects, identify stable cultivars, and identify mega-environments (Yan *et al*., 2000). Among them, the two commonly used statistical techniques for evaluating GEI are AMMI and GGE-biplot (Yan *et al*., 2000).

The presence of significant GEI for Arabica coffee yield in Ethiopia was reported by many authors (Afework, 2017; Argaw & Taye, 2018; Beksisa *et al*., 2018; Beksisa, 2021; Asefa, 2022; Legesse *et al*., 2022; Merga, 2022). According to their report, genotypes that showed better yield performance at one location did not show the same performance at other locations, indicating the inconsistency in yield performance over diverse environments. Hence, Ethiopia has an abundant genetic resource of Arabica coffee as well as diverse growing environments, conducting adaptation trials over different locations within the region is critical for identifying stable genotypes and increasing productivity and production in various environments. Most of the bean yield performance and stability studies over location were done in southwest Ethiopian growing ecologies using southwest materials, but little information was generated for wider adaptability and stability in south Ethiopian ecologies using south materials. Thus, investigation of southern Ethiopian genotypes in the main south Ethiopian coffee growing ecologies is an urgent task. Therefore, the main objective of this study was to analyze the extent of the effect of GEI on Arabica coffee bean yield and to identify the stable genotypes that exhibit wide adaptation across the diverse agroecologies of south Ethiopia.

4.2 Methodology

4.2.1 Study Area

The experiment was conducted at three major coffee-growing agro-ecological areas of south Ethiopia: the Awada Agricultural Research Sub-Center (AARSC), Wonago substation, and Shebedino trial field; all located in the south Ethiopian coffee-growing agro-ecology. The soil type, altitude, latitude, longitude, rainfall, and temperature of the experimental locations are indicated in Table 4.1. The experimental period lasted for four consecutive seasons, from 2018 to 2021. The location by yield harvest season combinations would have twelve environments Table 4.3.

Table 4. 1 Description of the experimental locations

Site	Latitude	Longitude	Altitude	Soil type	Rainfall	Temp Max	Temp Min
AARSC	6^{0}45'46"N	38^{0}22'36"E	1740masl	Clay	1269 mm	28.4^{0}C	11°C
Shebedino	6^{0}50'48"N	38^{0}27'06"E	1845masl	Clay	NA	NA	NA
Wonago	6^{0}17'54"N	38^{0}13'05"E	1850masl	Clay	NA	NA	NA

AARSC= Awada Agricultural Research Sub-center; NA = not available

4.2.2 Experimental/Plant materials

The experiment consisted of 17 Arabica coffee genotypes (14 south Ethiopian promising selections and 3 standard check varieties). The promising selections were promoted from different south Ethiopian coffee collection batches for their high yield potential and resistance to coffee leaf rust and coffee berry disease during a preliminary four-year evaluation carried out at Awada. The description of genotypes is specified in Table 4.2.

Table 4. 2 Description of experimental materials

Serial No.	Genotype Designation	Genotypes	Description	Source
1	G1	AW1777	Promising selection	AARSC
2	G2	AW1995	Promising selection	AARSC
3	G3	AW96/44	Promising selection	AARSC
4	G4	AW74/94	Promising selection	AARSC
5	G5	**Angafa**	**CRSCV**	AARSC
6	G6	AW9662	Promising selection	AARSC
7	G7	AW105	Promising selection	AARSC
8	G8	AW9622	Promising selection	AARSC
9	G9	AW9628	Promising selection	AARSC
10	G10	**74112**	**CRSCV**	JARC
11	G11	AW4994	Promising selection	AARSC
12	G12	AW5994	Promising selection	AARSC
13	G13	AW9641	Promising selection	AARSC
14	G14	AW3106	Promising selection	AARSC
15	G15	**Feyate**	**CRSCV**	AARSC
16	G16	AW7705	Promising selection	AARSC
17	G17	AW9623	Promising selection	AARSC

AARSC= Awada Agricultural Research Sub-center; JARC=Jima Agricultural Research Center; CRSCV= standard check variety (commercially released cultivar)

Table 4. 3 Description of Environments

Location	Harvesting Year	Environment designation
Awada	2018	E1
Awada	2019	E2
Awada	2020	E3
Awada	2021	E4
Shebedino	2018	E5
Shebedino	2019	E6
Shebedino	2020	E7
Shebedino	2021	E8
Wonago	2018	E9
Wonago	2019	E10
Wonago	2020	E11
Wonago	2021	E12

4.2.3 Experimental Design

The experimental field was established in August 2015 using a randomized complete block design in factorial arrangement (location and genotypes as factors) with three replications at

Awada, Wonago, and Shebedino. The experimental plots consisted of eight coffee trees planted in a single row for each genotype. The intra and inter-row spacing was 2 meters by 2 meters, whereas the spacing between replications was maintained at 4 meters by 4 meters at each of the three locations. The genotypes were evaluated from 2018–2021 for four consecutive yield seasons per three locations.

4.2.4 Experimental Procedures

Seed Preparation: Cherry picking from the middle nodes of the middle branches in the middle of the harvest season at the red-ripe stage from respective selections and check varieties, floater removal, hand pulping, mucilage removal, sorting, and drying up to a moisture content of 15–18% were carried out.

Seedling media preparation: Coffee seedlings were grown in dark-colored polyethylene tubes (width 16cm, length 22cm, thickness 0.06mm) filled with blends of topsoil (TS), compost (C), and sand (S) in a ratio of 2TS: 1C: 1S as per recommendation (Endale *et al*., 2008).

Seed Sowing: The coffee seeds were sown in the center of each bag to a depth of 1 to 1.5 cm.

Seedling management: Watering, mulching, overhead shade constriction and tinning, nutrient management, and acclimatization were carried out as per recommendation.

Land preparation: Field clearing, designing, hole digging, refilling, and relining were done as per the recommendations.

Planting: Healthy and vigorous seedlings were planted at three experimental sites in August 2015.

Field management: Weeding, nutrient management, permanent and temporary shedding, soil conservation, and sucker management were carried out as per recommendations.

4.2.5 Data collection

Cherry picking at the red-ripe stage during harvesting season was carried out annually from 2018 to 2021 for four consecutive harvesting years. The bean yields were weighted on a tree basis and converted to clean coffee in ton ha^{-1} for the statistical analyses.

4.2.6 Data analysis

A separate analysis was made for each of the environments to distinguish genetic variability among genotypes. The combined analysis of variance was carried to identify the possible interactions of genotypes with environments by using the SAS software (SAS version 9.4). Levene's test was used to attest the homogeneity of variances between environments to determine the validity of the data for a combined analysis of the variance. The effects of genotypes and environments as well as their interactions were determined by ANOVA. Following the presence of significant GEI effects, a multivariate analysis of genotype adaptability and stability across twelve environments (locations and years) was performed using the AMMI and GGE-biplot models.

The general model for AMMI and GGE-biplot is described as follow:

Additive Main Effect and Multiplicative Interaction (AMMI) Analysis

AMMI model was computed following the formula suggested by Gauch, (1992);

$$Yij=\mu+\alpha i+\tau j+¿$$

where: εij is the random error;

ρij the residual for the multiplicative components and,

t_{jk} is the j^{th} element of the k^{th} eigenvector;

a_{ik} is the i^{th} element of the k^{th} eigenvector;

λ_k is the singular value for the k^{th} interaction principal component axis (IPCA);

τj is the deviation of the j^{th} environment from the grand mean;

αi is the deviation of the i^{th} genotype from the grand mean;

μ is the grand mean;

Yij is the value of the i^{th} genotype in the j^{th} environment.

Genotype and genotype by environment interaction biplot (GGE-Biplot) analysis

The GGE biplot was built according to the formula given by Yan *et al*., (2000):

$$Yij-\mu-ej=\lambda 1\sigma i1hj1+\lambda 2\sigma i2hj2+\varepsilon ij$$

where:

εij=the error associated for the genotype i and environment j

$hj2$= eigen vectors of environment j for IPCA2;

$\lambda 2$=the singular value for principal component 2 (IPCA2);

$\sigma i2$= eigen vectors of genotype i for IPCA2 hj1= eigen vectors of environment j for IPCA1;

$\sigma i1$= eigen vectors of genotype i for IPCA1;

$\lambda 1$=the singular value for principal component 1(IPCA1);

ej= the mean effect of the j^{th} environment;

μ = is the grand mean;

$Yijr$= is the performance of the i^{th} genotype in the j^{th} environment and r^{th} replication.

AMMI's stability value (ASV) was computed following the formula proposed by Purchase (1997) as follows:

$$ASV=\sqrt{\left[\left(\frac{IPCA\,1\,ss}{IPCA\,2\,ss}\right)(IPCA\,1\,score)\right]^{2}+(IPCA\,2\,score)\text{¿ ¿}^{2}}$$

Where ASV is AMMI's stability value;

$\frac{IPCA\,1\,ss}{IPCA\,2\,ss}$ is is the weight given to the IPCA1 value by dividing the IPCA1 SS by the IPCA2 SS; and

the IPCA1 and IPCA2 scores are the genotypic scores in the AMMI model.

Cultivar superiority index (Pi) was calculated according to Lin and Binns (1988) formula as follows:

$$Pi=\frac{(Yij-Yij\max)^{2}}{2n}$$

Where,

Pi = cultivar superiority index

Y = the yield mean of the i^{th} genotype in the j^{th} environment;

Yij max = the average yield of the genotype with highest yield in the j^{th} environment; and

n = the number of environment.

The yield stability index (YSI) was computed using the Mahmodi *et al.*, (2011) formula as follow:

YSI =RY + RASV

Where, RASV = AMMI stability value, RY = the mean yield rank of genotypes across environments.

The AMMI and GGE-biplot analyses were done using R statistical software, version 4.2.0, by using the "metan'' packages (R Development Core Team, 2021).

4.3 Results and Discussion

In this study, a combined analysis of variance showed highly significant variation ($p\ 0.001$) among the environments, genotypes, and GEI for the yield trait (Table 4.4). The highly significant difference in F-value confirmed the presence of variability in the inherent genetic composition among the tested coffee genotypes, differences in climatic and edaphic conditions at different testing environments, and the differential response of tested genotypes across environments for the bean yield trait. Several authors also reported significant variation among genotypes, environment, and GEI for bean yield (Bacsi, 2019; Marie *et al*., 2020; Beksisa, 2021; Cheserek *et al*., 2021; Merga, 2021; Legesse *et al*., 2022; Ndikumana, 2022; Partelli *et al*., 2022).

The percentage of variation explained by environment, genotype, and GEI was 71.74%, 3.26%, and 17.52%, respectively. These results imply that the environmental main factors and GEI had a significant influence on the yield performance of genotypes as compared to the genotypic main effect, and it is an indication that the environment is the main factor that influences the yield performance of coffee genotypes in south Ethiopia. This finding is in agreement with many previous studies that show a high proportion of variation by the environment and the GEI component on Arabica coffee bean yield (Argaw and Taye, 2018; Beksisa *et al*, 2018; Marie *et al*., 2020; Beksisa, 2021; Cheserek *et al*., 2021; Merga, 2021; Legesse *et al*., 2022; Merga, 2022). When there is a significant GEI effect, one genotype's phenotypic expression may be highest in one environment but lowest in another. The breeding strategy is complicated by significant GEI. As a result, the superiority of genotypes across environments cannot be determined by examining their mean performance in a single environment. Therefore, breeders need to develop varieties that are adapted to specific environmental conditions or identify exceptional stable varieties that maintain their mean performance across diverse environments after multi-environment testing.

The AMMI model fitting method partitioned the differential response of genotypes across 12 environments into eleven possible interaction principal component axes (IPCA) along with their contributions in decreasing rank (Table 4.4). The eleven IPCAs were partitioned into seven significant and two non-significant IPCA. The first and second IPCA explained 30.8% and 24.2% of the variation, respectively. The sum of the first two IPCAs (IPCA1 and IPCA2) explained about 55% of the variation, which is greater than half of the variation, indicating the capability of the first two IPCAs for cross-validation of variation explained by GxE interaction. The third, fourth, and fifth IPCAs (IPCA3, IPCA4, and IPPCA5) explained 16.0%, 11.0%, and 8.2% of the variation of GEI, respectively. The first seven IPCAs (IPCA1–7) accounted for 97.2% of total GEI variation, leaving 2.8% of the variation (Table 4. 4). In line with current findings, many authors have also reported the high contribution of the top IPCAs on Arabica coffee yield (Demissie *et al*. 2011; Argaw and Taye, 2018; Beksisa *et al*., 2018 and Merga, 2022).

Table 4.4 Combined analysis of variance of AMMI model for 17 Arabica coffee genotypes yield (ton ha^{-1}) tested across 12 environment

Source	DF	SS	MS	F value	GEIPVE	CPVE
ENV	11	261.88	23.81	**	71.74%	
REP(ENV)	24	3.11	0.13	*	0.85%	
GEN	16	11.89	0.74	**	3.26%	
GEN:ENV	176	63.97	0.36	**	17.52%	
PC1	26	19.72	0.76	**	30.80%	30.80%
PC2	24	15.51	0.65	**	24.20%	55.10%
PC3	22	10.24	0.47	**	16.00%	71.10%
PC4	20	7.01	0.35	**	11.00%	82.00%
PC5	18	5.22	0.29	**	8.20%	90.20%
PC6	16	2.74	0.17	**	4.30%	94.50%
PC7	14	1.73	0.12	**	2.70%	97.20%
PC8	12	0.74	0.06	ns	1.20%	98.40%
PC9	10	0.57	0.06	ns	0.90%	99.20%
PC10	8	0.27	0.03	ns	0.40%	99.70%
PC11	6	0.21	0.04	ns	0.30%	100.00%
Residuals	384	24.21	0.06			
Total	787	429.03	0.55			

*, ** = Significant difference at P<0.01 and P<0.05 respectively; DF=degree of freedom; ENV = environment; GEN= Genotype; GEN:ENV = GEI; REP(ENV)=replication; SS= sum square; MS=mean square; GEIPVE=. present variation explained by GEI; and CPVE= Cumulative percent variation explained.

4.3.1 AMMI biplot analysis for Arabica coffee yield

To distinguish the GEI effect's contribution, an AMMI1-biplot was plotted using the environment and genotype mean yields versus their IPCA1 scores (Figure 4.1). The x-coordinate of the AMMI1-biplot indicates the average of the environments and genotypes, while the y-coordinate represents the IPC1. The environments and genotypes on the right side of the x-axis are high-yielding (above the mean yielding) genotypes and environments, while those on the left side are low-yielding genotypes and the environments are unfavorable environments (Yan and Tinker, 2006). Accordingly, E7, E1, E5, E3, and E11 were classified as high-yielding and favourable environments on the right side of the abscissa, whereas E12, E6, E10, E9, E4, E2, and E8 were classified as low-yielding and unfavourable environments. Likewise, the high-yielding genotypes that produce above-average means were G7, G5, G14, G15, G16, G12, G11, and G13 suited in the right side of the abscissa, whereas the low-yielding genotypes were G4, G3, G10, G8, G9, and G6 suited in the left side of the x - coordinate (Fig 4.1).

The higher the IPCA scores from the origin (either positive or negative), the more specific a genotype is to certain environments with the highest contribution to the GEI, or the more the IPCA scores approach zero, the more stable the genotype is over all the environments sampled (Abrha *et al*., 2022). Accordingly, G14, G5, G16, and G6 were discovered to be relatively more stable and above-average yielders; likewise, G8 was a stable and below-average yielder, whereas G13, G7, G9, and G4 were more unstable genotypes. Environments with scores near to zero have low GEI across genotypes and offer low discrimination among genotypes. Accordingly, E12, E6, E10, E8, E9, and E5 showed low GEI. Of the listed low GEI environments, only E5 was above the mean and a stable environment. In contrast, E3, E11, E7, and E4 with high interaction across genotypes provided the highest discrimination among genotypes. The environments E3, E11, and E7 with high yielding performance tended to have the highest contribution to GEI (Figure 4.1).

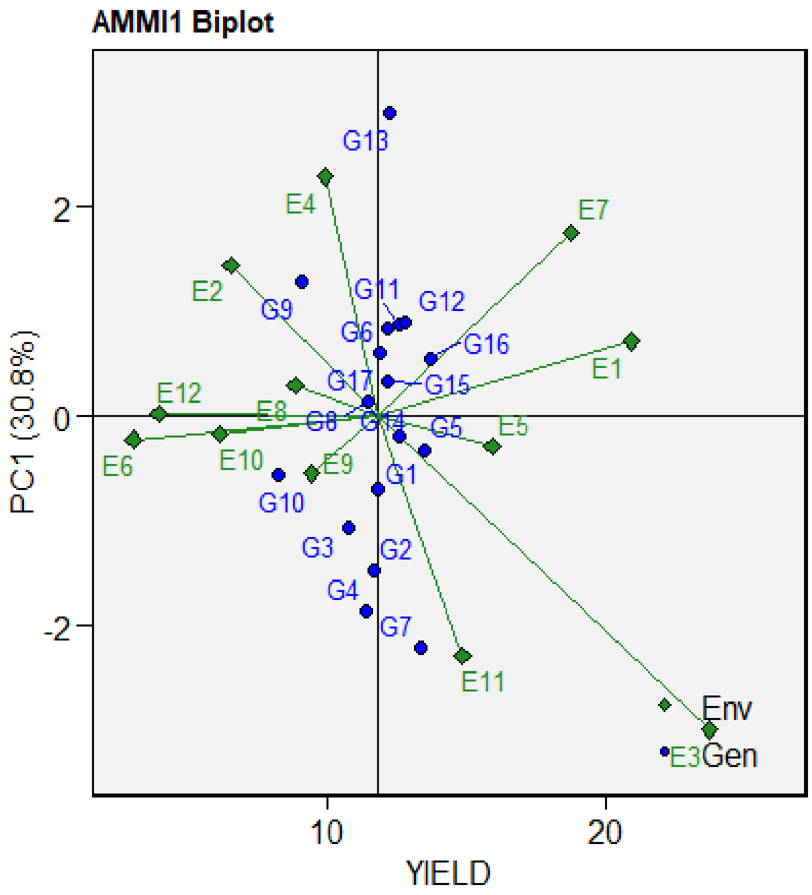

Figure 4. 1 AMMI biplot of the first IPC axis versus mean bean yield for 17 Arabica coffee genotypes grown in 12 environments

AMMI2 helps in the visual interpretation of the GEI pattern and identifies environments or genotypes that exhibit a high, medium, or low interaction effects (Hailemariam & Tesfaye, 2019). The biplot was generated to illustrate environment and genotype effects simultaneously with the first two IPCAs (IPCA1 and IPCA2) (Figure 4.2). IPCA1 and IPCA2 of bean yield accounted for 30.30% and 24.2% of interaction, respectively. Points near the origin have small interaction effects, and points near each other have similar interaction effects (Gauch, 1992). According to Purchase (1997), the genotypes that are positioned faraway from the center are more responsive or unstable, while genotypes and environments that are closer to the center of the biplot have higher stability performance. Accordingly, the genotypes G16, G15, G11, G6, G17, G5, and G3 were located close to the origin, proved highly stable, and expressed a low GE interaction (positive or negative), whereas the genotypes G13, G12, G8, G4, G7, and G1, located far away from the origin, were highly unstable and expressed a higher GE interaction (positive or negative) (Figure 4.2).

Environments with short spokes (length of arrow lines) do not exert strong interactive forces, whereas those with long spokes exert strong interaction. Thus, E1, E5, E3, E11, and E4 having long spokes (located far away from the biplot origin) were highly discriminative, meaning they contributed significantly to genotype-by-environment (G×E) interaction by effectively differentiating among genotypes. These environments likely had variable conditions that amplified genotypic differences. .Incontrast, environments like E10, E12, and E6 hadm short spoke (located close to the origin), indicating low discriminative ability and minimal G×E interaction. These environments were probably more stable or favorable, leading to uniform genotype performance.

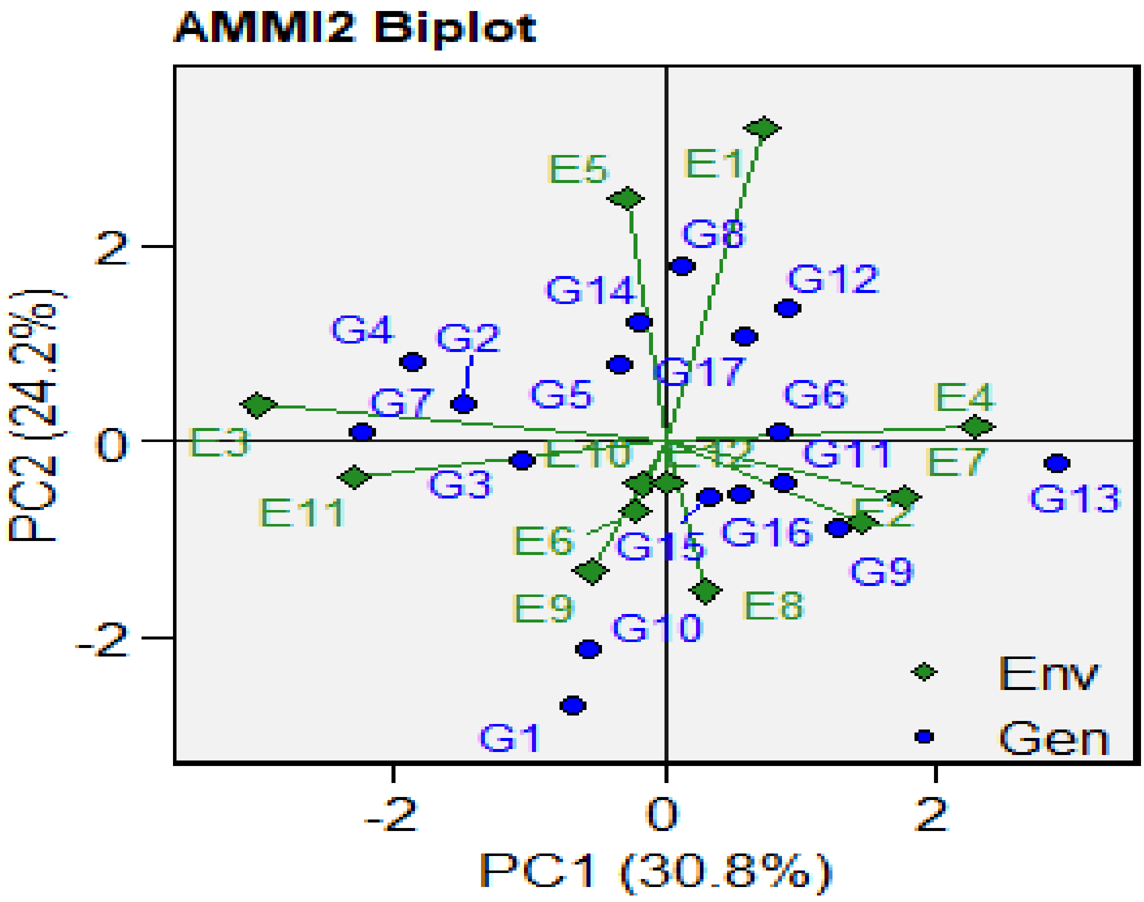

Figure 4. 2 IPCA1 vs IPCA2 for bean yield of 17 Arabica coffee genotypes over 12 environments.

4.3.2 GGE-biplot analysis

4.3.2.1 Which-won-where biplot

GGE-biplot analysis was carried out to identify the best genotype per each environment and to assess genotype stability. The which-won-where polygon view of the GGE-biplot for Arabica coffee yield (ton ha-1) was displayed in Figure 4.3. The GGE-biplot of the first two

IPCAs (IPCA1 and IPCA2) explained 28.7% and 25.97% of the variation, respectively, for yield in tons per hectare with 54.67% total variation (Figure 4.3).

The polygon was formed by connecting seven vertex genotypes, viz., G13, G12, G5, G7, G4, G10, and G9 (Figure 4.3). Seven sets of lines are drawn from the biplot's origin and intersecting the sides of the polygon at right angles (Figure 4.3), which divided the biplot into seven sections. As shown in the polygon, the twelve environments fell into five different coffee growing mega-environments with various winning genotypes. A mega-environment is a location, or a group of locations, where the same genotypes (or group of genotypes) consistently perform the best over a number of seasons (Yan and Rajcan, 2002).

The genotype of the vertices was the most responsive because it was the furthest away from the biplot origin and the one that gave the highest yield for the environments within that sector. According to this model, G7 gives the highest expected yields in E3, E11, E9, and E10. G13 is the winner in E4, E7, and E2, while G5 wins in E5, E1, and E12. G9 is the "winner" in E8, whereas G10 is the "winner" in E6. The points for E6 and E8 are slightly to the G10 and G9 sides of the dividing line, between G9 and G10, although E10 is slightly to the G5 and G7 sides of the dividing line. Environments E3 and E11 are most suited for G7; E1 and E5 are best suited for G5, and E4 is best suited for G13. In line with this study, many researchers have reported different mega-environments with different winning genotypes for Arabica coffee at different times (Beksisa *et al*., 2018; Beksisa, 2021; Beksisa , 2021; Asefa, 2022; Legesse *et al*., 2022)

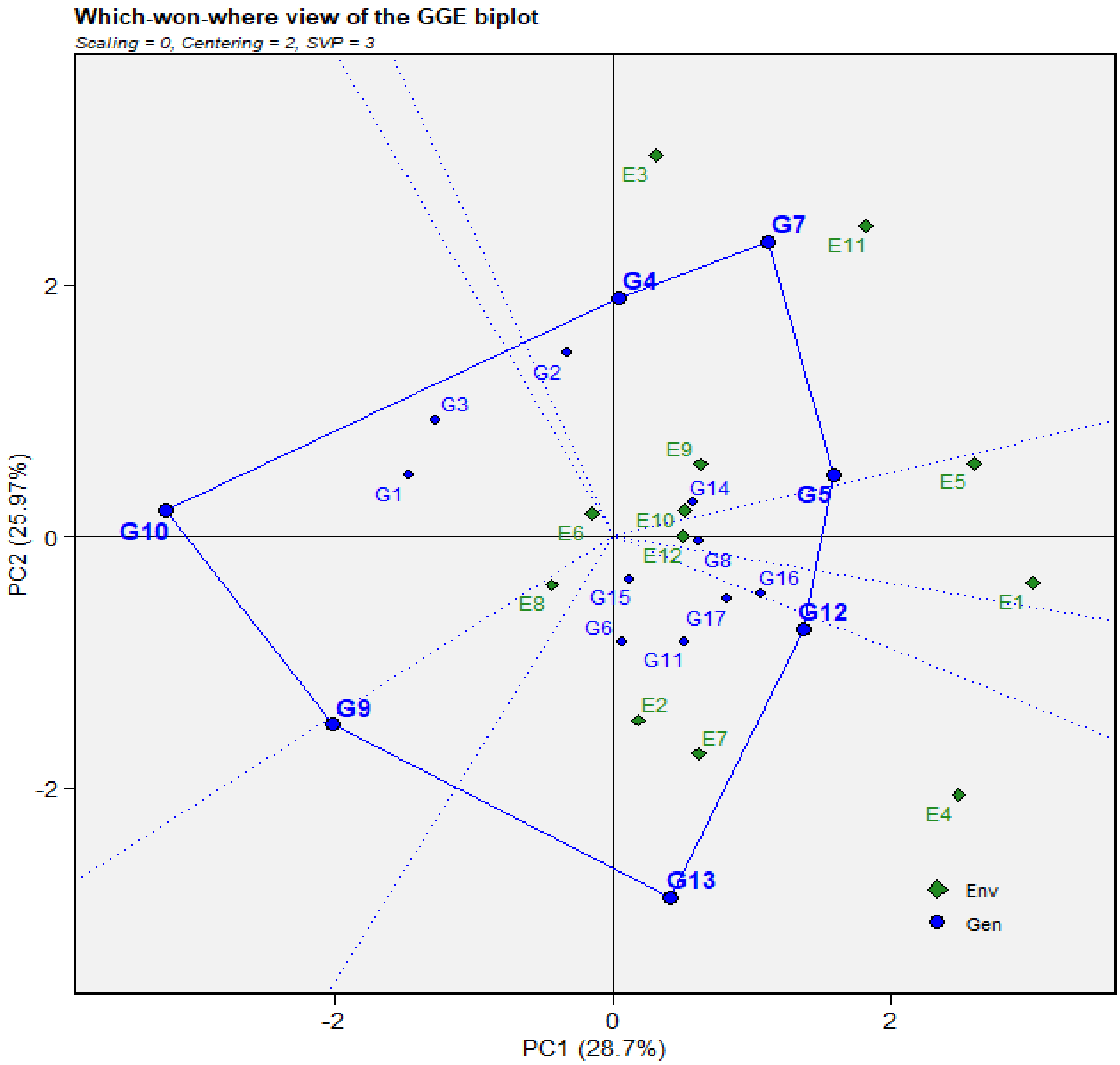

Figure 4. 3 Which -Won-Where view of GGE-biplot for the 17 genotypes in 12 environment centered bean yield data

4.3.2.2 **Test environment's representativeness and discriminating ability**

A successful breeding program for superior genotype selection requires the identification of the best-suited test environment. The discriminate versus representativeness biplot shown in figure 4.4 is an important GGE-biplot to measure the test environments. The concentric circles on a biplot are used to illustrate the length of the environment vectors and measure the discriminatory capacity of the environments. With the longest vectors from the origin, E4, E1, E5, E11, and E3 were the most discriminating of the genotypes. Conversely, E6, E12, E10, E9, and E8 provided little information regarding the genotype differences with the shortest vector from the origin.

The discriminate versus representativeness biplot view offers a brief description of the environmental interrelationships (Figure 4.4). According to Yan and Tinker, (2006), when two angles between two environment vectors are less than 90 degrees, then, these two environments are positively correlated; whereas, they are independent if the angle is 90 degrees and negatively correlated if the angle is greater than 90 degrees. Accordingly, E2, E7, E4, and E1 environments were positively correlated to each other since all of the angles among their vectors were smaller than 90°. In addition, E12, E5, E10, E9, E11, and E3 as well as E6 and E9 were positively correlated environments.

If the angle between the test environment and the line passing through the average environment is small, it means that this test environment is representative (Yan and Tinker, 2006). Thus, E1 and E5 showed a long vector that forms a small angle with the AEC abscissa line, indicating that these environments were the most representative and discriminative (Fig 4.4). The small angle between the E12 and E10, as well as the short line length of these environment vectors, indicated the least discriminating ability. Accordingly, E12 and E10 were non-discriminating test environments that offered little information regarding genotypes and should not be used as suitable environments. If the target environments are divided into mega-environments, discriminating but not representative test environments such as E3 and E4 can be used to select specifically adaptable genotypes.

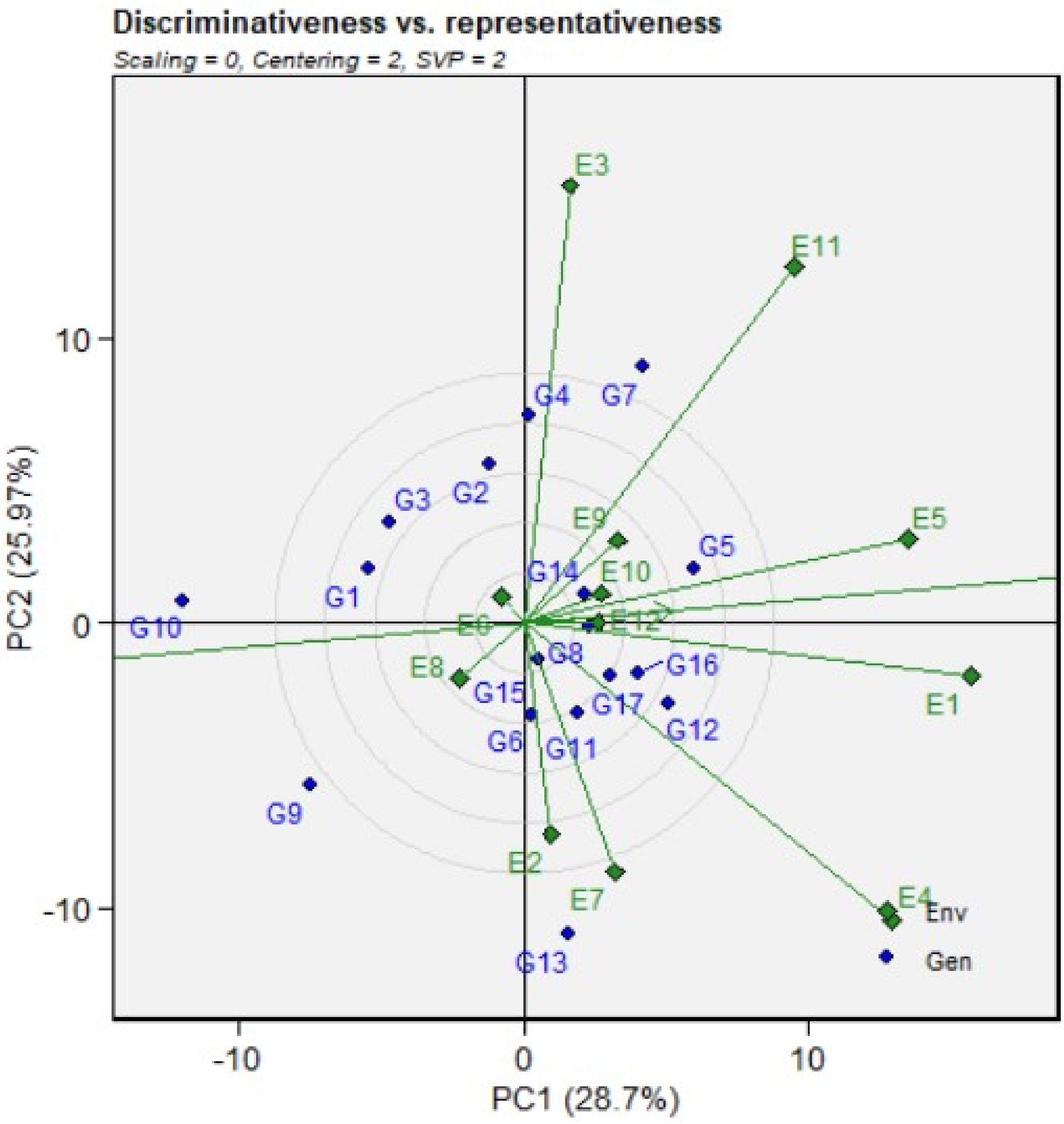

Figure 4. 4 Ranking of 12 Arabica coffee growing environments based on their discriminate and representativeness

4.3.2.3 Stability and Average Yield Performance of the Genotypes

The average environment coordination (AEC) method was used to evaluate the stability and average yield performance of the 17 Arabica coffee genotypes (Figure 4.5). The stability axis is the axis drawn as a double-headed arrow axis that passes at right angles to the AEC through the biplot origin. The genotype positioned further away from the AEC in the direction of either arrow is considered less stable across environments, while the genotype nearest to the AEC in the direction of either arrow is considered to be stable (Yan and Kang, 2003). Accordingly, the genotypes positioned nearest to the stability axis, or relatively stable genotypes, were G5, G16, G14, G8, G15, G1, and G10, whereas further genotypes (unstable genotypes) were G7, G13, G4, and G6 (Figure 4.5).

The AEC ordinate located on the left side of the biplot origin distinguishes genotypes with lower-than-average means, whereas the ordinate located on the right side of the biplot origin distinguishes genotypes with higher-than-average means. Thus, genotypes with higher-than-average means in this study were G5, G7, G12, G16, G17, G8, G14, G11, G13, and G15, while genotypes with lower-than-average means were G10, G10, G9, G1, G3, and G2 (Figure 4.5).

According to Farshadfar *et al*., (2012), the ideal genotype is the one with the highest yield and the highest stability among environments. Thus, G5 and G16 were both stable and high-yielding and therefore considered ideal genotypes. G7 and G12 were higher yielders, but they were unstable. Though G1 and G10 were relatively stable, they were low yielders, making them unsuitable for breeding programs.

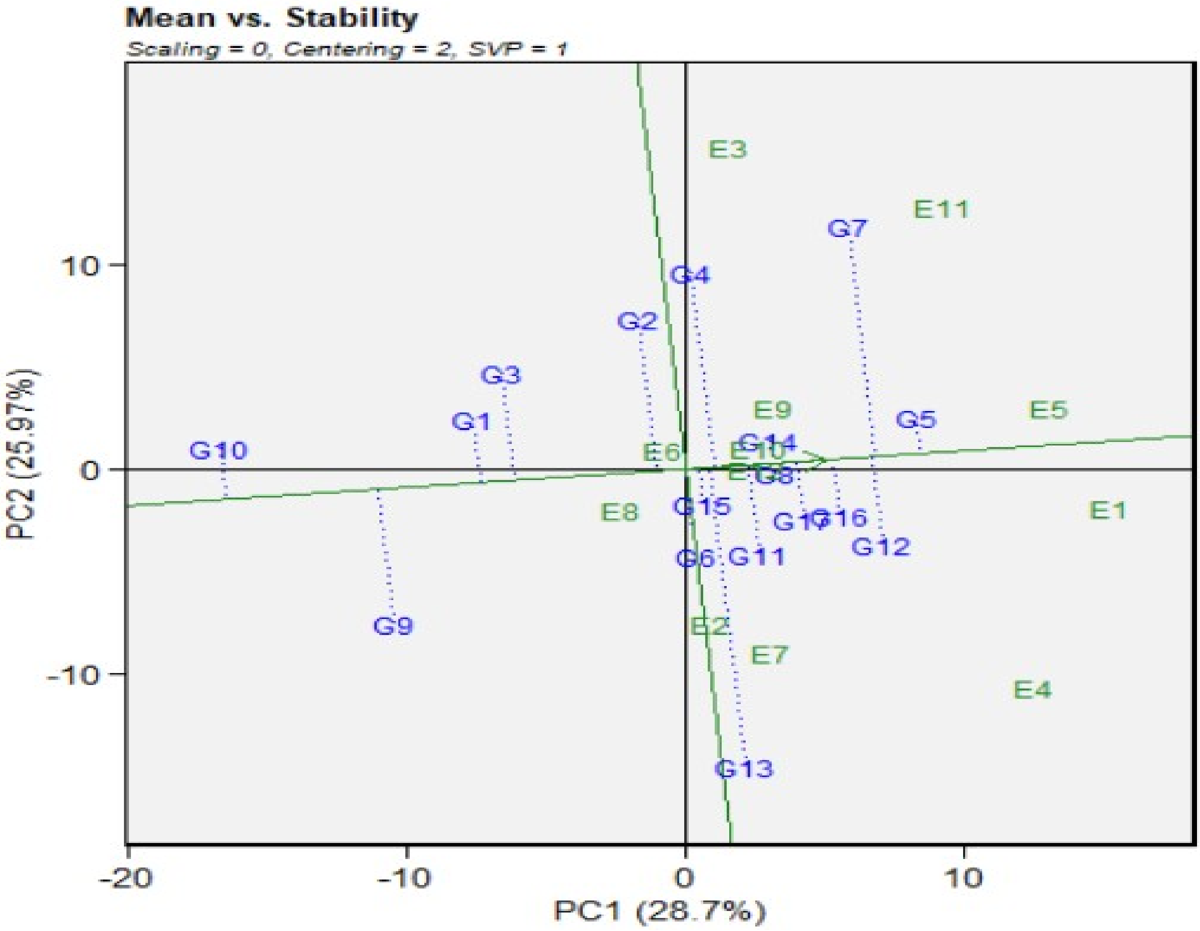

Figure 4. 5 Mean bean yield versus stability biplot for the 17 Arabica coffee genotypes and 12 environments

4.3.2.4 Ranking Genotypes Relative to the Ideal Genotype

Figure 4.6 shows the ranking of 17 Arabica coffee genotypes relative to the ideal genotypes using the GGE-biplot model across 12 environments. The AEC of the GGE-biplot view is used to rank genotypes with ideal genotypes. The AEC ordinate distinguishes genotypes with lower-than-average means from those with higher-than-average means. More data closer to the concentric circles indicates a higher mean yield. Accordingly, genotypes with the highest yielders were G5, G12, G16, G7, G14, G8, G17, G13, and G4.

The perpendicular line that passes through the origin to the AEC with double arrows represents the stability of genotypes. The genotypes with the highest stability and the highest mean yield are the best candidates for selection. In the biplot, they are close to the origin and have a shorter vector from the AEC. Regardless of the direction, a longer projection to the AEC indicates a genotype's inclination toward a higher GEI, which implies less environmental stability. The ideal genotype, which is situated at the center of the concentric circles, can be used as a benchmark for selection. If a genotype is nearby to the ideal genotype, it is more desirable. Thus, to illustrate the difference between each genotype and the ideal genotype, concentric circles were drawn around it. Genotypes situated nearest to the "ideal genotype" are more desirable than those located farther away. Accordingly, G6, G16, G14, G8, G17, G15, G11, and G7 were stable genotypes in decreasing order. In contrast, the genotypes G13, G9, G10, G1, G3, and G2 were far from AEC (long vector), indicating their least stability (Figure 4.6).

In this study, G5, G12, and G16 were located close to the concentric circle and regarded as desirable genotypes in further tests. On the other side, G13, G9, G10, G1, G3, and G2 were out of the concentric circle and could be rejected in early breeding cycles.

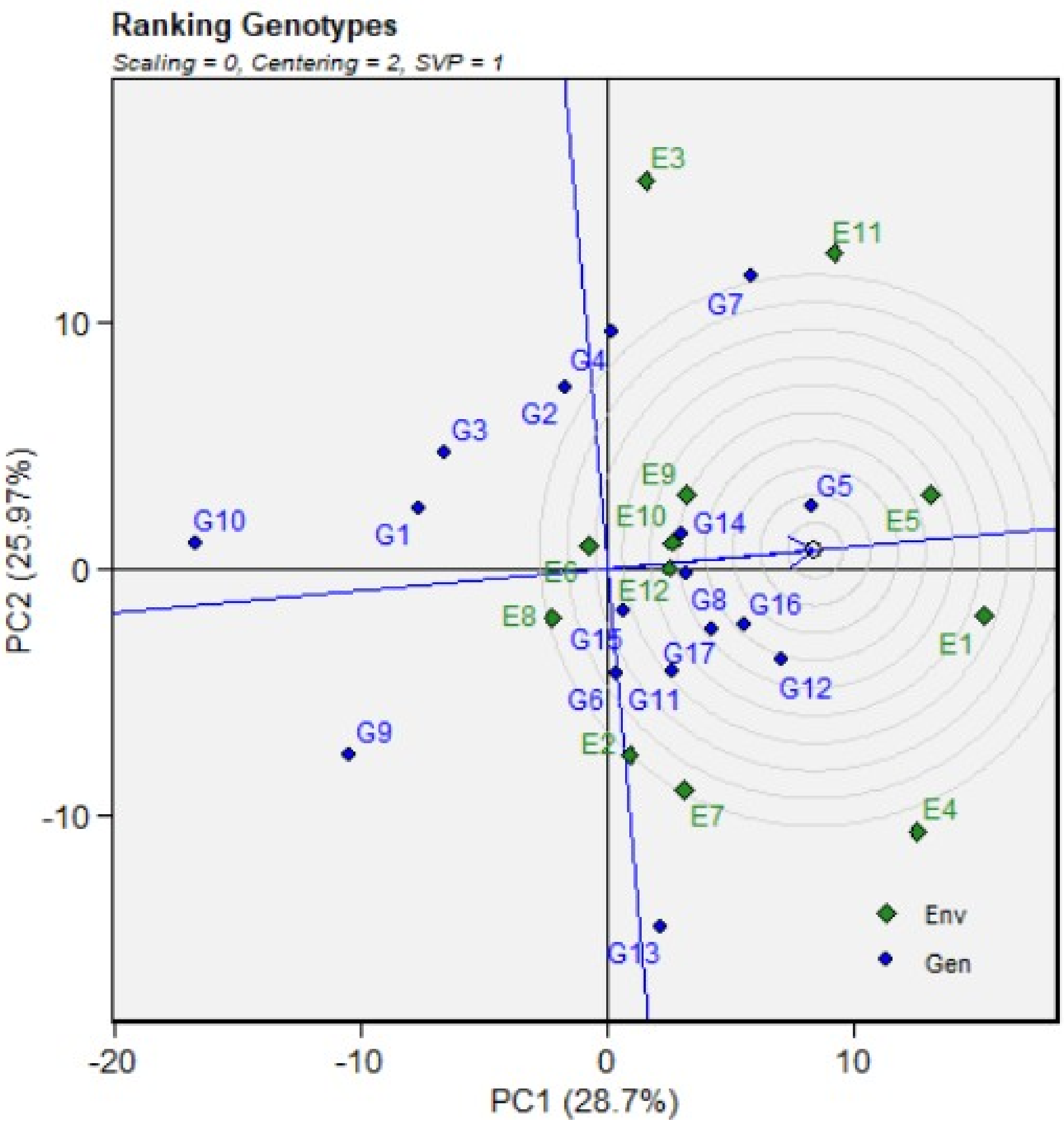

Figure 4. 6 Ranking of 17 Arabica coffee genotypes relative to the ideal genotypes using GGE-biplot model across twelve environments

4.3.2.5 Environmental Ranking in Relation to the Ideal Environment

An ideal environment is representative and has the highest discriminating power (Yan and Tinker, 2006). The most suitable environment is the one closest to the ideal environment, which is located in the first concentric circle of the environment-focused GGE-biplot (Yan *et al.*, 2000). Thus, among the environments, E1 and E5 were close to the ideal environment, and these environments were the most representative of the overall environments and the most powerful in discriminating genotypes; they were therefore identified as more desirable environments than the others. E4 and E11 were closer to the ideal environment and considered the second-most powerful to discriminate genotypes. Conversely, environments

E8, E3, E2, and E6 were far from the ideal environment and considered less powerful to discriminate genotypes (Figure 4.7).

Representativeness and discriminating ability are the key characteristics of a test location. An ideal location should be highly distinguishing for the genotypes tested while also being representative of the target location. Similar to the ideal genotype, the environment is more desirable and discriminating when located nearer to the center circle or an ideal environment. Thus, E1 and E5 show the highest representativeness and discriminating ability among the twelve environments.

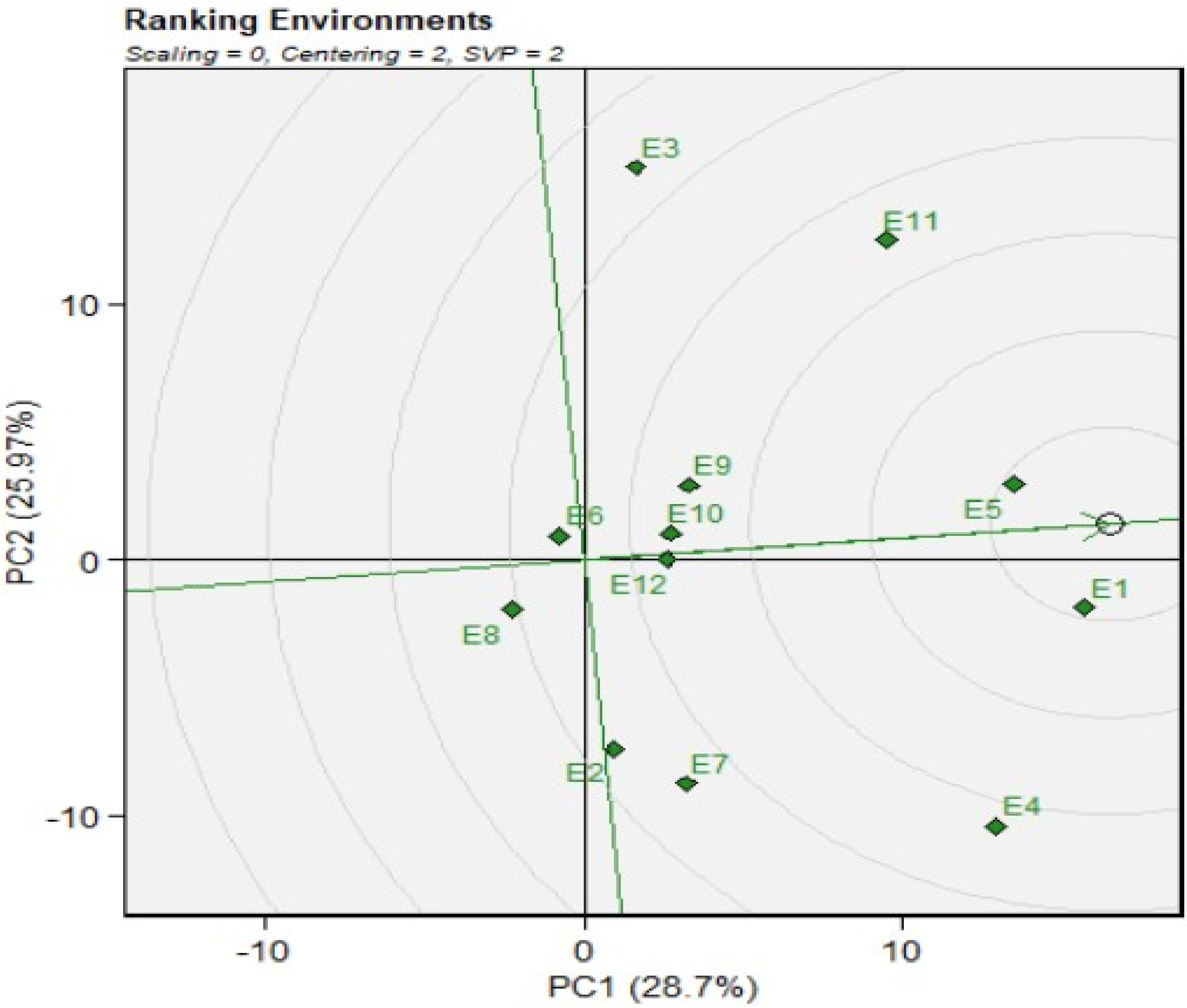

Figure 4. 7 GGE-biplot with environment-focused scaling for comparing 12 Arabica coffee growing environments to the ideal environment

4.3.3 Stability measurements

4.3.3.1AMMI stability value

The AMMI stability value (ASV) and its ranking orders for 17 coffee genotypes evaluated over twelve environments were presented in Table 5.5. In the ASV model, genotypes or varieties with the lowest ASV (either negative or positive) are the most stable, whereas those with the highest ASV are considered unstable (Purchase, 1997). Thus, G15, G16, G5, and G6 were found to be the top four stable genotypes in decreasing order; however, G13 was the most unstable genotype, followed by G1, G7, and G4. According to the ASV ranking, G15 was the first stable genotype, but its mean yield over tested environments was ranked 8th in bean mean yield among 17 genotypes. So, G15 could not be recommended over the location. G16 was the second-most stable genotype with the highest mean yield performance (ranked 1st in bean mean yield among 17 genotypes). G5 was also the third most stable and high-yielding genotype (ranked 3nd in mean yield performance) (Table 5.5). According to ASV and yield performance, G16 followed by G5 could be promoted for a development variety across the location (Table 5.5).

4.3.3.2 Cultivar superiority index (Pi)

The stability and mean performance is simultaneously evaluated by cultivar superiority index (Pi). According to Lin & Binns (1988), genotypes with low Pi are the most stable. In this study, G16, having the lowest cultivar superiority value, was considered a stable genotype. This genotype was also the high-yielding and reasonably important genotype for selection. G5, G7, and G12 were also considered stable genotypes in decreasing order according to Pi. Conversely, G10, G9, and G3 showed high Pi, indicating the instability of these genotypes (Table 4.5). Many authors used this stability parameter to identify stable and high-yielding coffee genotypes across different locations (Afework, 2017; Beksisa *et al*., 2018).

4.3.3.3 Yield stability index (YSI)

Farshadfar *et al*. (2011) developed the YSI parameter. Stability should not be the only selection criterion since the genotypes with the highest levels of stability may not always produce the highest yields. The YSI measures only deviations of yields from the yield trend, but it does not say anything about the actual level of the yield or the direction of the trend (increasing or decreasing). Therefore, for decision makers, both the trend of the yields and the YSI value should be taken into account. The optimal cropping technology should have high and stable yields. As a result, approaches that incorporate both stability and mean yield into a single index are required. The genotypes with low YSI would be considered stable and high-yielding genotypes. Accordingly, G16, G5, and G6 were ranked as the top three stable genotypes in ascending order, respectively. According to the YSI result, G16 and G5 were the top yielders and moreover stable genotypes that could be recommended for selection in decreasing order. In contrast, G10, G4, G9, and G1 showed high cultivar superiority values, indicating the greatest instability of these genotypes among the tested genotypes (Table 4). Many authors also used the YIS parameter to identify stable and high-yielding coffee genotypes across different locations (Mahmodi *et al.*, 2011; Afework, 2017; Beksisa *et al.*, 2018).

Table 4. 5 Mean yield (ton ha-1), stability measurements, and their ranking orders of 17 coffee genotypes evaluated over twelve environments

Gen	Bean Yield		ASV		YSI		pi	
	Value	Rank	Value	Rank	Value	Rank	Value	Rank
G1	1.19	11	2.841	16	27	14	0.311	13
G2	1.17	12	1.922	12	24	13	0.292	11
G3	1.07	15	1.357	8	23	11	0.388	15
G4	1.14	14	2.51	14	28	16	0.287	10
G5	1.35	3	0.987	3	4	2	0.154	2
G6	1.21	9	1.076	4	13	3	0.264	9
G7	1.37	1	2.822	15	18	8	0.176	3
G8	1.14	13	1.812	10	23	13	0.309	12
G9	0.91	16	1.848	11	27	15	0.548	16
G10	0.82	17	2.237	13	30	17	0.631	17
G11	1.26	5	1.189	5	10	5	0.238	6
G12	1.28	4	1.772	9	13	4	0.204	4
G13	1.22	7	3.677	17	24	7	0.339	14
G14	1.26	6	1.25	6	12	6	0.245	7
G15	1.22	8	0.706	1	9	9	0.236	5
G16	1.37	1	0.888	2	4	1	0.139	1
G17	1.19	10	1.318	7	17	10	0.246	8

Where Gen=Genotype, ASV = AMMI stability value, YSI= Yield stability index, Wi = Wricke's ecovalence, δ^2i = Shukla's stability variance, & Pi = cultivar superiority index

4.4 Conclusion

The present study indicated the existence of highly significant variation among Arabica coffee genotypes, environments, and GEI for Arabica coffee bean yield performance tested across different environments and seasons. The existence of significant variation confirmed the presence of variability in the inherent genetic make-up among the tested coffee genotypes, differences in climatic and edaphic conditions at different testing environments, and the differential response of tested genotypes across environments for the bean yield trait. The main proportion of the total difference in bean yield was explained by environmental factors (71.74%), followed by GEI (17.52%), indicating that the environment is the most important factor influencing the coffee bean yield performance in Ethiopia. As a result, testing genotypes in various environments prior to deciding on a variety to use in a larger agroecology is an important task for coffee breeders.

The multivariate analysis methods used in this study to explore GEI effects were analysis of variance, AMMI, and GGE-biplots analysis. Each method provided essential information on interaction structuring in a different manner but was complementary, thereby making them all useful. Furthermore, using multiple models is helpful to increase the reliability of genotypes' stability potential across different environments.

According to the AMMI biplot, E7, E1, E5, E3, and E11 were identified as high-yielding and favorable environments, whereas E12, E6, E10, E9, E4, E2, and E8 were the lower-yielding and unfavorable environments. From the high-yielding environments, E3, E11, and E7 tended to have the highest contributions to GEI. Likewise, the high-yielding genotypes that produce above-average means are G7, G5, G14, G15, G16, G12, G11, and G13, which are suited to the right side of the abscissa, whereas the low-yielding genotypes are G4, G3, G10, G8, G9, and G6. Among the high-yielding genotypes, G14, G5, and G16 were found to be more stable, whereas G13 and G7 were highly unstable.

The which-won-where polygon view of the GGE-biplot was formed by connecting seven vertex genotypes, viz., G13, G12, G5, G7, G4, G10, and G9. It partitions the twelve environments into five different coffee-growing mega-environments with different winning genotypes. According to the GGE-biplot model, E1 and E5 environments were the most representative and discriminative of the genotypes, whereas E6, E12, E10, E9, and E8 environments provided little information about the genotypes differences. In this model, G5 and G16 were both high-yielding, stable genotypes and therefore considered for further breeding programs.

In general, this study showed that it could be possible to increase the yield potential of coffee genotypes under its growing conditions either by using more widely adaptable coffee types or location-specific high-yielding genotypes under favorable environmental conditions. Despite the existence of high GEI, genotype (AW7705) was the highest bean yielder in tons per hectare and the stable genotype among all the tested genotypes by most of the stability measures used. Therefore, AW7705 was discovered to be a promising candidate, which can be considered desirable genotypes in further tests, to releasing a new variety for Arabica coffee growing regions of southern Ethiopia and other similar agro-ecologies elsewhere. Among the check varieties, Angafa (ranked the third highest yielder) with reasonably good stability was found to be the best check variety, whereas 74112 was the least yielder (ranked seventh out of seventeen tested genotypes) and was also highly oscillating (unstable). Accordingly, south Ethiopian coffee growers need to watch for other varieties in place of 74112 for cultivation as well as coffee breeders for comparison. Hence, the environment and GEI explained a large amount of variation for coffee bean yield; testing genotypes across diverse environments prior to deciding on any variety to use under wider agro-ecology is recommended for coffee breeders to identify high-yielding and stable improved coffee varieties to boost production and productivity of coffee in Ethiopia.

4.5 Reference

Abrha, G. W., Kebede, S. A., Bedada, L. T., Berecha Yadessa, G., & Adugna Gutu, A. (2022). Genotype by Environment Interaction and Yield Stability of Coffee (Coffea arabica L.) Genotypes Evaluated in Western Ethiopia. Plant Production Science, 25(4), 467–483. https://doi.org/10.1080/1343943X.2022.2136722

Afework Legesa, 2017. Genotype x Environment Interaction and Stability Analysis of Some Promising Ilu Ababora Coffee (Coffea arabica L.) genotypes for yield and yield related traits in Southwestern Ethiopia. An M.Sc. Thesis submitted to Jimma University department of horticulture and plant science, Ethiopia.

Annicchiarico, P. (2002). Genotype x Environment Interactions: Challenges and Opportunities for Plant Breeding and Cultivar Recommendations. FAO Plant Production and Protection Paper 174.

Argaw, T., & Taye, G. (2018). Genotype by Environment Interaction Analysis of Arabica Coffee Bean Yield. 6(October), 509–519. https://doi.org/10.14662/ARJASR2018.090

Asefa, G. (2022). GGE Biplot Analysis of Genotype by Environment Interaction and Yield Stability Analysis of Arabica Coffee (Coffea arabica l .) Diallel Genotypes in South Western Ethiopia. GSJ,,ISSN 2320-9186, 10(3), 1082–1100.

Ayele, A., Worku, M., & Bekele, Y. (2021). Heliyon Trend , instability and decomposition analysis of coffee production in Ethiopia (1993 – 2019). Heliyon, 7(September), e08022. https://doi.org/10.1016/j.heliyon.2021.e08022

Bacsi, Z. (2019). A YIELD STABILITY INDEX AND ITS APPLICATION FOR CROP. 13(1), 11–20. https://doi.org/10.14232/analecta.2019.1.11-20

Bacsi, Z., & Fekete-farkas, M. and I. M. M. . (2022). Coffee Yield Stability as a Factor of Food Security. Foods, 19. https://doi.org/10.3390/%0Afoods11193036

Beksisa, L. (2021). GGE Biplot Analysis of Genotype x Environment Interaction and Bean Yield Stability of Arabica Coffee (Coffee arabica L .) Genotypes in Southwestern Ethiopia. 9(3), 110–115. https://doi.org/10.11648/j.ajbio.20210903.16

Beksisa, L., Alamerew, S., Ayano, A., & Daba, G. (2018). Genotype environment interaction and yield stability of Arabica coffee (Coffea arabica L.) genotypes. African Journal of Agricultural Research, 13(4), 210-219. https://doi.org/10.5897/AJAR2017.12788

Cheng, Y.-L., Lee, C.-Y., Huang, Y.-L., Buckner, C. A., Lafrenie, R. M., Dénommée, J. A., Caswell, J. M., Want, D. A., Gan, G. G., Leong, Y. C., Bee, P. C., Chin, E., Teh, A. K. H., Picco, S., Villegas, L., Tonelli, F., Merlo, M., Rigau, J., Diaz, D., …

Mathijssen, R. H. J. (2018). Genotype × Environment Interaction: A Prerequisite for Tomato Variety Development. Intech, 11(tourism), 23. http://dx.doi.org/10.5772/intechopen.76011

Cheserek, J. J., Ngugi, K., Muthomi, J. W., Omondi, C. O., & Ezekiel, K. N. (2021). Green bean biochemical attributes of Arabusta coffee hybrids from Kenya using HPLC and soxhlet extraction methods. Australian Journal of Crop Science, 15(2), 201–208. https://doi.org/10.21475/ajcs.21.15.02.p2581

Crossa, J., Hg, G., Jr, & Zobel, R. W. (1990). Additive main effects and multiplicative interaction analysis of two international maize cultivar trials. Crop Science, 30(3), 493–500. https://doi.org/10.2135/cropsci1990.0011183X003000030003x

Demissie M., M. K. and G. T. (2011). Additive main effects and multiplicative interaction analysis of coffee germplasms from southern Ethiopia. Ethiop. J. Sci., ISSN: 0379–2897; 34(1):63–70

Egea-Gilabert, C., Pagnotta, M. A., & Tripodi, P. (2021). Genotype × environment interactions in crop breeding. Agronomy, 11(8), 2–5. https://doi.org/10.3390/agronomy11081644

EIAR (Ethiopian Institute of Agricultural Research), 2017. National Coffee Commodity Research Strategy of Fifteen Years (2016 -2030). Addis Ababa, Ethiopia.

Endale, T., T. Kufa, A. Nestre, T. Shimber, A. Yilma and T. Ayano, 2008. Research on coffee field management. pp.187-195. In: Girma Adugna, Bayetta Belachew, Tesfaye Shimber, Endale Taye and Taye Kufa (eds.). Proceedings of the workshop on four decades of coffee research and development in Ethiopia: A National workshop, 14-17 August 2007, Addis Ababa, Ethiopia.

Enyew M, FeyissaT, GeletaM, TesfayeK,Hammenhag C, CarlssonAS (2021). Genotype byenvironment interaction, correlation, AMMI, GGEbiplot and cluster analysis for grain yield and other agronomic traits in sorghum(Sorghum bicolor L.Moench).PLoSONE 16(10):e0258211. ttps://doi.org/10.1371/journal.pone.0258211

Falconer, D. S., & Mackay, T. F. C. (1996). Introduction to Quantitative Genetics (4th ed.). Longman.

FAO (Food and Agricultural Organization of United Nations) publications catalogue, (2021). FAO office of communications. publications catalogue 2021. Retrieved from https://doi.org/10.4060/cb4402en

Farshadfar, E., Mahmodi, N., & Yaghotipoor, A. (2011). AMMI stability value and simultaneous estimation of yield and yield stability in bread wheat (Triticum aestivum L.). Australian Journal of Crop Science, 5(13), 1837–1844.

Farshadfar, E., Safari, H., Jamshidi, B. (2012). GGE biplot analysis of adaptation in wheat substitution lines. International Journal of Agriculture and Crop Science, 4(13): 877–881.

Gauch H.G. (1992): Statistical analysis of regional yield trials: AMMI analysis of factorial designs. Department of Soil, Crop & Atmospheric Sciences, College of Agriculture & Life Sciences, Cornell University, Ithaca, NY, USA. Elsevier Science Publishers, Netherlands

Gomez, K. A., & Gomez, A. A. (1984). Statistical Procedures for Agricultural Research (2nd ed.). New York: John Wiley & Sons.

Hailemariam, M., & Tesfaye, A. (2019). Genotype X Environment Interaction by AMMI and GGE-Biplot Stability Analysis in Grain Yield for Soybean [(Glycine Max L .) Merrill] in Ethiopia. 5(4), 10–21. DOI: http://dx.doi.org/10.20431/2454-9487.0504002

Hongyu K., Silva F.L., Oliveira A.C.S., Sarti D.A, Araújo L.C., Dias C.T.S. (2015): Comparação entre os modelos AMMI e GGE Biplot para os dados de ensaios multi-ambientais. Rev. Bras. Biom., São Paulo 33(2): 139-155

ISO (International Coffee Organization), (2021). Coffee market report. https://www.ico.org/news/cmr-0121-e.pdf

Khan, M. M. H., Rafii, M. Y., Ramlee, S. I., Jusoh, M., & Al Mamun, M. (2021). AMMI and GGE biplot analysis for yield performance and stability assessment of selected Bambara groundnut (Vigna subterranea L. Verdc.) genotypes under the multi-environmental trails (METs). Scientific Reports, 11(1), 1–17. https://doi.org/10.1038/s41598-021-01411-2

Legesse, A., Alamrew, S., & Tesfaye, A. (2022). Stability Analysis of Coffee (Coffea arabica L .) Bean Yield Using GGE Biplot in South Western Ethiopia. 8(1), 36–42. https://doi.org/10.11648/j.ajbes.20220801.15

Lin, C. S., & Binns, M. R. (1988). A superiority measure of cultivar performance for cultivar× location data. Canadian journal of plant science, 68(1), 193-198 https://doi.org/10.4141/cjps88-018

Luthra O. P. & Singh R. K., (1974). A comparison of different stability models in wheat. Theoret. Appl. Gen. 45: 143–149.

Mahmodi, N., Yaghotipoor, A., & Farshadfar, E. (2011). AMMI stability value and simultaneous estimation of yield and yield stability in bread wheat ('Triticum aestivum'L.). Australian Journal of Crop Science, 5(13), 1837-1844

Marie, L., Abdallah, C., Campa, C., Courtel, P., Bordeaux, M., Navarini, L., Lonzarich, V., Bosselmann, A. S., Turreira-García, N., Alpizar, E., Georget, F., Breitler, J. C., Etienne, H., & Bertrand, B. (2020). G × E interactions on yield and quality in Coffea arabica: new F1 hybrids outperform American cultivars. Euphytica, 216(5). https://doi.org/10.1007/s10681-020-02608-8

Merga, W. (2021). An overview of genotype x environment interaction and yield stability analysis in applied plant breeding: great emphasis given to coffee (Coffea arabica L.). 11(2)(2224–0616), 117–123. https://www.banglajol.info/index.php/IJARIT

Merga, W. (2022). Bean ield stability analysis of coffee (Coffea arabica L) genotypes in southwestern Ehiopia. Ternational Journal of Recent Scientific Research, 13(ISSN: 0976-3031), 1–8. https://doi.org/10.24327/IJRSR

Miranda G.V., Souza L.V., Guimarães L.J.M., Namorato H., Oliveira L.R., Soares M.O. (2009): Multivariate analyses of genotype x environment interaction of popcorn. Pesq. agropec. bras., Brasília 44(1): 45-50

Motebayenore, E. (2022). Review on Coffee Production Constraints and Opportunities in Ethiopia. Journal of Horticulture, 9(1), 1–7.

Ndikumana, J. (2022). Agro-morphological characterization of arabica coffee cultivars in burundi. Thesis Submitted to Graduate Studies of Jomo kenyatta university, Nirobi, Keniya.

Neisse, A. C., & Kirch, J. L. (2018). AMMI and GGE Biplot for genotype × environment interaction : a medoid – based hierarchical cluster analysis approach for high – dimensional data. 55(2), 97–121. https://doi.org/10.2478/bile-2018-0008

Purchase, J.L. (1997). Parametric analysis to describe genotype x environment interaction and yield stability in winter wheat. PhD. Thesis. University of the Orange Free State.

Tadesse H, Abay F. (2011). Additive main effects and multiplicative interactions analysis of yield performance of sesame genotypes across environments in Northern Ethiopia. J Drylands.;4:259–266.

Tadesse, T., Tesfaye, B., & Abera, G. (2020). Coffee production constraints and opportunities at major growing districts of southern Ethiopia Coffee production constraints and opportunities at major growing districts of southern Ethiopia. Cogent Food & Agriculture, 6(1). https://doi.org/10.1080/23311932.2020.1741982

USDA (The United States Department of Agriculture), (2023). Ethiopia Coffee Annual Report, GAIN Report Number: ET2023-0014 (2023), 1-7, https://apps.fas.usda.gov.

WEF (World Economic Forum), (2021). Which country produced the most coffee in 2020? retrieved on March 20, 2023 and available at https://www.weforum.org/agenda/2021/10/which-country-produced-the-most-coffee-in-2020/

Yan W, Hunt LA, Sheng Q, *et al*., (2000). Cultivar evaluation and mega-environment investigation based on the GGE biplot. Crop Sci.;40(3):597–605.

Yan W, Rajcan I. (2002). Biplot Analysis of Test Sites and Trait Relations of Soybean in Ontario. Crop Sci. Jan;42(1):11-20. doi: 10.2135/cropsci2002.1100. PMID: 11756248.

Yan W., Hunt L.A., Sheng Q., Szlavnics Z. (2000): Cultivar evaluation and mega-environment investigation based on the GGE biplot. Crop Science 40(3) :597-605.

Yan W., Kang M.S. (2003): GGE Biplot Analysis: A Graphical Tool for Breeders,Geneticists, and Agronomists. CRC Press, Boca Raton, FL, USA, 271p.

Yan W., Kang M.S., Ma B., Woods S., Cornelius P.L. (2007): GGE biplot vs.AMMI analysis of genotype-by-environment data. Crop Sci. 47 : 643-655.

Yan, W. and Hunt, L.A. (2001) Interpretation of Genotype x Environment Interaction for Winter Wheat Yield in Ontario. Crop Science, 41, 19-25. https://doi.org/10.2135/cropsci2001.41119x

Yan, Y. 2003. Automatic Qualitative Model Abstraction from Numerica Simulation Model. In Proceedings of the Fourteenth International Workshop on Principles of Diagnosis DX'03, 199-206, Washington DC (USA).

Yilma Yemane Teferaa, 2017. A guide to coffee production in Ethiopia. Addis Ababa Ethiopia. P 1-280.

Zenebe M, Hussien M. (2009). Study on genotype environment interaction of oil content in sesame (Sesamum indicum L.). Middle East J Sci Res. 2009;4(2):100–104. Zobel RW, Wright MJ, Gauch HG. Statistical analysis of a yield trial. Agron J. 1988;80(3):388–393.

CHAPTER FIVE

5. Evaluation of Physical and Chemical Characteristics of Bean and Cup Quality of Arabica Coffee Genotypes Grown in Southern Ethiopia

Abstract

The study aimed to investigate physical characteristics, cup quality, and biochemical content variability among thirty South Ethiopian Arabica coffee genotypes over three locations. The results revealed the existence of statistically significant variation among genotype, location, and GEI effects for all studied traits. The overall coffee quality score for most of the tested genotypes in three locations was above 80%. Therefore, in terms of quality, most of the tested genotypes can be used to produce specialty coffee in the study areas. A wider range of caffeine (0.52% dwb to 1.53% dwb) was recorded among the studied genotypes. Accordingly, the low caffeine contenting genotypes could be a promising candidate for the development of low caffeine varieties through selection and hybridization. Cluster analysis grouped genotypes into different clusters based on quality trait variation and similarity among genotypes. According to the PCA, caffeine content (0.35), chlorogenic acid (0.34), aromatic quality (0.31), trigonelline (0.29), acidity (0.28), astringency (0.28), color (0.27) in the first PCA, flavor (-0.48), and screen size (0.46) in the second PCA were the important variables contributing more to the variation, and these traits could be considered for effective parent selection in quality improvement programs. Genotype AW9648 achieved the highest score in overall quality attributes at all three locations and could be promoted as a promising candidate and best parent for hybridization in terms of quality. Hence, genotype by environment interaction was significant, the coffee quality improvement program should give due attention to incorporating genetic and environmental influences by using a multi-locational selection strategy.

Key words: Coffee quality, genotypic variability, growing environment

5.1 Introduction

Coffee is the second most traded commodity and the most beloved non-alcoholic beverage in the world (FAO, 2021). It is important in the lives of billions of people on the globe by giving pleasure and satisfaction to the consumer through flavor, aroma, and desirable physiological and psychological effects (Samoggia and Riedel, 2019). Although coffee is primarily consumed for its pleasant flavor and stimulating properties, recent investigations assert the health benefits of the beverage due to the presence of caffeine, trigonelline, and chlorogenic acids (Depaula and Farah, 2019; Zarebska *et al*., 2022).

Coffee is a well-known pillar of the Ethiopian economy, which accounts for 34% of its export earnings (USDA, 2019). Growing, processing, trading, transporting, as well as marketing of coffee provide livelihood to about 25 million people directly or indirectly (Yirga *et al.,* 2021). Coffee is the defining feature and sphere of Ethiopian culture, politics, economy, social life, and identity, with about 55% of the production consumed domestically (USDA, 2021). The cultural ceremony of Ethiopian coffee beverage preparation and drinking is unique and stunning.

Ethiopia is the origin and center of genetic diversity for Arabica coffee. The country has collected over 12,452 accessions from various coffee-growing ecology and maintained them in in-situ and ex-situ gene banks (Yirga *et al.,* 2021). Different studies witnessed the existence of abundant genetic diversity among Arabica coffee genotypes (Dawit *et al., 2018;* Bor´em *et al*., 2019; Fonseca *et al*., 2019; Adem *et al*., 2020; Dalazen *et al*., 2020; Marie, *et al*., 2020; Mengistu *et al*., 2020; Merga *et al*., 2021; Merga *et al*., 2022; Ndikumana, 2022). Ethiopia has an ideal and suitable environment for the production of coffee in both quality and quantity (CSA, 2015). Because of the suitable altitude, abundant rainfall, ideal temperature, fertile soil, and sufficient labor, the potential for coffee production is very high (Tadesse, 2020).

Despite the importance of coffee in Ethiopia’s economy and the presence of ample genetic resources, favorable climate and soil types, cup and physical quality as well as biochemical evaluation is not yet done for most of the collections maintained in the gene bank including the south Ethiopian collection (EIAR, 2017; Benti, 2017). Therefore, an assessment of Ethiopian Arabica coffee genetic resources for quality have to be done for the development of superior-quality cultivars by exploiting the existing resources.

The diverse genetic basis for Arabica coffee and a wide range of ecological conditions in Ethiopia prevail for the production of different quality types. Ethiopian coffee is known for its unique characteristics including Sidama, Yirga Chefe, Harerige, Limu, Keffa, Gimbi, Amaro, Gujji, and Jinka coffee types. The country produces specialty coffee with a wide range of aromas and characteristic flavors. Specialty coffee currently accounts for 20% of Ethiopia's coffee exports, and there is enormous potential to increase its share of the global market (Tolessa, *et al.,* 2017). The country is privileged by a strong potential and opportunity to boost the specialty coffee market by producing the finest specialty coffee ever.

Caffeine, trigonelline, and chlorogenic acids are some of the coffee biochemical compounds that have been used to characterize coffee genotypes (Tolessa *et al., 2016).* Different studies noted a significant variation in the caffeine, chlorogenic acids, and trigonelline contents of Arabica coffee (Vignoli *et al 2014;* Sualeh *et al.,* 2020). Coffee's biochemical components affect the organoleptic characteristics that contribute to cup quality, which is a major factor in determining its market value and use (Woelore, 1993). As a result, the assessment of biochemical components in Arabica coffee genotypes is critical in the development of the best quality cultivar.

Green bean physical character and beverage quality assessment by a professional coffee cupper is crucial to examine the coffee quality and identify characteristic variations of the genotype. In the Ethiopian coffee research program, a coffee-cupping protocol was developed for the evaluation of *C. arabica* genotypes with specific evaluation criteria for the physical characteristics of green beans and cup quality (Woelore, 1993). The main bean physical characteristics are bean odor, color, and shape, whereas cup quality attributes are flavor, acidity, body, aromatic quality, aromatic intensity, astringency, bitterness, and overall cup quality. To evaluate Arabica coffee genotypes physical character and beverage quality across different growing locations, the current study was conducted according to Ethiopian coffee coffee-cupping protocol (Gichimu *et al.,* 2014).

One of Ethiopia's specialty coffee-producing areas is the south Ethiopian growing ecology, which contributes to more than 36% of the national market for coffee production Milligan and Cooper, 1998). South Ethiopian coffee, with its unique and internationally branded spicy and floral flavor, is one of the main commercially available coffees among Ethiopian specialty coffees. Awada Agricultural Research Sub-Center, in collaboration with the Jimma Agricultural Center Coffee Research Team, collected over 700 coffee accessions from the southern Ethiopian regions (Sidama, Gedeo, Amaro, Gamo Gofa, Jinka, and Gujji). These accessions are being evaluated and characterized, and 27 promising selections have been promoted for variety development. Through preliminary evaluation carried out at the Awada sub-center, these promising selections were promoted for their high yield potential, resistance to coffee berry disease, and resistance to coffee leaf rust. Even though these promising selections have been evaluated for disease resistance and yield performance, their cup and physical quality, as well as biochemical evaluation, are not yet done. In addition to this, the genotypic stability and wider adaptability in the different growing environments were not carried out. Therefore, this study was conducted with the objective of investigating the variation in green bean physical characteristics, cup quality, and biochemical content among 30 south Ethiopian coffee genotypes; assessing the effect of environment on green bean physical characteristics, cup quality, and biochemical content; and identifying the best quality coffee genotypes for the southern Ethiopian growing environment.

5.2 Materials and Methods

5.2.1 Experimental Sites

The field experiments were established at three research sites, namely Awada Agricultural Research Sub-Center (AARSC) ($6^0$45'46"N, $38^0$22'36"E, and 1740 masl); Shebedino trial field ($6^0$50'48"N, $38^0$27'06"E, and 1845 masl), and Wonago substation ($6^0$17'54"N, $38^0$13'05"E, and 1945 masl). All three sites are found in the south Ethiopian specialty coffee growing agro-ecology.

5.2.2 Experimental Materials

A total of 30 Arabica coffee genotypes, comprising twenty-seven promising selections and three standard check varieties, were used for this experiment (Table 1). The promising selections were promoted from different south Ethiopian coffee collection batches for their resistance to coffee berry disease (CBD), resistance to coffee leaf rust (CLR), and high yield performance during a preliminary evaluation carried out at the Awada agricultural research sub-center (Table 1).

Table 5. 1 **List of *C. arabica* genotypes evaluated for green bean physical characters, cup quality, and biochemical composition**

Serial No.	Genotypes	Source of collection
1	AW1777	South Ethiopian collection
2	AW4994	South Ethiopian collection
3	AW5994	South Ethiopian collection
4	AW7494	South Ethiopian collection
5	AW1995	South Ethiopian collection
6	AW9641	South Ethiopian collection
7	AW9644	South Ethiopian collection
8	AW9662	South Ethiopian collection
9	AW9622	South Ethiopian collection
10	AW9623	South Ethiopian collection
11	AW9628	South Ethiopian collection
12	AW7705	Sidama Collection
13	AW105	Sidama Collection
14	AW3106	Sidama Collection
15	AW4083	South Ethiopian collection
16	AW695	South Ethiopian collection
17	AW9610	South Ethiopian collection
18	AW9611	South Ethiopian collection
19	AW9617	South Ethiopian collection
20	AW9640	South Ethiopian collection
21	AW9648	South Ethiopian collection
22	AW9658	South Ethiopian collection
23	AW9660	South Ethiopian collection
24	AW4305	Sidama Collection
25	AW8105	Sidama Collection
26	AW12305	Sidama Collection
27	AW8806	Sidama Collection
28	Feyate	Standard check variety
29	Angafa	Standard check variety
30	74112	Standard check variety

5.2.3 Experimental Design

Thirty genotypes (27 selections and 3 standard check varieties) were planted in August 2015 in a population of 8 trees per plot with 3 replications and 2 meter by 2 meter spacing per location by using a randomized complete block design (Gomez and Gomez 1984). All agronomic management practices were applied as per the recommendations per location.

The laboratory experiment was arranged in a completely randomized design (CRD) with three replications per location (Gomez and Gomez 1984).

5.2.4 Sample Preparation

During peak harvesting time in October 2021, about eight kilograms of healthy and fully matured red-ripened coffee cherries were handpicked from the 30 genotypes per 3 replication per each three locations. Therefore, 90 samples were prepared per location. Prior to processing, over-mature and immature cherries and foreign materials were sorted out from all samples.

The samples were pulped on the day of harvest using a single-disc manual pulper to separate beans from the skin and pulp. The wet parchment coffee was left in the fermentation tank for 40 hours to facilitate the breakdown of mucilage. After fermentation, the parchment coffee was properly washed and under gone further 24 hours of soaking and washing. The fermentation process was done according to Woelore, (1993) fermentation protocols for Arabica coffee under Ethiopian conditions (Sualeh, *et al*., 2020). Following fermentation, the samples were placed on a raised mesh wire under the sun for drying. During drying, the amount of moisture in the parchment coffee was measured using a moisture tester H-E50 to keep the moisture level consistent at 10.5–11.0% for all samples. About 500-gram samples of green coffee beans per treatment were prepared and leveled for evaluation of physical character, cup quality, and biochemical content.

5.2.5 Bean physical character assessment

Three Q-grade certified and experienced cuppers assessed cup quality and bean physical character in the Ethiopian commodity exchange (ECX) laboratory at the Hawassa branch (Apendix 2 and 3). All three professional and certified Q-grade coffee cuppers are permanent employees of the ECX authority at the Hawassa branch. The cup quality and physical character of the bean were assessed using Sualeh and Mekonnen's coffee-cupping protocol (Sualeh and Mekonnen, 2015).

About 300g of green bean sample from each of the 270 experimental units (30 genotypes x 3 locations x 3 replications) was used for assessment of screen size, shape and make, color, and odor according to Sualeh and Mekonnen's coffee-cupping protocol (Sualeh and Mekonnen, 2015) based on scale values given in Table 2. Screen size determination was carried out using a rounded and perforated screen plate. The percentage of coffee beans retained above the screen-size holes of 14 (1/64 inch of 14) was recorded. The shape and make of coffee samples were evaluated based on 1–5 scales as very good, good, fair good, average, mixed, and small. Color was evaluated out of 15 using a 1–6 scale as bluish, grayish, greenish, coated, faded, and white. Odor was evaluated out of 10 using a 1–5 scale as clean, fair clean, trace, light, light, moderate, and strong (Table 2).

5.2.6 Cup quality assessment

About 100 grams of green coffee beans were prepared for each of the 270 samples (30 genotypes with 3 replications at each of the 3 locations). The roaster machine probatBRZ6 was heated first to about 200°C. Coffee samples were roasted medium roast (eight minutes on average) and tipped out into the cooling tray. Cold air was blown through the roasted coffee to produce rapid cooling and then stored in an airtight glass jar for about 12 hours before grinding. The loose silver skins were removed with a blaster before grinding. The samples were ground using a Mahlkoing electrical grinder to medium sized grounds, placed in ceramic cups, and covered. Eight grams of coffee powder were put into each cup, which has 180 ml of capacity (Sualeh and Mekonnen, 2015). Then, boiling water was poured onto the ground coffee up to about halfway in the cup. Soon after, the volatile aromatic quality and intensity parameters were recorded using sniffing. Then, the content of the cup was stirred to ensure an infusion of all coffee grounds. The cup was then completely filled with boiled water at about 92^0c (Sualeh and Mekonnen, 2015). The brew was made and ready for panelists within 8 minutes at the ECX Laboratory Hawassa branch.

Cup quality analysis was carried out once the beverage cooled to around 60°C (drinkable temperature). Three cups per sample per cupper were prepared for the tasting session. The coffee type and the replicates were arranged at random. Cup quality attributes such as aromatic quality, aromatic intensity, astringency, and bitterness were scored on a scale of 0 to 5, and acidity, body, flavor, and overall cup quality were scored on a scale of 0 to 10 (Sualeh and Mekonnen, 2015), as shown in Table 2. Each experienced and certified Q-grade cuppers gave their independent judgment for each sample.

Table 5. 2 Form completed by sensory evaluator for green bean character and cup quality

No	Characters	Scale Value Description
	Cup quality Characteristics	
1	Aromatic Quality (5)	5 (Excellent), 4 (V. good), 3 (Good), 2 (Regular), 1(Bad), 0(Nil)
2	Aromatic Intensity (5)	5 (Very strong), 4 (Strong), 3 (Medium), 2 (Light), 1 (Very Light), 0 (Nil)
3	Acidity (10)	10 (Pointed), 8 (Medium pointed), 6 (Medium), 4 (Light), 1 (Lacking), 0 (Nil)
4	Astringency (5)	5 (Nil), 4 (Very light), 3 (Light), 2 (Medium), 1 (Strong), 0 (Very Strong)
5	Bitterness (5)	5 (Nil), 4 (Very light), 3 (Light), 2 (Medium), 1 (Strong), 0 (Very Strong)
6	Body (10)	10 (Full), 8 (Medium full), 6 (Medium), 4 (Light), 2 (Very Light), 0 (Nil)
7	Flavour (10)	10 (V. good), 8 (Good), 6 (Average), 4 (Fair), 2 (Bad), 0 (Nil)
8	Overall cup Quality (10)	10 (Excellent), 8 (V. good), 6 (Good), 4 (Regular), 2 (Bad), 0 (Unacceptable)
	Green Bean Physical Characteristics	
1	Shape and Make (15%)	15 (V. good), 12 (Good), 10 (Fair), 8 (Average), 6 (Mixed) 4 (Small)
2	Color (15%)	15 (Bluish), 12 (Greyish), 10 (Greenish), 8 (Coated), 6 (Faded) 4 (White)
3	Oder (10)	10 (Clean), 8(Fair clean), 6 (Trace), 4(Light), 2(Light moderate), 0 (Strong)

Source: Sualeh and Mekonnen, (2015)

5.2.7 Caffeine, trigonelline and chlorogenic acid analysis

Analysis of caffeine, trigonelline, and chlorogenic acid was performed in the food science and nutrition laboratory of the Ethiopian Institute of Agricultural Research (EIAR). Caffeine, trigonelline, and chlorogenic acid contents were analyzed as described by Vignoli *et al.* (2014). Ground green coffee beans (0.5 g) were subjected to direct solvent extraction with 50 mL of distilled water (hot water, 95°C) and stirred for 20 min on a hot plate. Then, the solvent extract was filtered through No. 4 Whatman filter followed by a 0.45 µm PTFE filter prior to injection into the HPLC having a prevail C18 (250×4.6 mm, i.d. 5 µm, 25°C) column. The mobile phase was composed of 5% acetic acid in water (v/v) (solvent A) and acetonitrile (solvent B). The injection volume was 10 uL, and the flow rate was 0.5 ml min^{-1}. Caffeine and trigonelline were detected by a UV detector at 280 and 320 nm wavelength respectively, whereas total chlorogenic acid was detected at 320 nm. Calibration curves of caffeine, trigonelline, and chlorogenic acid standards were used for the quantification of those compounds. Caffeine, trigonelline, and chlorogenic acid were identified by comparing the retention times of the caffeine standard, trigonelline standard, and chlorogenic acid standard and their concentrations calculated from peak areas using calibration equations. Calibration curves were made using the standard concentration and area of the sample, which were subsequently used to calculate the composition of the respective biochemical component using the area generated after a retention time (Gichimu *et al.*, 2015).

5.2.8 Data Analysis

The data were subjected to analysis of variance (ANOVA) for each location separately using the Statistical Analysis System (SAS 9.4 version statistical package) to examine the presence of statistically significant differences among genotypes in their performance for bean physical characteristics, cup quality, and biochemical composition traits. Combined analyses were performed to determine genotypic effects, location effects, and genotype by environment interaction effects. Levene's test was used to test the homogeneity of variances between environments to determine the validity of the combined ANOVA on the data. The mean separation was carried out using Turkey test at a 5% level of significance. Principal component analysis (PCA) was done using SAS software to determine the relative importance of the traits responsible for variation among the sensory attributes and chemical composition. Coffee genotypes were grouped by the ward technique of clustering, which groups and arranges genotypes into clusters to generate a dendrogram using SAS software. The number of clusters was decided by following the approach suggested by Milligan and Cupper (1998) by the cubic clustering criteria (ccc), pseudo F statistics combined with values of pseudo t^2 statistics for the next cluster fusion.

5.3 Results and Discussion

5.3.1 Variability of quality characters among coffee genotypes

A separate ANOVA revealed significant differences ($p<0.05$ & $p<0.05$) among genotypes in all locations for all the studied characters (Table 3). The significant difference indicates the presence of a considerable amount of variation for the green bean physical, cup quality, and biochemical composition traits among the studied Arabica coffee genotypes, and this variation could be exploited to bring coffee quality improvement through selection and hybridization. Many studies also reported the existence of statistically significant variation among Arabica coffee genotypes for different traits at different times (Yigzow, 2005; Sualeh *et al*., 2014; Abdulfeta, 2018; Dawit, 2018; Desalegn, 2018; Tolessa *et al*., 2019; Adem *et al*., 2020; Mengistu *et al*., 2020; Merga *et al*., 2021; Cheserek *et al*., 2022 and Ndikumana, 2022). The existence of significant variation among the traits indicated the presence of a considerable amount of variation among the studied Arabica coffee genotypes, and this variation could be exploited to improve coffee quality through selection and hybridization.

A combined ANOVA revealed significant ($p<0.01$ & $p<0.05$) variation among the main effects (genotypes and environment) for all characteristics except bitterness. Likewise, significant genotype by environment interaction (GEI) was also observed for the studied traits except for bitterness (Table 3). The presence of significant GEI indicates the inconsistency in the performance of the genotypes for green bean physical attributes, cup quality traits, and biochemical composition across environments. This suggested that one genotype's phenotypic expression might be superior to another genotype in one environment but inferior in another environment. Therefore, the coffee quality improvement program should give due attention to incorporating genetic and environmental influences into the development of market-competent specialty coffee varieties. In line with this result, different researchers reported differential performance of genotypes when grown in different environments on quality-related traits in Arabica coffee (Cheng *et al*., 2016; Redi *et al*., 2018; Borém *et al*., 2019; Fonseca *et al*., 2019; Dalazen *et al*., 2020; Marie *et al*., 2020; Mengistu *et al*., 2020; Nugroho *et al*., 2020; Merga *et al*., 2021).

Table 5.3 Analysis of variance (mean squares) for the 18 characters of 30 Arabica coffee genotypes grown at Awada, Shebedino and Wonago

Traits	Awada			Shebedino			Wonago			Combined				
	Gen (df=29)	Error (df=58)	CV	Gen (29)	Error (df=58)	CV	Gen (df=29)	Error (df=58)	CV	Gen (df=29)	Loc (df=2)	Gen*Loc (df=58)	Error (df=178)	CV
SS	4.98**	0.42	0.67	2.55**	0.35	0.61	7.09**	0.84	0.95	9.66**	6.72**	2.48**	0.55	0.8
SM	2.76**	0.35	4.8	3.50**	0.19	3.5	1.46**	0.04	1.68	3.79**	0.55*	0.84**	0.19	3.5
Col	3.80**	0.89	8.56	5.75**	1.80	11.59	1.69**	0.48	6.19	7.39**	7.45**	1.92**	1.07	9.2
Od	2.37**	0.09	3.19	1.53**	0.40	6.69	2.13**	0.04	0.44	1.55**	6.32**	1.39**	0.18	4.4
RwT	13.53**	1.20	3.35	14.00**	2.05	4.28	5.19**	0.49	2.09	22.57**	17.91**	5.07**	1.25	3.4
AQ	0.99**	0.15	9.63	0.34*	0.15	10.1	0.32*	0.14	9.48	0.76**	1.25*	0.44**	0.16	10
AI	0.75**	0.21	11.58	0.28**	0.05	5.78	0.52**	0.2	11.79	0.95**	0.71*	0.29**	0.17	11
Ac	0.59**	0.16	4.88	2.09**	0.17	5.01	1.07*	0.76	10.53	0.86*	2.02**	0.87**	0.38	7.3
AS	0.29*	0.19	9.94	0.32*	0.17	9.26	0.29*	0.08	10.65	0.51*	0.21^{ns}	0.35*	0.22	11
Bit	0.59*	0.25	12.15	0.18*	0.10	7.59	0.68*	0.41	15.27	1.00**	0.41^{ns}	0.22^{ns}	0.26	12
Bod	0.17**	0.03	2.08	0.11**	0.04	2.62	0.56**	0.04	2.6	0.69**	0.07^{ns}	0.08**	0.04	2.4
Flav	1.32*	0.55	12.72	1.14**	0.23	6.03	1.98**	0.73	10.74	1.80**	0.90^{ns}	1.32**	0.66	10
OQL	0.47*	0.14	7.22	0.50**	0.13	4.64	1.75**	0.51	8.95	1.44**	1.40*	0.64**	0.33	7.2
TCQ	16.59**	4.00	4.08	1.23**	13.16	2.3	29.14**	4.69	4.45	35.98**	9.12^{ns}	11.46**	3.33	3.8
TRC	40.63**	5.16	2.78	40.49**	2.74	2.02	46.02**	4.4	2.55	100.44**	4.14^{ns}	13.35**	4.16	2.5
Tri	0.14**	0.002	1.43	0.04**	0.003	8.73	0.03**	0.001	3.4	0.06**	4.44**	0.07**	0.001	4.8
Chl	1.17**	0.002	1.13	1.08**	0.004	1.49	1.08**	0.003	1.1	0.77**	11.56**	1.28**	0.003	1.2
Caf	2.07**	0.001	2.07	0.07**	0.001	1.17	0.06**	0.000	2.29	0.10**	0.13**	0.07**	0.002	4.7

Where: Gen=Genotype, Loc=Location, Gen*Loc=Genotype by environment interaction, df=degree of freedom, *& ** = Significant at P<0.05 and P<0.01 respectively, ns=non-significant, CV (%) =coefficient of variation in percent; Numbers in parenthesis stands for the degree of freedom, SS=screen size, SM=shape & make, Col=color, od=odour, RawT=raw total, AQ=aromatic quality, AI=aromatic intensity, Aci=acidity, AS=astringency, Bit=Bitterness, Bod=body, Fla=flavor, OQL=overall quality, TCQ=Total Cup Quality, TRC=Total Raw and Cup quality, Tri=Trigonelline Chl=Chlorogenic acid, Caf=Caffeine.

5.3.2 Green Bean Physical Characteristics

Green Bean Screen Size

The main effects as well as the interaction of genotype and location revealed highly significant (p<0.01) variation for green bean screen size (Table 3). Bold and large bean sizes were recorded for Angafa (99%) grown at Awada; Angafa, Feyate, AW105, AW9622, AW1995, AW7705, AW4994, AW5994, and AW9610 (98%) all grown at Wonago; and Angafa (98.22%) grown at Shebedino. In line with this study, a wider variation in screen size for the Arabica coffee genotype was reported: 98.09–90.33% (Mengistu *et al*., 2020), 99.13–91.10 (Sualeh *et al*., 2014), 98.50–96.03 (Tesfa, 2019), and 98.75–96 (Tesfa *et al*., 2021).

Bean size is an important trait for roasters since the homogeneity of the coffee beans avoids the burning of smaller grains (Bicho *et al*., 2012). It is one of the most important physical characteristics of green beans and plays a significant role in coffee marketing (Yigzaw, 2005). According to the Ethiopian coffee grading system, more than 85% of the coffee beans must have a screen size of 14 in order to meet the standard requirements (ECX, 2010). Accordingly, the beans of the tested genotypes from three environments were over 85% screen size, accepted for grading, and fulfilled the Ethiopian coffee grading standard in terms of bean screen size.

Bean Shape and Make

In this study, the shape and make of green beans ranged from very good (15%) to fair (10%). AW9648 and Angafa (15) grown at Awada, AW9622 (14) grown at Wonago, and AW5994 (13.3667) grown at Shebedino revealed significantly highest green bean shape and make (Table 4). Sualeh *et al*., (2014) also reported the presence of significant variation for 16 Ethiopian Arabica coffee hybrids and 1 check variety for bean shape and make tested in three south Ethiopian growing environments. The shape and make indicate the boldness and uniformity of the bean in a sample. It is an important physical characteristic of coffee, which affects the roasting process and cup quality. Uniform coffee beans are the most preferred and are usually priced highly (Sualeh and Mekonnen,, 2015). In this study, most of the genotypes rated very good to good and preferred by trained panelists.

Green Bean Color

The location, genotype, and their interaction significantly (P<0.01) influenced the color of green beans (Table 3). The color rate varies from bluish (15%) to faded to coated (7.33%)

(Table 4). The maximum rate for green bean color was recorded for AW9648 (15%) grown at Awada, AW9648 (13.22%) at Wonago; and AW9648, AW12305, and AW8105 (14%) all grown at Shebedino. In line with these results, variation in color of coffee beans among the Arabica coffee genotype and the differential response of genotype in the different growing environments were also reported by different studies (Severa *et al*., 2012; Sualeh *et al.*, 2014; Tesfa *et al*., 2021; Mengistu *et al.*, 2020).

Bean Odder

According to this study result, odder rated from clean odder (10%) to fair clean (8%), and most of the genotypes rated clean odder (10%) at all three locations (Table 4). When we consider the growing environment, the highest mean score (9.91%) was recorded at Wonago, whereas the lowest (9.42%) was recorded at Awda growing environment. Accordingly, the Wonago growing environment is more suitable to produce clean odder beans. Many studies also reported differential responses of genotypes across different growing environments for bean odders (Severa *et al*., 2012; Sualeh *et al.*, 2014; Cheng *et al.*, 2016: Tesfa *et al*., 2021; Mengistu *et al.*, 2020).

Total Row Quality

Growing environment and genotype interaction influenced significantly total row quality (Table 3). In this study, genotype AW9648 was best performed with highest row total score mean values of (40), (36.22), and (37.33) at Awada, Wonago, and shebedino respectively over other genotypes including check varieties (Table 4) and could be used as a best parent for selection and hybridization to improve physical quality.

Table 5.4 Mean value of 30 Arabica coffee genotypes for 18 characteristics at Awada, Wonago, Shebedino, and combined over locations

Location	Treatment	SS	SM	Col	Od	RawT	AQ	AI	Acid	AST	Bit	Bod	Flav	OQL	Total R	RCup	Trigo	Ch
Awada	AW1777	95.00	12.00	9.33	8.00	29.33	4.00	3.67	8.67	4.67	4.00	8.20	9.33	8.00	50.53	79.86	1.38	4
Awada	AW105	97.00	11.33	12.00	8.00	31.33	4.00	4.00	8.00	4.00	3.67	8.00	8.67	8.00	48.33	79.67	0.84	3
Awada	AW12305	96.67	12.00	10.67	10.00	32.67	3.33	3.33	8.00	4.33	4.00	8.52	7.33	8.00	46.85	79.52	0.98	3
Awada	AW1995	96.00	12.00	11.33	8.00	31.33	4.33	4.00	8.00	4.33	4.00	8.30	8.67	8.67	50.30	81.63	1.39	3
Awada	AW3106	95.00	12.00	11.33	10.00	33.33	3.67	3.67	8.00	4.33	4.00	8.00	8.00	8.00	47.67	81.00	0.79	3
Awada	AW4083	96.00	12.00	10.00	10.00	32.00	4.33	4.33	8.67	4.33	4.33	8.00	7.33	8.00	49.34	81.34	0.92	4
Awada	AW4305	97.00	10.00	10.00	8.00	28.00	4.00	4.00	8.00	4.33	4.00	8.00	8.00	8.00	48.33	76.33	0.91	3
Awada	AW4994	96.33	12.00	10.00	10.00	32.00	5.00	4.67	8.00	4.67	4.33	8.00	9.33	8.00	52.00	84.00	0.98	3
Awada	AW5994	96.00	12.00	10.67	9.33	32.00	3.67	3.67	8.00	4.00	3.00	8.00	8.00	7.33	45.67	77.67	0.81	2
Awada	AW695	97.00	12.00	12.00	10.00	34.00	4.33	4.33	8.00	4.00	4.00	8.00	8.00	8.00	48.67	82.67	0.93	3
Awada	AW7494	97.00	12.00	10.00	10.00	32.00	5.00	5.00	8.00	5.00	5.00	7.93	8.67	8.67	53.26	85.26	0.87	3
Awada	AW7705	97.00	12.00	12.00	10.00	34.00	4.00	4.00	8.00	4.00	4.00	8.00	8.00	8.00	48.00	82.00	0.86	4
Awada	AW8105	95.67	12.00	10.00	10.00	32.00	4.00	4.00	8.00	4.33	4.00	8.10	8.00	8.67	49.10	81.10	1.24	4
Awada	AW8806	94.67	12.00	12.00	9.33	33.33	4.67	4.33	8.00	4.67	4.33	8.07	8.67	9.33	52.07	85.41	1.11	4
Awada	AW9610	98.00	12.00	11.33	10.00	33.33	4.00	4.00	8.00	4.67	4.67	8.00	8.00	8.00	49.33	82.67	1.28	3
Awada	AW9611	98.00	13.00	11.00	10.00	34.00	4.33	4.33	8.67	4.00	4.00	8.00	8.00	8.00	49.34	83.34	1.49	3
Awada	AW9617	98.00	14.00	10.00	10.00	34.00	3.67	3.67	8.00	4.00	4.00	7.93	7.33	8.67	47.26	81.26	1.02	4
Awada	AW9622	97.00	12.00	10.67	8.00	30.67	3.00	3.00	8.67	4.00	3.33	8.00	8.00	8.00	46.00	76.67	1.49	3
Awada	AW9623	97.00	12.00	10.67	10.00	32.67	4.33	4.33	8.67	4.33	4.33	8.20	7.33	8.00	49.53	82.20	1.07	3
Awada	AW9628	95.00	12.00	11.33	8.00	31.33	3.00	3.33	8.00	4.67	3.33	7.93	6.67	8.00	44.93	76.26	0.73	4
Awada	AW9640	93.00	12.00	10.00	10.00	32.00	4.67	4.33	8.45	4.67	4.67	8.10	8.00	8.00	50.88	82.88	1.09	5
Awada	AW9641	95.00	12.00	11.33	8.00	31.33	3.33	3.33	8.00	4.33	3.67	8.20	8.00	7.33	46.20	77.53	1.00	3
Awada	AW9644	96.00	12.00	10.00	10.00	32.00	4.00	3.33	8.00	4.00	4.00	8.00	8.00	8.00	47.33	79.33	1.28	3
Awada	AW9648	96.0	15.00	15.0	10.00	40.00	4.67	4.67	10.00	4.33	4.67	9.19	8.00	8.67	54.19	94.19	1.04	3

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Awada	AW9658	97.00	12.00	10.67	10.00	32.67	3.67	3.67	8.00	4.00	4.00	8.20	8.00	8.00	47.53	80.20	0.90	4
Awada	AW9660	96.00	12.00	12.00	10.00	34.00	4.00	4.00	8.00	4.33	4.00	8.20	6.67	8.00	47.20	81.20	0.98	4
Awada	AW 9662	95.00	12.00	10.00	10.00	32.00	3.00	3.33	8.00	4.67	3.67	8.00	8.67	8.00	47.33	79.33	0.97	3
Awada	74112	94.00	12.00	10.67	10.00	32.67	5.00	4.67	8.67	4.67	4.00	8.00	8.00	8.00	51.00	83.67	1.02	3
Awada	Angafa	99.00	15.00	12.00	10.00	37.00	4.00	4.00	8.67	4.67	4.67	8.00	8.00	8.00	50.00	87.00	1.42	5
Awada	Feyate	96.00	13.00	12.33	8.00	33.33	4.67	4.67	8.67	5.00	4.67	8.10	9.33	8.00	53.10	86.43	0.93	5
Awada Mean		96.21	12.24	11.01	9.42	32.68	4.06	3.99	8.26	4.38	4.08	8.11	8.07	8.11	49.04	81.72	1.06	3
StD		1.29	0.96	1.12	0.89	2.13	0.58	0.50	0.45	0.32	0.45	0.24	0.66	0.40	2.36	3.68	0.22	0
CV		0.67	4.80	8.56	3.19	3.35	9.63	11.58	4.88	9.94	12.15	2.08	12.72	7.22	4.08	2.78	1.43	1
Wonago	AW1777	97.00	12.00	11.11	9.33	32.45	3.67	3.33	8.00	4.33	4.00	8.00	6.67	8.00	46.00	78.45	0.62	4
Wonago	AW105	98.00	12.00	11.56	10.00	33.56	3.67	3.67	8.00	4.33	4.33	8.00	7.33	7.33	46.67	80.22	0.71	5
Wonago	AW12305	92.00	12.00	11.78	10.00	33.78	4.00	4.00	8.00	4.67	4.00	9.33	8.00	8.00	50.00	83.78	0.60	4
Wonago	AW1995	98.00	12.00	11.55	10.00	33.55	4.00	4.00	8.67	5.00	5.00	8.00	8.00	8.00	50.67	84.22	0.63	4
Wonago	AW3106	96.00	12.00	11.33	10.00	33.33	3.33	3.67	8.00	4.33	4.33	8.00	7.33	8.00	47.00	80.33	0.87	5
Wonago	AW4083	96.00	12.33	11.11	10.00	33.44	4.00	3.67	10.00	4.67	4.67	8.00	8.00	8.00	51.00	84.44	0.66	4
Wonago	AW4305	97.00	11.00	9.33	10.00	30.33	3.00	2.67	7.33	3.67	3.00	8.00	6.00	5.00	38.67	69.00	0.71	3
Wonago	AW4994	98.00	12.00	10.89	10.00	32.89	3.67	3.67	8.00	4.00	4.00	8.00	8.00	7.33	46.67	79.55	0.68	5
Wonago	AW5994	98.00	13.11	11.11	10.00	34.22	3.67	3.33	8.00	4.33	4.00	8.00	6.67	8.00	46.00	80.22	0.85	4
Wonago	AW695	94.00	11.33	11.33	10.00	32.67	3.67	3.67	8.67	5.00	4.00	8.00	8.67	8.67	50.33	83.00	0.55	4
Wonago	AW7494	97.00	12.00	11.66	10.00	33.66	4.00	3.67	8.67	4.67	5.00	8.00	8.00	8.00	50.00	83.66	0.72	5
Wonago	AW7705	98.00	12.00	11.89	10.00	33.89	3.67	4.00	8.67	5.00	4.67	8.00	8.00	8.00	50.00	83.89	0.85	5
Wonago	AW8105	97.00	13.00	11.34	10.00	34.34	4.33	4.33	8.00	4.33	4.00	8.00	8.00	8.00	49.00	83.34	0.71	4
Wonago	AW8806	95.33	12.00	12.33	10.00	34.33	4.00	3.67	8.00	4.33	4.00	8.00	8.00	8.00	48.00	82.33	0.68	5
Wonago	AW9610	98.00	12.67	10.45	10.00	33.11	4.33	4.33	8.00	4.33	4.33	8.00	8.00	8.00	49.33	82.45	0.83	4
Wonago	AW9611	97.00	12.33	10.78	10.00	33.11	4.33	4.00	8.00	4.33	4.00	8.00	8.67	8.00	49.33	82.44	0.87	4
Wonago	AW9617	97.00	12.00	10.67	10.00	32.67	4.00	4.00	8.00	4.67	4.33	8.00	8.00	8.00	49.00	81.67	0.67	4

Wonago	AW9622	98.00	14.00	10.67	10.00	34.67	4.00	3.67	8.00	4.00	3.67	8.00	8.00	7.33	46.67	81.33	0.73	4
Wonago	AW9623	96.00	12.00	11.55	10.00	33.55	4.00	4.00	8.67	4.67	5.00	8.00	8.67	8.00	51.00	84.55	0.57	3
Wonago	AW9628	96.00	12.00	11.55	10.00	33.55	3.67	3.33	7.33	4.33	3.33	8.00	6.67	7.33	44.00	77.55	0.60	4
Wonago	AW9640	96.00	11.00	10.22	10.00	31.22	4.00	3.67	8.67	5.00	3.67	8.00	9.33	8.67	51.00	82.22	0.56	3
Wonago	AW9641	96.00	13.67	11.11	10.00	34.78	3.67	4.00	8.00	4.33	4.33	8.00	8.00	7.33	47.67	82.45	0.57	5
Wonago	AW9644	97.00	13.00	11.11	10.00	34.11	4.00	4.00	8.67	4.67	4.67	8.00	8.67	8.00	50.67	84.78	0.76	5
Wonago	AW9648	92.67	13.00	13.22	10.00	36.22	4.33	4.33	10.00	4.33	4.33	10.00	10.00	10.00	57.33	93.55	0.69	4
Wonago	AW9658	96.00	12.00	9.78	8.00	29.78	4.00	3.67	8.00	4.33	4.00	8.00	7.33	8.67	48.00	77.78	0.65	4
Wonago	AW9660	95.00	12.00	11.33	10.00	33.33	4.00	4.00	8.00	4.33	4.00	8.00	8.00	8.00	48.33	81.67	0.76	3
Wonago	AW 9662	96.00	13.00	10.89	10.00	33.89	3.67	3.33	8.00	4.33	4.33	8.00	8.00	8.00	47.67	81.56	0.70	5
Wonago	74112	95.67	12.00	10.67	10.00	32.67	4.00	4.00	8.67	4.33	3.67	8.00	8.67	8.00	49.33	82.00	0.58	4
Wonago	Angafa	97.67	13.00	11.89	10.00	34.89	4.67	5.00	8.67	4.67	4.67	8.00	8.67	8.67	53.00	87.89	0.58	4
Wonago	Feyate	98.00	13.00	12.00	10.00	35.00	4.00	3.67	8.00	4.67	4.67	8.00	8.00	8.00	49.00	84.00	0.72	3
Wonago Mean		96.44	12.31	11.21	9.91	33.43	3.91	3.81	8.29	4.47	4.20	8.11	7.98	7.94	48.71	82.14	0.69	4
StD		96.45	12.31	11.21	9.91	33.44	3.92	3.82	8.30	4.46	4.20	8.11	7.98	7.94	48.71	82.15	0.69	4
CV		0.95	1.68	6.19	0.44	2.09	9.48	11.79	10.53	10.65	15.27	2.60	10.74	8.95	4.45	2.55	3.40	1
Shebedino	AW1777	96.67	12.00	12.00	10.00	34.00	3.00	3.33	8.00	4.33	4.00	8.15	8.00	8.00	46.48	80.48	0.62	4
Shebedino	AW105	97.67	12.11	11.33	10.00	33.44	3.67	3.89	8.00	5.00	4.22	8.00	8.00	8.00	48.89	82.33	0.60	4
Shebedino	AW12305	95.56	12.33	14.00	10.00	36.33	3.67	3.78	8.67	4.00	4.00	8.22	6.67	7.33	46.56	82.89	0.68	3
Shebedino	AW1995	97.00	11.78	11.33	10.00	33.11	3.33	4.00	8.00	4.33	4.44	8.22	8.00	8.00	48.34	81.45	0.44	3
Shebedino	AW3106	96.00	12.00	12.00	10.00	34.00	4.00	3.78	8.00	4.67	4.11	8.07	8.00	8.00	48.78	82.78	0.51	4
Shebedino	AW4083	96.33	12.11	12.00	10.00	34.11	4.00	4.33	8.00	4.00	4.56	8.00	8.00	8.00	49.56	83.67	0.88	4
Shebedino	AW4305	97.33	11.00	7.33	8.00	26.33	3.00	3.22	6.00	4.00	3.67	8.00	6.00	6.00	39.67	66.00	0.67	5
Shebedino	AW4994	97.44	12.33	11.33	8.67	32.33	3.67	4.11	8.00	4.67	4.33	8.00	8.00	8.00	48.67	81.00	0.66	3
Shebedino	AW5994	97.33	13.37	11.33	8.00	32.70	4.00	3.67	8.00	4.67	3.78	8.00	8.00	8.00	48.44	81.15	0.57	3
Shebedino	AW695	96.33	11.33	11.33	8.67	31.33	4.00	4.00	8.00	4.33	4.11	8.00	7.33	8.00	47.78	79.11	0.57	3
Shebedino	AW7494	97.3	12.33	13.0	9.33	34.67	3.67	4.22	8.00	4.00	4.56	7.78	7.33	7.33	46.67	81.33	0.69	4

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Shebedino	AW7705	97.67	12.00	10.67	10.00	32.67	3.67	4.00	8.00	4.00	4.22	8.00	8.00	8.00	47.89	80.55	0.81	3
Shebedino	AW8105	96.89	12.66	14.00	10.00	36.66	3.67	3.78	8.00	4.00	4.00	8.07	8.00	8.00	46.74	83.41	0.65	4
Shebedino	AW8806	94.00	12.33	13.00	10.00	35.33	4.00	4.00	8.00	4.00	4.22	8.22	8.00	8.00	48.44	83.78	0.88	3
Shebedino	AW9610	97.89	12.22	10.00	8.00	30.22	4.00	4.11	8.00	4.33	4.33	8.00	8.00	8.00	48.67	78.89	0.60	4
Shebedino	AW9611	97.67	12.44	10.67	10.00	33.11	4.00	4.11	10.00	4.33	4.11	8.00	8.00	8.00	50.44	83.55	0.67	4
Shebedino	AW9617	97.67	12.67	11.33	9.33	33.33	4.00	3.89	9.33	4.33	4.22	7.78	8.00	8.00	49.66	83.00	0.71	3
Shebedino	AW9622	97.67	13.34	10.67	8.67	32.67	4.00	3.56	8.00	5.00	4.00	8.00	8.00	8.00	49.00	81.67	0.81	3
Shebedino	AW9623	97.00	12.00	12.00	10.00	34.00	4.00	4.11	8.00	4.33	4.44	8.15	8.00	8.00	48.93	82.93	0.68	4
Shebedino	AW9628	95.89	12.33	12.00	10.00	34.33	3.33	3.22	10.00	5.00	3.78	7.78	7.33	8.00	48.22	82.55	0.79	3
Shebedino	AW9640	95.67	11.45	10.00	10.00	31.45	4.33	4.00	8.00	4.33	4.22	8.07	8.00	8.00	48.96	80.41	0.90	4
Shebedino	AW9641	96.33	13.22	10.67	9.33	33.22	4.33	3.78	8.00	4.67	4.22	8.15	10.00	8.00	51.37	84.59	0.51	3
Shebedino	AW9644	97.00	13.33	12.00	9.33	34.66	3.67	3.78	8.00	4.67	4.44	8.00	7.33	7.33	47.44	82.11	0.66	3
Shebedino	AW9648	95.56	13.33	14.00	10.00	37.33	4.00	4.33	10.00	4.33	4.56	8.89	8.00	8.00	51.78	89.11	0.57	4
Shebedino	AW9658	97.00	12.00	10.00	8.67	30.67	4.00	3.78	8.67	4.00	3.89	8.15	8.00	8.00	48.70	79.37	0.67	3
Shebedino	AW9660	96.33	12.00	11.33	10.00	33.33	4.00	4.00	8.00	4.67	4.11	8.15	8.00	8.00	48.93	82.26	0.69	4
Shebedino	AW 9662	96.00	13.33	12.00	8.67	34.00	3.67	3.56	8.00	4.33	4.00	8.00	8.00	8.00	48.00	82.00	0.46	3
Shebedino	74112	95.89	12.33	10.67	10.00	33.00	3.67	4.22	8.67	4.67	4.11	8.00	8.00	8.00	49.11	82.11	0.60	2
Shebedino	Angafa	98.22	13.33	13.00	10.00	36.33	4.00	4.33	8.00	4.67	4.56	8.00	8.00	8.00	49.22	85.56	0.52	3
Shebedino	Feyate	97.33	13.00	12.33	10.00	35.33	4.33	4.11	10.00	4.00	4.44	8.07	8.00	8.00	50.85	86.19	0.71	4
Shebedino Mean		96.76	12.40	11.58	9.49	33.47	3.82	3.90	8.31	4.39	4.19	8.06	7.87	7.87	48.41	81.87	0.66	3
StD		0.92	0.64	1.38	.71	2.16	0.33	0.30	0.83	0.34	0.24	0.20	0.62	0.41	2.09	3.68	0.12	
CV		0.61	3.50	11.59	6.69	4.28	10.10	5.78	5.01	9.26	7.59	2.62	6.03	4.64	2.30	2.02	8.73	1
Combined	AW1777	96.22	12.00	10.82	9.11	31.93	3.56	3.44	8.22	4.44	4.00	8.12	8.00	8.00	47.67	79.60	0.87	4
Combined	AW105	97.56	11.81	11.63	9.33	32.78	3.78	3.85	8.00	4.44	4.07	8.00	8.00	7.78	47.96	80.74	0.71	4
Combined	AW12305	94.74	12.11	12.15	10.00	34.26	3.67	3.70	8.22	4.33	4.00	8.69	7.33	7.78	47.80	82.06	0.75	3
Combined	AW1995	97.00	11.93	11.41	9.33	32.67	3.89	4.00	8.22	4.56	4.48	8.17	8.22	8.22	49.77	82.43	0.82	3

Combined	AW3106	95.67	12.00	11.56	10.00	33.56	3.67	3.70	8.00	4.44	4.15	8.02	7.78	8.00	47.81	81.37	0.73	4
Combined	AW4083	96.11	12.15	11.04	10.00	33.18	4.11	4.11	8.89	4.33	4.52	8.00	7.78	8.00	49.96	83.15	0.82	4
Combined	AW4305	97.11	10.67	8.89	8.67	28.22	3.33	3.30	7.11	4.00	3.56	8.00	6.67	6.33	42.22	70.45	0.76	4
Combined	AW4994	97.26	12.11	10.74	9.56	32.41	4.11	4.15	8.00	4.44	4.22	8.00	8.44	7.78	49.11	81.52	0.78	4
Combined	AW5994	97.11	12.83	11.04	9.11	32.97	3.78	3.56	8.00	4.33	3.59	8.00	7.56	7.78	46.70	79.68	0.74	3
Combined	AW695	95.78	11.55	11.56	9.56	32.67	4.00	4.00	8.22	4.44	4.04	8.00	8.00	8.22	48.93	81.59	0.68	3
Combined	AW7494	97.11	12.11	11.55	9.78	33.44	4.22	4.30	8.22	4.56	4.85	7.90	8.00	8.00	49.98	83.42	0.76	4
Combined	AW7705	97.56	12.00	11.52	10.00	33.52	3.78	4.00	8.22	4.33	4.30	8.00	8.00	8.00	48.63	82.15	0.84	4
Combined	AW8105	96.52	12.55	11.78	10.00	34.33	4.00	4.04	8.00	4.22	4.00	8.06	8.00	8.22	48.28	82.61	0.87	4
Combined	AW8806	94.67	12.11	12.44	9.78	34.33	4.22	4.00	8.00	4.33	4.19	8.10	8.22	8.44	49.51	83.84	0.89	4
Combined	AW9610	97.96	12.30	10.59	9.33	32.22	4.11	4.15	8.00	4.44	4.44	8.00	8.00	8.00	49.11	81.33	0.90	4
Combined	AW9611	97.56	12.59	10.81	10.00	33.41	4.22	4.15	8.89	4.22	4.04	8.00	8.22	8.00	49.70	83.11	1.01	4
Combined	AW9617	97.56	12.89	10.67	9.78	33.33	3.89	3.85	8.44	4.33	4.18	7.90	7.78	8.22	48.64	81.97	0.80	4
Combined	AW9622	97.56	13.11	10.67	8.89	32.67	3.67	3.41	8.22	4.33	3.67	8.00	8.00	7.78	47.22	79.89	1.01	3
Combined	AW9623	96.67	12.00	11.41	10.00	33.41	4.11	4.15	8.44	4.44	4.59	8.12	8.00	8.00	49.82	83.23	0.77	3
Combined	AW9628	95.63	12.11	11.63	9.33	33.07	3.33	3.30	8.44	4.67	3.48	7.90	6.89	7.78	45.72	78.79	0.71	4
Combined	AW9640	94.89	11.48	10.07	10.00	31.56	4.33	4.00	8.37	4.67	4.19	8.06	8.44	8.22	50.28	81.84	0.85	4
Combined	AW9641	95.78	12.96	11.04	9.11	33.11	3.78	3.70	8.00	4.44	4.07	8.12	8.67	7.56	48.41	81.52	0.69	3
Combined	AW9644	96.67	12.78	11.04	9.78	33.59	3.89	3.70	8.22	4.44	4.37	8.00	8.00	7.78	48.48	82.07	0.90	4
Combined	AW9648	94.74	13.78	14.07	10.00	37.85	4.33	4.44	10.00	4.33	4.52	9.36	8.67	8.89	54.43	92.28	0.77	4
Combined	AW9658	96.67	12.00	10.15	8.89	31.04	3.89	3.70	8.22	4.11	3.96	8.12	7.78	8.22	48.08	79.12	0.74	3
Combined	AW9660	95.78	12.00	11.56	10.00	33.56	4.00	4.00	8.00	4.44	4.04	8.12	7.56	8.00	48.15	81.71	0.81	3
Combined	AW 9662	95.67	12.78	10.96	9.56	33.30	3.44	3.41	8.00	4.44	4.00	8.00	8.22	8.00	47.67	80.96	0.71	4
Combined	74112	95.19	12.11	10.67	10.00	32.78	4.22	4.30	8.67	4.56	3.93	8.00	8.22	8.00	49.81	82.59	0.73	3
Combined	Angafa	98.30	13.78	12.30	10.00	36.07	4.22	4.44	8.45	4.67	4.63	8.00	8.22	8.22	50.74	86.82	0.84	4
Combined	Feyate	97.11	13.00	12.22	9.33	34.55	4.33	4.15	8.89	4.56	4.59	8.06	8.44	8.00	50.98	85.54	0.79	4
Combined Mean		96.4	12.32	11.2	9.61	33.19	3.93	3.90	8.29	4.41	4.16	8.09	7.97	7.97	48.72	81.91	0.80	4

	7		7														
StD	1.40	0.65	0.91	0.42	1.58	0.29	0.33	0.47	0.15	0.33	0.28	0.45	0.40	.020	3.34	0.08	0
CV	0.80	3.50	9.20	4.40	3.40	10.00	11.00	7.30	11.00	12.00	2.40	10.00	7.20	3.80	2.50	4.80	1

Where; SS=screen size, SM=shape & make, Col=color, od=odour, RawT=raw total, AQ=aromatic quality, AI=aromatic intensity, Acid=acidity, AST=astringency, Bit=Bitterness, Bod=body, Fla=flavor, OQL=overall quality, TotalR=Total Cup Quality, RCup=Total Raw and Cup quality, Trigo=Trigonelline, Chloro=Chlorogenic acid, Caff=Caffeine, StD=Standard deviation, and CV=coefficient of variation

5.3.3 Cup Quality

Aromatic Quality

The interaction of genotype and growing environment significantly influenced ($p<0.01$) aromatic quality of coffee taste (Table 3). An excellent aromatic quality score (5) was recorded for genotypes AW7494 and AW4994 at Awada growing environment. When we consider growing environments, the highest aromatic score (4.06) was scored at Awada, followed by (3.91) at Wonago, and (3.82) at Shebedino (Table 4). Aromatic quality is an important cup quality parameter that determines the magnitude of the aroma of the coffee beverage and influences the sense organs of the cuppers (Bhumiratana, *et al.* 2011). In this study, all the genotypes studied scored good to excellent at all three locations. Accordingly, the studied genotype and growing environments are suitable to produce a good-quality aroma beverage. However, genotypes AW7494 and AW4994, as well as the Awada growing environment are more likely to produce excellent aromatic-quality coffee.

Aromatic Intensity

A very strong aromatic intensity score (5) was recorded for AW7494 grown at Awada and Angafa grown at Wonago (Table 4). Aromatic intensity is one of the most important cup quality parameters, which determines the magnitude of the aroma of the coffee beverage and influences the sense organs of the cuppers (Sualeh and Mekonnen, 2015; Mengistu *et al.*, 2020). In line with this study, the influence of environmental conditions and coffee varieties was reported in a previous study (Sualeh *et al*., 2014; Mengistu *et al*., 2020; Assa *et al*., 2021; Tesfa *et al*., 2021). Except genotype AW4305 grown at Wonago, all the studied genotypes at all three locations scored medium to very strong aromatic intensity.

Acidity

According to this study result, the acidity of coffee genotypes brew at three locations was rated from pointed to medium level. The pointed coffee brew acidity (10%) was recorded for AW9648 grown at Awada; AW9648 and AW4083 both grown at Wonago; and AW9648, Feyate, AW9628, and AW9648 all grown at Shebedino (Table 4). Acidity is a sensation of dryness that the coffee brew produces under the edges of the tongue and on the back of the palate (Lingle, 2011). Acidic intensity is a desirable feature, appreciated in coffee, as there is a positive relationship between the intensity of the acid impression and coffee quality. High acidity coffee has a pointed sharp pleasing flavor (Sualeh and Mekonnen, 2015).

Astringency

The nil astringency (5%) was recorded for AW7494, AW9628, and Feyate at Awada; AW7705, AW695, AW1995, and AW9640 at Wonago; and AW105, AW9622, and AW9628 at Shebedino (Table 4). Astringency is a complex sensation accompanied by a shrinking, drawing, or puckering mucosal surface in the mouth, produced by tannins. Coffee beans with a low content of astringency have generally high quality (Sultan and Mekonin, 2015; Mengistu *et al.,* 2020). The astringency of the studied coffee genotypes from three locations is scaled between very light and nil, which indicates a relatively low quantity of astringency and thus good quality.

Bitterness

In this investigation, there was a highly significant difference ($p<0.01$) in bitterness score among genotypes, but the effects of the growing environment and the genotype x environment interaction were not significant (Table 3). Genotypic performance for the bitterness score ranges from 3.48 to 4.85 with a mean of 4.16 (Table 4). Bitterness is the perception of coffee brew on the tongue of panelists during cup tasting, and it is the opposite of sweetness (Sualeh *et al.,* 2015). In general, good coffee should have low bitterness and a medium to full body (Sultan and Mekonin, 2015; Mengistu *et al.,* 2020), which is what almost all Arabica coffee genotypes tested in this study fulfilled. However, AW7494 and Angafa are the most preferred genotypes to produce very low bitterness.

Total Cup Quality

The total cup quality of coffee brews was computed by the summation of aromatic intensity, aromatic quality, acidity, astringency, bitterness, body, flavor, and overall standard, which is evaluated from 60%. Growing environment, genotype, and their interaction significantly influenced the total cup quality of the coffee brew (Table 3). The highest total cup quality score values were recorded for AW9648 (54.19) followed by AW7494 (53.26) both grown at Awada; AW9648 (57.3), followed by Angafa (53) at Wonago; and AW9648 (51.78) followed by AW9641 (51.37) at Shebedino, whereas the lowest rate was recorded for AW4305 (38.67) at Wonago and AW4305 (39.67) at Shebedino (Table 4). In this study, the total cup quality of all studied genotypes' brew is very good except for genotype AW4305.

Total Row and Cup Quality

The overall cup quality evaluation was used for the final coffee cup quality judgment from good to excellent. It was calculated from 100% as a sum of raw (40%), and cup quality (60%) (Gichimu *et al.,* 2014). In this study, the highest overall cup quality score values were recorded for genotype AW9648 (94.19%), followed by Angafa (87.00%) and Feyate (86.43%) at Awada; genotype AW9648 (93.55%), followed by Angafa (87.89%) and Feyate (86.77%) at Wonago; and genotype AW9648 (89.11%) followed by Feyate (86.19%) and Angafa (85.56%) at Shebedino. On the other side, the lowest total raw and cup quality values were recorded for genotypes AW9628 (76.26%) and AW4305 (76.33%) at Awada; genotype AW4305 (69.00%) followed by AW9628 (77.55) at Wonago; and genotype AW4305 (66.00%) followed by AW9610 (78.89%) grown at Shebedino (Table 4).

Combined analysis results revealed that the highest mean total raw and cup quality over locations was recorded for genotype AW9648 (92.28%), followed by Angafa (86.82%), whereas the lowest was recorded for AW4305 (70.45%), followed by AW9628 (78.79%) (Table 4). According to this study result, most of the genotypes received more than 80% scores, except AW4305, AW9658, AW9610, and AW695. Accordingly, most of the genotypes in the present study could be used as sources of a desirable parent for South Ethiopian coffee cup quality improvement through selection and hybridization. Out of all tested genotypes, AW9648 achieved the highest score in all three growing environments, whereas AW4305 was very poor in quality. Angafa is a South Ethiopian released variety that is known for its superior quality and yield performance. Accordingly, it was used as a parent for south Ethiopian coffee quality improvement, and two hybrid varieties were registered from the cross of it. According to this study result, genotype AW9648 is superior even from Angafa. Therefore, AW9648 could be used as the best selection and best parent for hybridization for south Ethiopian coffee quality improvement if its yielding ability and disease resistance are reasonably good in the respective study area and similar coffee growing agroecology.

5.3.4 Biochemical Composition

Green Bean Trigonelline

Trigonelline content ranges from 0.73 to 1.49% dwb, 0.44 to 0.9% dwb, and 0.55 to 0.87% dwb, with a mean of 1.06% dwb, 0.67% dwb, and 0.69% dwb at Awada, Shebedino, and Wonago, respectively (Table 4). Considering the genotypic effect, the highest value was recorded for AW9611 (1.49% dwb), followed by Angafa (1.416% dwb) grown at Awada, whereas the lowest was recorded for AW1995 (0.442% dwb) grown at Shebedino (Table 4). The highest mean trigonelline content over location was recorded for AW9622 (1.01% dwb), whereas the lowest was recorded for AW695 (0.68% dwb) and AW9641 (0.69% dwb) (Table 4). Different authors (Gichimu *et al*., 2015; Cheserek *et al*., 2021; Ogutu *et al*., 2022) reported significant variations among genotype and location in Arabica coffee trigonelline content.

Trigonelline, which is a pyridine alkaloid, is an important component of the coffee bean, which acts as a reservoir of nicotinic acid in plants (Belay, 2011). It is known to contribute to the formation of the appreciated coffee flavor, and the higher trigonelline contents could partially explain the better flavor observed (Gichimu *et al*., 2015).

Green Bean Chlorogenic Acid

Chlorogenic acid ranges from 2.908 to 5.897% dwb, 2.778% dwb to 5.475% dwb, and 3.444 to 5.643% dwb, with a mean of 3.914% dwb, 3.976% dwb, and 4.563% dwb at Awada, Shebedino, and Wonago, respectively (Table 4). The highest green bean chlorogenic acid content was observed for Angafa (5.90% dwb) grown at Awada, followed by AW105 (5.61) grown at Wonago, whereas the lowest was recorded for 74112 (2.78% dwb) at Shebedino (Table 4). According to the combined analysis result, the highest mean green bean chlorogenic acid content was recorded for Angafa (4.887% dwb), followed by AW4083 (4.53% dwb), whereas the lowest was recorded for AW5994 (3.58% dwb) (Table 4). Many authors reported a significant variation among genotypes and genotypes by environment interaction for chlorogenic acid in Arabica coffee (Cheserek *et al*., 2021; Gichimu *et al*., 2015).

Chlorogenic acids are phenolic compounds commonly found in green coffee beans (Belay, 2011). Chlorogenic acid is of great interest because of its possible positive impact on human health (Tajik *et al*., 2017). They also play a significant role in the defensive mechanism of plants, particularly when bacterial and fungal disease invasions are verified (Belay, 2011). Chlorogenic acids are responsible for the astringency and bitterness of coffee brews (Gichimu *et al*., 2015).

Green Bean Caffeine

Caffeine content ranges from 0.60 to 1.53, 0.52 to 1.06, and 0.62 to 1.12 with means of 0.89, 0.82, and 0.898 at Awada, Shebedino, and Wonago, respectively (Table 4). Among all genotypes, AW5994 (0.52% dwb) and AW9641 (0.56% dwb) grown at Awada, AW1995 (0.60% dwb) grown at Shebedino, and AW9623 (0.62% dwb) grown at Wonago had the lowest green bean caffeine content. On the other side, Feyate (1.53% dwb) at Awda, AW9623 (1.06% dwb) at Shebedino, and AW9622 (1.12% dwb) at Wonago had the highest green bean caffeine content (Table 4). The highest mean green bean caffeine content over location was recorded for Feyate (1.17), whereas the lowest was recorded for AW5994 (0.64% dwb), followed by AW1995 (0.69% dwb) (Table 4). In line with the present study, several studies reported coffee genetic variability and environmental influence on caffeine content variation (Yigzaw *et al*., 2008; Gebeyehu *et al*., 2015; Gichimu *et al*., 2015; Tolessa *et al*., 2019; Cheserek *et al*., 2021; Ogutu *et al*., 2022). The existence of wider variation in caffeine content among South Ethiopian coffee genotypes provides a great privilege for the development of low-caffeine-contenting varieties that have superior cup quality and aroma.

Moderate caffeine consumption is considered to be a safe habit and beneficial (Depaula & Farah, 2019). However, there are negative aspects linked to excess caffeine intake that must be considered (Depaula & Farah, 2019; Zou *et al*., 2022). Caffeine in excess use can cause a state of excitement and anxiety, as well as negative side effects such as tachycardia, headache, palpitations, insomnia, restlessness, nervousness, gastrointestinal disturbance, palpitation, and increasing blood pressure and tremor (Ludwig *et al*., 2014; Zou *et al*., 2022). Due to this adverse effect of excess caffeine, the demand for decaffeinated coffee increased around the world. As a result, instant coffee manufacturers have developed methods for artificially removing caffeine from coffee. However, decaffeination is an expensive process that modulates the amount of flavor components and precursors (Yigzaw *et al.*, 2008; Zou *et al*., 2022). By developing coffee varietals that are naturally high in quality and low in caffeine, it is possible to avoid the expensive decaffeination process while retaining the coffee's original flavor and precursors (Yigzaw *et al.*, 2008).

Many studies reported different caffeine levels in Arabica coffee; 14.1 -142 g kg-1 Assa et al (2021); 1.05 - 1.52 g kg-1 Sualeh *et al*., (2020); 6.2 - 12 g kg-1 Redi *et al.,* (2018); 9.1 - 13.2 g kg-1 Yigzaw *et al*., (2008); 7.7 – 16.8 g kg-1 Kathurima, (2013); and 9.3-14.4 g kg-1 Getachew *et al*., (2020). In the current investigation, a very low caffeine content range was registered for genotypes AW5994, AW1995, AW12305, AW4305, AW 9641, and AW9610. Therefore, these low caffeine-containing genotypes could be used as a parent for the development of low caffeine varieties and it is a great privilege for the nation and the coffee industry to fulfill the customer need and boost the specialty coffee market without decaffeination.

5.3.5 Principal component analysis

The PCA of 30 Arabica coffee genotypes based on a correlation matrix of combined data for 15 characters was performed to estimate the relative contribution of each attribute to the observed variability, and the results are presented in Table 5. The first and second principal components explained 54.42% and 12.78% of variation, respectively, and 67% of total variation (Table 5). The relative weight given to the variables in each component and important variables are those that possess high positive and high negative weights (Abbas *et al.*, 2019). Based on this suggestion, the most important traits contributing more to the variation in first PCA were observed for caffeine content (0.35), chlorogenic acid (0.34), aromatic quality (0.31), trigonelline (0.29), acidity (0.28), astringency (0.28), and color (0.27), in decreasing order. In the second PCA, high variation was contributed by flavor (-0.48) and screen size (0.46), whereas in the third and fourth PCAs, high variation was accredited by bitterness (0.79) and screen size (0.57), respectively (Table 5).

From the PCA result of the present study, it may be concluded that important variables in Arabica coffee genotype with respect to bean physical, cup quality, and biochemical traits were caffeine, chlorogenic acid, aromatic quality, trigonelline, acidity, astringency, flavor, and screen size. These variables might be taken into consideration for the effective selection of parents.

Table 5.5 Principal component analysis of 30 Arabica coffee genotypes for 15 green bean row, cup quality and biochemical content parameters

Character	Eigenvectors			
	Prin1	Prin2	Prin3	Prin4
Screen Size	-0.06	0.46	0.57	-0.13
Shape & Make	0.22	-0.12	0.61	0.11
Color	0.27	-0.29	0.08	0.15
Oder	0.23	0.03	-0.40	0.10
Aromatic Quality	0.31	-0.21	0.19	0.16

Aromatic Intensity	0.27	0.31	-0.17	-0.25
Acidity	0.28	0.31	-0.16	-0.22
Astringency	0.28	-0.16	0.07	-0.12
Bitterness	0.14	0.18	-0.11	0.79
Body	0.25	0.34	-0.04	-0.08
Flavour	0.18	-0.48	-0.04	-0.37
Overall cup Quality	0.25	0.20	0.11	-0.03
Trigonelline	0.29	-0.06	-0.05	0.08
Chlorogenic acid	0.34	0.09	-0.04	-0.09
Caffeine	0.35	-0.05	0.07	0.02
Eigenvalue	8.16	1.92	1.27	1.03
Present variation explained	54.42	12.78	8.47	6.84
Cumulative present variation explained	54.42	67.2	75.68	82.52

5.3.6 Correlation of Arabica coffee genotype green bean physical characters, cup quality and biochemical contents

The correlation analysis among Arabica coffee green bean physical characteristics and cup quality as well as bean caffeine, chlorogenic acid, and trigonelline contents were assessed and the results are given in Table 6. All cup quality characteristics (except astringency vs body were positively associated among themselves (Table 6). In addition, all green bean physical characteristics had a positive correlation between themselves and all cup quality attributes. This finding is in agreement with Cheserek *et al.*, (2022), who reported positive significant associations of organoleptic traits with each other.

Green bean chlorogenic acid and trigonelline content had a non-significant positive correlation with all green bean physical characteristics and cup quality attributes (except trigonelline vs color (r=-0.04) & chlorogenic acid vs body (r=-0.15). This finding partly agreed with Gichimu *et al.*, (2015) and Cheserek *et al.*, (2022) who reported a significant positive correlation between cup quality character with trigonelline (Gichimu *et al.*, 2015; Cheserek *et al.*, 2022). Caffeine content was non-significantly and negatively associated with most of the quality traits; shape and make (r=-0.5), odour (r=-0.06), total row (r=-0.03), astringency (r=-0.07), body (r=-0.15) and overall quality (r=-0.04). Many authors (Gichimu *et al.*, 2015; Cheserek *et al.*, 2022) reported a negative correlation between cup quality traits and caffeine. Trigonelline content was positively and non-significantly correlated with chlorogenic acid (r=0.08) while negatively and significantly associated with caffeine. Green bean caffeine content was positively and significantly correlated with chlorogenic acid (r=0.59) (Table 6).

Understanding the relationships between different characteristics is critical for designing an effective breeding program for selecting breeding materials for improving complex characters through indirect selection (Fellahi *et al*., 2018). Cup quality is economically important but its evaluation method is subjective and difficult. Based on correlation repeatability and sensitivity analyses, Yigzaw (2005), recommended flavour rating as the best selection criterion for genetic improvement of cup quality in Arabica coffee. In this study, flavour showed positive, statistically highly significant, and relatively high correlation coefficients with all other cup quality characteristics. Therefore, selection using liquor flavour alone would enable all-rounded cup quality improvement in Arabica coffee. However, this study showed that all the sensory variables analyzed in this study using a trained panel of tasters were important in determining the overall quality of a coffee.

Table 5.6 Correlation coefficients among green bean physical characters, cup quality, and green bean caffeine, chlorogenic acid, and trigonelline contents

	SS	SM	Col	Od	RwT	AQ	AI	Ac	AS	Bit	Bod	Flav	OQL	TCQ	TRC	Tri	Chl	Caf
SS	1.00	0.15^{ns}	-0.29^{ns}	-0.29^{ns}	-0.18^{ns}	-0.01^{ns}	0.08^{ns}	-0.21^{ns}	-0.10^{ns}	0.16^{ns}	-0.48^{*}	-0.04^{ns}	-0.25^{ns}	-0.15^{ns}	-0.17^{ns}	0.29^{ns}	0.03^{ns}	0.15^{ns}
SM		1.00	0.54^{*}	0.17^{ns}	0.77^{**}	0.28^{ns}	0.27^{ns}	0.56^{*}	0.18^{ns}	0.27^{ns}	0.32^{ns}	0.49^{*}	0.47^{*}	0.52^{*}	0.68^{**}	0.34^{ns}	0.11^{ns}	-0.05^{ns}
Col			1.00	0.48^{*}	0.92^{**}	0.36^{*}	0.46^{*}	0.60^{**}	0.29^{ns}	0.42^{*}	0.61^{**}	0.35^{ns}	0.65^{**}	0.64^{**}	0.82^{**}	-0.04^{ns}	0.10^{ns}	0.01^{ns}
Od				1.00	0.60^{**}	0.53^{*}	0.64^{**}	0.44^{*}	0.28^{ns}	0.49^{*}	0.21^{ns}	0.27^{ns}	0.53^{*}	0.58^{*}	0.63^{**}	0.03^{ns}	0.33^{ns}	-0.06^{ns}
RwT					1.00	0.46^{*}	0.54^{*}	0.69^{**}	0.31^{ns}	0.48^{*}	0.53^{*}	0.47^{*}	0.71^{**}	0.74^{**}	0.91^{**}	0.12^{ns}	0.19^{ns}	-0.03^{ns}
AQ						1.00	0.91^{**}	0.55^{*}	0.26^{ns}	0.68^{**}	0.19^{ns}	0.65^{**}	0.59^{**}	0.83^{**}	0.72^{**}	0.23^{ns}	0.32^{ns}	0.18^{ns}
AI							1.00	0.54^{*}	0.28^{ns}	0.76^{**}	0.23^{ns}	0.58^{*}	0.57^{*}	0.83^{**}	0.75^{**}	0.17^{ns}	0.32^{ns}	0.08^{ns}
Ac								1.00	0.23^{ns}	0.42^{*}	0.57^{*}	0.46^{*}	0.67^{**}	0.80^{**}	0.81^{**}	0.12^{ns}	0.20^{ns}	0.02^{ns}
AS									1.00	0.35^{ns}	-0.15^{ns}	0.33^{ns}	0.32^{ns}	0.38^{*}	0.38^{*}	0.02^{ns}	0.33^{ns}	-0.07^{ns}
Bit										1.00	0.17^{ns}	0.56^{*}	0.47^{*}	0.75^{**}	0.68^{**}	0.17^{ns}	0.31^{ns}	0.25^{ns}
Bod											1.00	0.22^{ns}	0.38*	0.47*	0.54*	0.03^{ns}	-0.15^{ns}	-0.15^{ns}
Flav												1.00	0.60^{**}	0.79^{**}	0.69^{**}	0.36^{ns}	0.13^{ns}	0.01^{ns}
OQL													1.00	0.82^{**}	0.83^{**}	0.19^{ns}	0.24^{ns}	-0.04^{ns}
TCQ														1.00	0.95^{**}	0.23^{ns}	0.27^{ns}	0.05^{ns}
TRC															1.00	0.20^{ns}	0.25^{ns}	0.01^{ns}
Trig																1.00	0.08^{ns}	-0.22^{ns}
Chl																	1.00	0.59^{**}
Caf																		1.00

Where: *& ** = Significant at $P<0.05$ and $P<0.01$ respectively; ns=non-significant, Sc=screen size, SM=shape & make, Col=color, od=odour, RawT=raw total, AQ=aromatic quality, AI=aromatic intensity, Aci=acidity, AS=astringency, Bit=Bitterness, Bod=body, Fla=flavor, OQL=overall quality, TCQ=Total Cup Quality, TRC=Total Raw and Cup quality, TRIG=Trigonelline Chloro=Chlorogenic acid, Caff=Caffeine

5.3.7 Cluster analysis

To investigate the genetic relationship among 30 Arabica coffee genotypes, cluster analysis was conducted, and a dendrogram was generated using the ward method for cup quality, green bean physical characteristics, caffeine, trigonelline, and chlorogenic acid content traits (Figures 5.1–3). In the Awada growing environment, genotypes were grouped into two main clusters (Figure 5.1). The first main clusters contained two genotypes, namely, Angafa, which is a released variety that maintains a spicy and floral flavor, and AW9648, the top-rated, promising selection. These two genotypes were characterized by excellent cup quality and desirable green bean physical characteristics. The second main cluster comprised 28 genotypes and was bifurcated into two different sub-clusters. The first sub-cluster of the second main cluster consists of eleven genotypes (7412, Feyate, AW9640, AW8806, AW7494, AW4994, AW9644, AW8105, AW9662, AW3106, AW1777) that were characterized by good cup quality and desirable green bean physical characteristics. The second sub-cluster of the second main cluster consisted of seventeen coffee genotypes that were further subdivided into two sub-sub-clusters. The first sub-sub-cluster of the second sub-cluster consisted of seven genotypes, namely, AW9641, AW9628, AW4305, Aw9622, AW5994, AW1995, and AW105, which were characterized by average cup quality. The second sub-sub-cluster consisted of 10 coffee genotypes, namely, AW9617, AW9611, AW9610, AW9660, AW7705, AW695, AW9623, AW4083, AW9658, and AW12305, which were characterized by poor cup quality (Figure 5.1).

In the Wonago growing condition, the genotypes were grouped into two clusters. The first main cluster consisted of only one genotype, namely AW4305, which was characterized by its poor quality. The second cluster was grouped into two sub-cluster. The first sub-cluster of the second main cluster consisted of only one genotype (AW9648), which was characterized by its top quality. The second sub-cluster of the second main cluster was further grouped into two sub-sub-clusters. The first sub-sub-cluster of the second main cluster consisted of twenty genotypes (Angafa, Feyate, AW8105, AW9644, AW9623, AW4083, AW7494, AW7705, AW1995, 74112, AW9617, AW9611, AW9610, AW9662, AW9641, AW9660, AW8806, AW9640, AW695, and AW2305), which had good quality. The second sub-sub-cluster of the first main cluster consisted of eight genotypes (AW9658, AW9628, AW9622, AW5994, AW3106, AW4994, AW105, and AW1777) that had poor to moderately good quality (Figure 5.2).

The genotypes were clustered into two main clusters in the Shebedino growing environment. The first main cluster was grouped into two sub-clusters. The first sub-cluster of the first main cluster consisted of only one genotype, AW9648, which is characterized by its top quality and is recommended as a superior genotype for quality improvement. The second sub-cluster of the first main cluster consisted of seven genotypes, viz., Angafa, 74112, AW9640, Feyate, AW8806, AW7494, and AW4994, which are categorized by their best quality. The second main cluster was grouped into two sub-clusters. The first sub-cluster of the second main cluster consisted of five genotypes (AW9628, AW9622, AW9641, AW5994, and AW4305), whereas the second sub-cluster of the second main cluster consisted of sixteen genotypes (AW9617, AW9611, AW9623, AW9610, AW7705, AW695, AW8105, AW4083, AW9660, AW3106, AW9662, AW9644, AW9658, AW2305, AW1995, AW105, and AW1777) (Figure 5.3). Genetic variation among coffee genotypes and inconsistency in the performance of genotypes when grown in different environments might contribute to the occurrence of different clusters within each environment. Therefore, the breeding program should give due attention to those top quality genotypes in their respective locations for quality improvement through crossing and selection in south Ethiopia.

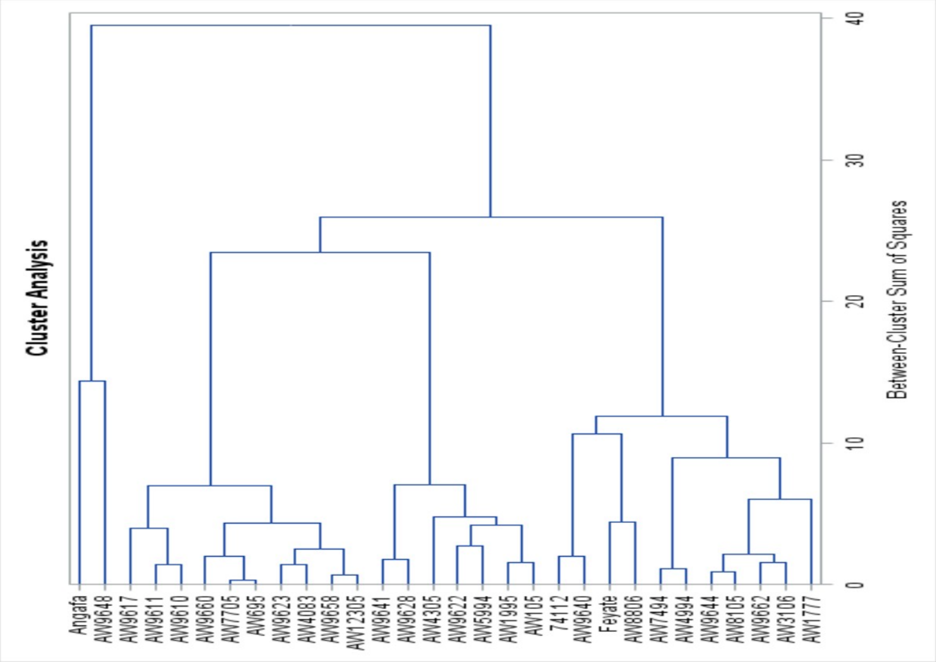

Figure 5. 1 Cluster dendrogram describing variation among 30 genotypes of Arabica coffee for quality traits at Awada

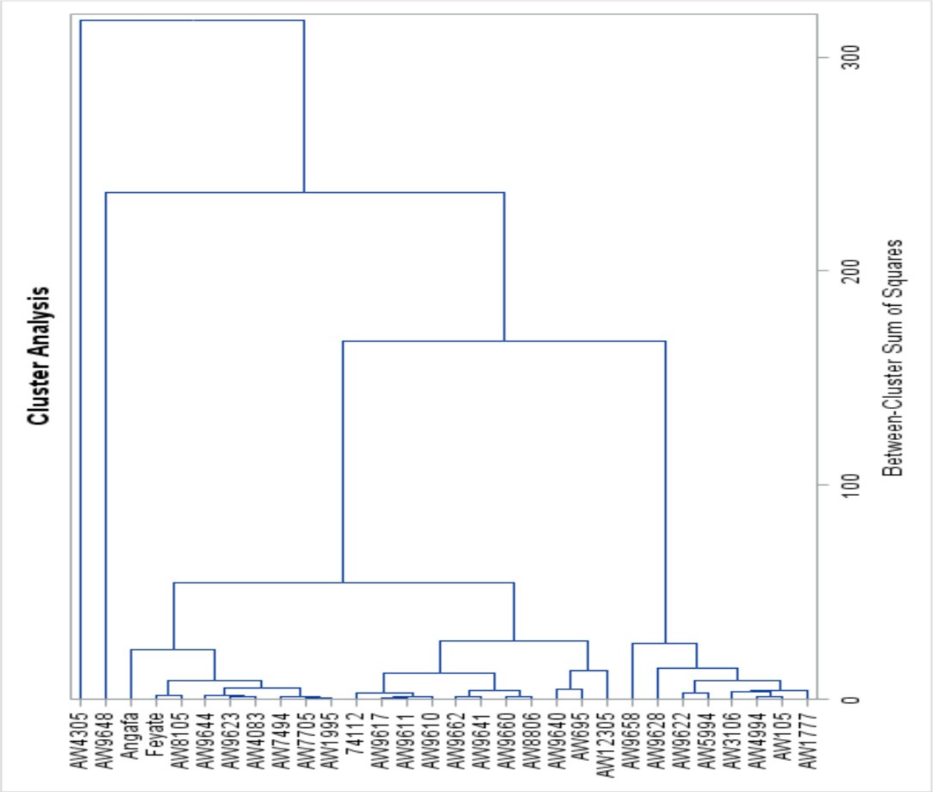

Figure 5. 2 Cluster dendrogram describing variation among 30 genotypes of Arabica coffee for quality traits at Wonago

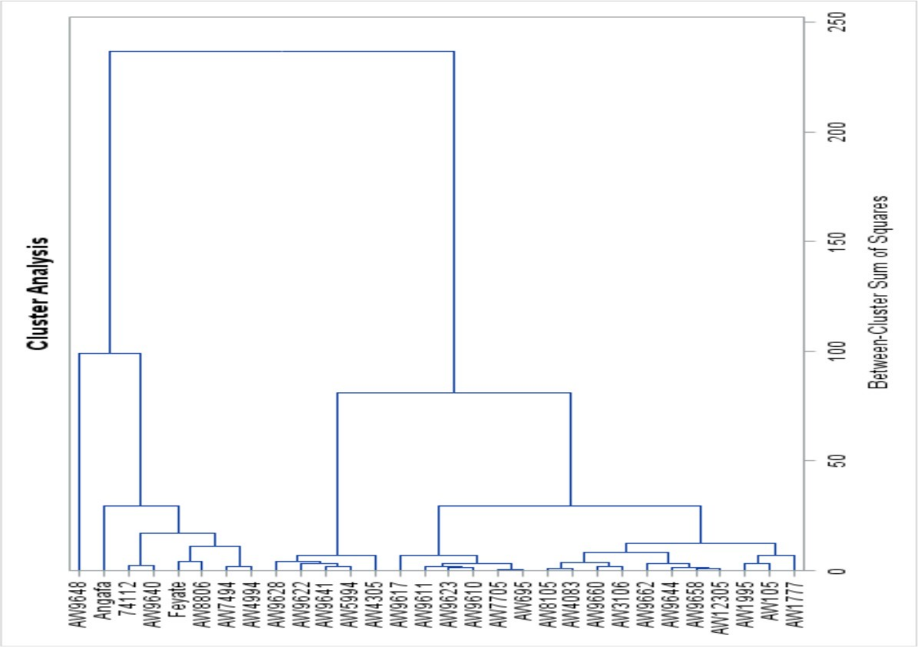

Figure 5. 3 Cluster dendrogram describing variation among 30 genotypes of Arabica coffee for quality traits at Shebedino

5.4 Conclusion

The present study confirmed the existence of significant variation among south Ethiopian Arabica coffee genotypes, growing environments, and genotype by environment interaction for physical character, cup quality, and biochemical composition traits. Therefore, the studied genetic resources should be properly utilized in the quality improvement program through selection and hybridization for the emerging specialty coffee markets, and the coffee quality improvement program should give due attention to incorporating genetic and environmental influences by using a multi-locational selection strategy.

The overall coffee quality score for tested genotypes in three locations were above 80% for most of the studied genotypes. Therefore, in terms of quality, most of the tested genotypes can be used to produce specialty coffee in the study areas. Out of all tested genotypes in this study, AW9648 achieved the highest score in green bean physical attributes and cup quality parameters at all three-tested locations. Therefore, AW9648 could be used as the best selection and best parent for hybridization for South Ethiopian coffee quality improvement.

From the PCA, it may be concluded that important variables in the Arabica coffee genotype with respect to bean physical, cup quality, and biochemical traits were caffeine, chlorogenic acid, aromatic quality, trigonelline, acidity, astringency, flavor, and screen size. These variables might be taken into consideration for the effective selection of parents.

5.5 Reference

Abbas, F.M., Alkarkhi, Wasin, A., Alqaraghuli, A. (2019). Principal Components Analysis, Academic Press, Easy Statistics for Food Science with R. Pages 125-141, ISBN 9780128142622. https://doi.org/10.1016/B978-0-12-814262-2.00008-X.

Abdulfeta, T. K. (2018). Characterization and Yield Performance Evaluation of Coffee (Coffea arabica L.) Germplasm Accessions from Tepi, Southwestern Ethiopia MSc. Thesis summated to Hawassa University College of Agriculture. 71p.

Adem, A., Mohammed, H., & Ayana, A. (2020). Phenotypic Diversity in Arabica Coffee Genotypes from Eastern Ethiopia. 5(4), 42–47. https://doi.org/10.11648/j.ijee.20200504.11

Assa, A., Loppies, J. E., Amalia, A. N., Indriana, D., Mamang, U. R. R., Ariyanti, M., & Winaldi, A. (2021). Chemical compounds and sensory characteristics of arabica coffee (Coffea arabica) as a novel specialty coffee from sinjai regency, indonesia. Food Research, 5, 107–112. https://doi.org/10.26656/fr.2017.5(S2).001

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

Benti, T. (2017). Progress in Arabica Coffee Breeding in Ethiopia: Achievements, Challenges and Prospects. International Journal of Sciences: Basic and Applied Research, 33(2), 15–25. http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Bicho, N. C. C., Leitão, A. E., Ramalho, J. C., Alvarenga N. B. and Lidon, F. C. (2012). Application of colour parameters for roasting coffee assessment. Ciência e Tecnologia dos Alimentos 32(2):1-7. https://doi:10.9755/ejfa.v26i1.17190

Borém, F. M., Luz, M. P. S., Sáfadi, T., Volpato, M. M. L., Alves, H. M. R., Borém, R. A. T., & Maciel, D. A. (2019). Meteorological variables and sensorial quality of coffee in the mantiqueira region of Minas Gerais. Coffee Science, 14(1), 38–47. https://doi.org/10.25186/cs.v14i1.1522

Cheng, B., Furtado, A., Smyth, H.E., Henry, R.J. (2016). Influence of genotype and environment on coffee quality. Trends in Food Science & Technology 57:20-30. https://doi.org/10.1016/j.tifs.2016.09.003

Cheserek, J. J., Ngugi, K., Muthomi, J. W., & Omondi, C. O. (2022). Genetic Variability and Correlation of Biochemical and Sensory Characteristics of Coffee. 14(2), 95–103. https://doi.org/10.5539/jas.v14n2p95

Cheserek, J. J., Ngugi, K., Muthomi, J. W., Omondi, C. O., & Ezekiel, K. N. (2021). Green bean biochemical attributes of Arabusta coffee hybrids from Kenya using HPLC and soxhlet extraction methods. Australian Journal of Crop Science, 15(2), 201–208. https://doi.org/10.21475/ajcs.21.15.02.p2581

CSA (Central Statistical Agency). (2019). Agricultural sample survey: report on area and production of major crops of private peasant holdings for meher season of 2018/19. 58. Addis Ababa, Ethiopia: Central statistical Agency.

CSA (Central Statistical Authority). (2015). Federal Republic of Ethiopia, Central Statistical Agency, Agricultural Sample Survey Report on Area and Production of Major Crops, 2014/2015, Vol. 1, Statistical Bulletin 578, May 2015, Addis Ababa, 121P.

Dalazen, Rodrigues, J., Rocha, R.B., Pereira, L.L., Alves, E.A., Espindula, M.C., and de Souza, C.A. (2020). Beverage Quality of Most Cultivated Coffea Canephora Clones in the Western Amazon. Coffee Science 15 (1): 1–10. https://doi.org/10.25186/.v15i.1711

Dawit, (2018). Genetic Diversity Analysis among Promising Wollega Coffee (Coffea arabica l.) Selections in Western Ethiopia. MSc. Thesis of Hawassa University. Thesis Submitted to Graduate Studies of in partial fulfillment of Hawassa University, Hawassa Ethiopia. P38.

Depaula, J., & Farah, A. (2019). Caffeine consumption through coffee: Content in the beverage, metabolism, health benefits and risks. Beverages, 5(2). https://doi.org/10.3390/beverages5020037

Desalegn, A. (2018). Genetic Variability and Character Association of Amaro Coffee (Coffea arabica l.) Accessions at Awada, Southern Ethiopia. Thesis Submitted to Graduate Studies of Jimma University, Jimma, Ethiopia.

ECX (Ethiopian Commodity Exchange), (2010). Daily market bulletin, in 125 Quarter I, Ethiopian Commodity Exchange, Addis Ababa, Ethiopia.

EIAR (Ethiopian Institute of Agricultural Research), (2017). National Coffee Commodity Research Strategy of Fifteen Years (2016 -2030). Addis Ababa, Ethiopia.

FAO (Food and Agricultural Organization of United Nations) publications catalogue, (2021). FAO office of communications. Publications catalogue 2021. Retrieved from https://doi.org/10.4060/cb4402en

Fellahi, Z. E. A., Hannachi, A., & Bouzerzour, H. (2018). Analysis of Direct and Indirect Selection and Indices in Bread Wheat (Triticum aestivum L.) Segregating Progeny. International Journal of Agronomy, 2018. https://doi.org/10.1155/2018/8312857

Fonseca, A. S., Lima, J. S. de S., Silva, S. de A., Dardengo, M. C. J. D., & Xavier, A. C. (2019). Fuzzy logic in the spatial and temporal distribution in the qualitya of the beverage in conilon coffee. Coffee Science, 14(2), 163–172. https://doi.org/10.25186/cs.v14i2.1563

Gebeyehu, B.T. and Bikila, S.L. (2015). Determination of caffeine content and antioxidant activity of coffee. Am. J. Appl. Chem. 3, 69–76

Getachew, Sentayehu, A., Leta, T., & Gezahegn, B. (2020). Genetic Variability of Ethiopian Coffee (Coffea arabica L.) Accessions Collected from East Wollega Zone for Bean Biochemical Constituents. Ethiopiopian Journal of Agricultural Science, 30(3), 77–97.

Gichimu, B. M., Gichuru, E. K., Mamati, G. E., & Nyende, A. B. (2015). Biochemical Composition Within Coffea arabica cv . Ruiru 11 and Its Relationship With Cup Quality. Canadian Center of Science and Education. https://doi.org/10.5539/jfr.v3n3p31

Gichimu, B.M., Gichuru, E.K., Mamati, G.E., Nyende, A.B., (2014). Biochemical composition with in Coffea arabica cv. Ruiru 11 and its relationship with cup quality. J. Food Res. 3 (3), 31–44.

Gomez, K. A., & Gomez, A. A. (1984). Statistical Procedures for Agricultural Research (2nd ed.). New York: John Wiley & Sons.

Kathurima, C W. (2013). Characterization of the Diversity of Coffee Genotypes in Kenya by Genetic, Biochemical and Beverage Quality Profiles. A Thesis Submitted in Partial Fulfilment for the Degree of Doctor of Philosophy in Food Science and Technolog. PhD Thesis.

Lingle, T. R. (2011). The Coffee Cuppers Handbook: A Systematic Guide to the Sensory Evaluation of Coffee's Flavor. Specialty Coffee Association of America (SCAA), Long Beach, CA.

Ludwig, I. A., Clifford, M. N., Lean, M. E. J., Ashihara, H., & Crozier, A. (2014). Coffee: Biochemistry and potential impact on health. Food and Function, 5(8), 1695–1717. https://doi.org/10.1039/c4fo00042k

Marie, L., Abdallah, C., Campa, C., Courtel, P., Bordeaux, M., Navarini, L., Lonzarich, V., Bosselmann, A. S., Turreira-García, N., Alpizar, E., Georget, F., Breitler, J. C., Etienne, H., & Bertrand, B. (2020). G × E interactions on yield and quality in Coffea arabica: new F1 hybrids outperform American cultivars. Euphytica, 216(5). https://doi.org/10.1007/s10681-020-02608-8

Mengistu, M. W., Workie, M. A., & Mohammed, A. S. (2020). Physical and Cup Quality Attributes of Arabica Coffee (Coffea arabica L.) Varieties Grown in Highlands of Amhara Region, Northwestern Ethiopia. International Journal of Agronomy, 6(1). https:// https://doi.org/10.1155/2020/6420363

Merga, D., Mohammed, H., & Ayano, A. (2021). Estimation of Genetic Variability , Heritability and Genetic Advance of Some Wollega Coffee (Coffea arabica L .) Landrace in Western Ethiopia Using Quantitative Traits. 9(4), 182–191. https://doi.org/10.11648/j.jps.20210904.18

Milligan, G.W., Cooper, M.C. (1988). A study of standardization of variables in cluster analysis. Journal of Classification 5, 181–204. https://doi.org/10.1007/BF01897163

Ndikumana, J. (2022). Agro-morphological characterization of arabica coffee cultivars in burundi. Thesis Submitted to Graduate Studies of Jomo kenyatta university, Nirobi, Keniya.

Nugroho, D., Basunanda, P., & Yusianto, Y. (2020). Performance of Biochemical Compounds and Cup Quality of Arabica Coffee as Influenced by Genotype and Growing Altitude. Pelita Perkebunan (a Coffee and Cocoa Research Journal), 36(1), 1–23. https://doi.org/10.22302/iccri.jur.pelitaperkebunan.v36i1.409

Ogutu, C., Cherono, S., Ntini, C., Wang, L., & Han, Y. (2022). Comprehensive analysis of quality characteristics in main commercial coffee varieties and wild Arabica in Kenya. Food Chemistry: X, 14(November 2021), 100294. https://doi.org/10.1016/j.fochx.2022.100294

Redi, M., Chandravanshi, B. S., Mustefa, A., & Mamo, H. (2018). Correlation between caffeine contents of green coffee beans and altitudes of the coffee plants grown in southwest Ethiopia. April. https://doi.org/10.4314/bcse.v32i1.2

Samoggia, A. & Riedel, B. (2019). Consumers' perceptions of coffee health benefits and motives for coffee consumption and purchasing. Nutrients, 11(3). https://doi.org/10.3390/nu11030653

Severa, L., Buchar, J., and Nedomov, S. (2012). Shape and size ´ variability of roasted arabica coffee beans. International Journal of Food Properties, vol. 15, no. 2, pp. 426–437.

Sualeh, A., and Mekonnen, N. (2015). Manual for Coffee. Quality Laboratory. ISBN: 978999446605410-8 . PP. 1-24. https://doi.org/10.13140/RG.2.2.18203.49441

Sualeh A., Mekonnen, N., & Degefa, M. (2014). Hybrid Coffee (Coffea arabica L) Genotypes Quality Evaluation under different Environment. Greener Journal of Agricultural Sciences Vol. 4 (6), pp. 245-251 http://dx.doi.org/10.15580/GJAS.2014.6.0523014244

Sualeh, A., Tolessa, K., & Mohammed, A. (2020). Biochemical composition of green and roasted coffee beans and their association with coffee quality from different districts of southwest Ethiopia. Heliyon, 6(12), e05812. https://doi.org/10.1016/j.heliyon.2020.e05812

Tadesse, T., Tesfaye, B., & Abera, G. (2020). Coffee production constraints and opportunities at major growing districts of southern Ethiopia Coffee production constraints and opportunities at major growing districts of southern Ethiopia. Cogent Food & Agriculture, 6(1). https://doi.org/10.1080/23311932.2020.1741982

Tajik, N., Tajik, M., Mack, I., & Enck, P. (2017). The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: a comprehensive review of the literature. European Journal of Nutrition, 56(7), 2215–2244. https://doi.org/10.1007/s00394-017-1379-1

Tesfa, M. (2019). Effect of Drying Methods on Raw Quality of Selected Cultivars of Arabica Coffee (Coffea arabica L .) Grown in South West, Ethiopia. 8(11), 1412–1420.

Tesfa, M., Abera, S., & Sualeh, A. (2021). Effect of Drying Methods on Cup Quality of Selected Cultivars of Arabica Coffee (<i>Coffea arabica L</i>.) Grown in South

West, Ethiopia. Agriculture, Forestry and Fisheries, 10(2), 52. https://doi.org/10.11648/j.aff.20211002.13

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

Tolessa, K., Alemayehu, D., Belew, D., & Boeckx, P. (2019). Biochemical composition of Ethiopian coffees (Coffea arabica L .) as influenced by variety and postharvest processing methods. 13(February), 48–56. https://doi.org/10.5897/AJFS2018.1770

Tolessa, K., Rademaker, M., Baets, B. De, & Boeckx, P. (2016). Prediction of specialty coffee cup quality based on near infrared spectra of green coffee beans Talanta Prediction of specialty coffee cup quality based on near infrared spectra of green coffee beans. 367–374. https://doi.org/10.1016/j.talanta.2015.12.039

USDA (The United States Department of Agriculture), (2019). Ethiopia Coffee Annual Coffee Annual Report, GAIN Report Number: ET1904, 6p. https://apps.fas.usda.gov

USDA (The United States Department of Agriculture), (2021). Ethiopia Coffee Annual Report, GAIN Report Number: ET2021-0013, 6p. https://apps.fas.usda.gov.

Woelore, W.M. (1993). Optimum fermentation protocols for arabica coffee under Ethiopian conditions. pp. 727-733. Proceedings of the 15th International Scientific Colloquium on Coffee (ASIC). Montpellier, France, June 6-11 1993.

Yigzaw, D. (2005). Assessment of cup quality, morphological, biochemical and molecular diversity of C. arabica L. genotypes of Ethiopia, A PhD Dissertation, University Free State.

Yigzaw, D., Labuschagne, M. T., Osthoff, G., & Herselman, L. (2008). Genetic diversity and correlation of bean caffeine content with cup quality and green bean physical characteristics in coffee (Coffea arabica L .). May 2015. https://doi.org/10.1002/jsfa.3271

Yirga, M., Gebreselassie, W., & Tesfaye, A. (2021). Genetic Diversity of Coffee (Coffea arabica L .) Collections for Morpho-agronomic Traits in Southwestern Ethiopia. East African Journal of Sciences. Volume 15 (2): 141-154.

Zarebska, M., Stanek, N., Barabosz, K. *et al*., (2022). Comparison of chemical compounds and their influence on the taste of coffee depending on green beans storage conditions. Sci Rep 12, 2674. https://doi.org/10.1038/s41598-022-06676-9

Zou, Y., Gaida, M., Franchina, F. A., Stefanuto, P. H., & Focant, J. F. (2022). Distinguishing between Decaffeinated and Regular Coffee by HS-SPME-GC×GC-TOFMS, Chemometrics, and Machine Learning. Molecules, 27(6). https://doi.org/10.3390/molecules27061806

Vignoli, J. A., Viegas, M. C., Bassoli, D. G., & Benassi, M. D. T. (2014). The roasting process affects differently the bioactive compounds and the antioxidant activity of arabica and robusta coffees. Food Research International, 61, 279–285.

6. Genetic Diversity and Population Structure of South Ethiopian Arabica Coffee [*Coffea arabica* L.] Genotypes Using ISSR Markers

Abstract

Arabica coffee is originated and diversfied in Ethiopia. Despite its considerable diversity, it was not much utilized. The present study was conducted to determine the genetic diversity and population structure of 50 Arabica coffee genotypes representing five populations (Sidama, Amarao, Jinka, Gujji, and Improved varities) using inter-simple sequence repeat markers. The population under study produced 74 distinct bands. The number of private bands and the lowest number of common bands (≤50%) were higher for improved variety, with the highest value of 8 and 6, respectively. The band frequency varied from 8.62% (Gujji) to 25.86% (improved varieties) with an average of 17.93%. The analysis of genetic diversity reveled that the number of alleles per population, the number of effective alleles, shannon's information index, observed diversity, and unbiased diversity varied from 0.276 to 0.672, 1.063 to 1.149, 0.052 to 0.12, 0.036 to 0.082, and 0.039 to 0.092, with averages of 0.434, 1.101, 0.092, 0.061, and 0.067, respectively. The analysis of molecular variance (AMOVA) also revealed the presence of significant genetic variability, with the highest value of genetic variation found among populations (67%), whereas a low value of genetic variance was observed within populations (33%). The first, second, and third axes of principal coordinate analysis (PCA) explained 19.45%, 14.73%, and 8.78% of genetic variation, respectively, with the explained total variation of 42.96%. Hierarchical cluster analysis with UPGMA classified the genotypes into four clusters: I, II, III and IV each of which consisted of 20% (10 genotypes), 28% (14 genotypes), 12% (6 genotypes), and 40% (20 genotypes), respectively. In the clustering analyses, genotypes from the same populations were found to be clustered similarly. In conclusion, the analysis of genetic diversity and population structure among South Ethiopian Arabica coffee genotypes using ISSR markers revealed the presence of genetic variation, which is critical for the conservation and improvement of this vital crop. In the future, it is important to conduct more genetic variability studies utilizing high resolution markers and a broader range of accessions.

Keywords: Arabica Coffee, Genetic Polymorphism, Population Stracture, ISSR Markers

6.1 Introduction

Coffea arabica is the only allotetraploid (2n=4x=44) and self-fertile species in the Coffea genus, whereas the rest species are diploid, highly self-incompatible and allogamous (out crossing) (Lachenmeier, 2023). It is evergreen plant, which grows within the belt lies equidistant from the equator (23.5° N) in the north and (23.5° S) in the south, typically in tropical regions of Africa, Asia, Oceania, South America (Brazil and Colombia), Central America and Mexico (ICO, 2016). It is the most important agricultural commodity upon which the economy of more than 80 producer countries depend on (Bayetta, 2015) and a foreign currency earner. It plays a key role in creating direct employment for 125 million people in worldwide (Fairtrade, 2022). It is a vital and delightful beverage that is frequently drank due to its stimulatory effects, desirable aroma, and flavor. Its beverage contains antioxidant phenols, which may provide health benefits such as a lower risk of cancer, cardiovascular disease, diabetes, and Parkinson's disease (Higdon and Frei, 2006; Nkondjock, 2009; Bøhn *et al*., 2012; Gökcen and Şanlier, 2019).

Coffee Arabica has enormous economic, tourism, social, and cultural value in Ethiopia (Geremew *et al*., 2022). From smallholder coffee growers to coffee exporters, the coffee industry provides employment and boost economic growth (Muhie, 2022). Coffee tourism allows the tourists to explore the birthplace of coffee, strongly connect with the country's rich cultural heritage, witness vibrant and ritualistic traditional coffee ceremonies, and drink the flavorsome brews captured the world's attention (Woyesa & Kumar, 2021). The Ethiopian coffee ceremonies bring people together; promote unity, and create a sense of togetherness among individuals (Geremew *et al*., 2022).

Ethiopia is the original homeland and center of genetic diversity of coffee Arabica (Vavilov, 1941; Lashermes *et al* 1996). Many different types of coffee trees can be found within small areas (Yilma Y., 2017). Even though Arabica coffee has much important and it has high genetic diversity, Ethiopian coffee improvement program is solely dependents on conventional breeding approach and needed to be supplemented with modern molecular breeding techniques (EIAR, 2017; Benti, 2017).

The genetic diversity of *Coffea arabica* arises from its origin as an allotetraploid hybrid between *C. canephora and C. eugenioides*, which introduced a novel genetic combination (Lashermes *et al.,* 1999). Although *C. arabica* is predominantly self-pollinating, significant variation persists, especially in Ethiopia, maight be due to its outcrossing by insects and humanmade activities. This diversity is maintained by natural selection across diverse agro-ecological zones, traditional farming practices that promote seed propagation and genetic recombination, and long-term in situ conservation by smallholder farmers (Tesfaye *et al*., 2007; Labouisse *et al*., 2008). Human-mediated selection for yield, quality, and resilience has further shaped this diversity over generations (Aga *et al*., 2005; Anthony *et al*., 2002). Despite the species' narrow genetic base compared to outcrossing crops, these combined factors contribute to the rich genetic variability observed in Arabica coffee.

Conventional coffee breeding in Ethiopia was started in 1952 with the major objectives of collection and conservation of coffee germplasm, development of cultivars that combine high yield, disease resistance, improved cup quality, and wider environmental adaptable (Belachew, 1968). Since then, a number of achievements reported in germplasm collection and conservation, development of high yielder and CBD resistant having good cup quality selections and heterotic hybrids varieties. Despite its many merits, conventional coffee breeding in Ethiopia faced a number of challenges. Firstly, it takes long time (at least 21 years) to develop a new coffee cultivar (Benti, 2017). Secondly, highly influenced by growing environmental factors (Adem *et al*., 2020: Merga *et al*., 2021: Beksisa 2021; Merga 2022: Gebreselassie *et al*., 2024) and growth stage of the plant (Weising *et al*., 2005). Thirdly, the coffee Arabica genetic diversity is low due to its narrow genetic base associated with autogamy, evolutionary history, and domestication (Anthony *et al*., 2002; Silvestrini *et al*., 2007; Setotaw *et al*, 2013; Meyer and Purugganan, 2013: Scalabrin *et al*, 2020; Weldemichael, 2023). Fourthly, phenotypically similar individuals may be genetically distinct, which reduces the selective efficiency.

Use of molecular markers associated with important traits can make coffee breeding more precise, rapid, allow the detection of variations in DNA level, overcome environmental factors and growth stage of the plant, and probably cost effective in comparison to phenotypic selection (Henry, 1997; *Ni et al*., 2008; Sousa *et al*, 2017). Some of the DNA based markers that have been extensively used in coffee genetic diversity studies includes Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Randomly Amplified Polymorphic DNAs (RADP), Sequence-related Amplified Polymorphism (SRAP) and Simple Sequence Repeats (SSR), single-nucleotide polymorphisms (SNPs) markers (Maluf *et al*, 2005; Mishra *et al*., 2011; Sousa *et al.*, 2017; Oktavioni *et al*., 2020; Yan *et al*, 2020; Benti *et al*., 2021).

Inter Simple Sequence Repeat (ISSR) markers are widely used in assessing genetic diversity in *Coffea arabica* due to their simplicity, cost-effectiveness, and ability to generate high polymorphism without prior genomic information (Raina *et al.,* 2011). They are particularly useful for identifying genetic variation, evaluating population structure, and fingerprinting cultivars (Raina *et al.,* 2011). However, ISSRs are dominant markers, meaning they only reveal the presence or absence of bands and cannot distinguish between homozygous and heterozygous loci, which limits their effectiveness for detailed heterozygosity or allelic frequency analysis (Zietkiewicz *et al.,*1994). This contrasts with co-dominant markers like SSRs or SNPs, which provide more detailed genetic information and are preferred for studies requiring precise genotypic data. Despite this limitation, ISSRs have been effectively applied in Arabica coffee diversity studies (Aga *et al*., 2005; Benti *et al*., 2020), and combining them with co-dominant markers is often recommended for comprehensive genetic analysis (Powell *et al*., 1996; Raina *et al*., 2001; Selvakumar *et al*., 2010; Alkimim et al., 2020). Due to shortage of budget ISSR marker is used in this study and feture research will be expected co-dominat markers.

Eeventho many studies were conducted on Arabica coffee genetic diversity, the coffee genetic study program, particularly the south Ethiopian coffee populations, has not benefited a lot from the development of the recent molecular markers. Because of this, little is known about the genetic structure and pattern of south Ethiopian coffee (EIAR, 2017), which in turn has limited the use of its diverse gene pool in the improvement program (EIAR, 2017). Accordingly, the present study was conducted estimate the genetic diversity and population genetic structure of South Ethiopian coffee Arabica genotypes for successful conservation of genetic resources for future improvement using ISSR markers.

6.2 Materials and methods

6.2.1 Plant Material

The study employed fifty Arabica coffee genotypes that were collected from four growing zones (Sidama, Gujji, Amaro, Jinka) and improved varieties (Table 6.1). The coffee trees of the genotypes were *ex-situ* conserved at the field gen bank of Awada Agricultural research sub-center, Ethiopia.

Table 6. 1 Description of Arabica coffee germplasm used for molecular genetic diversity study

Acc.	Population	Region	Loction	Specific location	Altitude	Mainainer
Ak-4	Gujji	Oromiya	Dole	Mendere	1746	AARSC/EIAR
Ak-5	Gujji	Oromiya	Dole	Mendere	1741	AARSC/EIAR
Ak-6	Gujji	Oromiya	Dole	Akoku	1755	AARSC/EIAR
Ak-10	Gujji	Oromiya	Obadola	kumina	1698	AARSC/EIAR
Ak-12	Gujji	Oromiya	Obadola	kumina	1720	AARSC/EIAR
Ak-13	Gujji	Oromiya	Obadola	kumina	1709	AARSC/EIAR
Ak-23	Gujji	Oromiya	Chembe	keca eba	1655	AARSC/EIAR
Ak-25	Gujji	Oromiya	Dida guda	rohe	1519	AARSC/EIAR
Ak-27	Gujji	Oromiya	Chembe	keca eba	1563	AARSC/EIAR
Ak-23	Gujji	Oromiya	Qebesa	Sageji Ela Bedessa	1962	AARSC/EIAR
Ak-9	Amaro	South Ethiopia	Golbe	Golbe	1880	AARSC/EIAR
Ak-10	Amaro	South Ethiopia	Golbe	Golbe	1880	AARSC/EIAR
Ak-16	Amaro	South Ethiopia	Kerma	Dogodo-2	1660	AARSC/EIAR
Ak-23	Amaro	South Ethiopia	Danobulto	Shashe	1500	AARSC/EIAR
Ak-37	Amaro	South Ethiopia	Kele town	Kele-02	1600	AARSC/EIAR
Ak-38	Amaro	South Ethiopia	Kele town	Kele	1600	AARSC/EIAR
Ak-44	Amaro	South Ethiopia	Sharo	Angushi	1600	AARSC/EIAR
Ak 47	Amaro	South Ethiopia	Darba	mane na	1700	AARSC/EIAR
Ak 52	Amaro	South Ethiopia	Tifata	Tsilalo omo	1680	AARSC/EIAR
Ak 57	Amaro	South Ethiopia	Tifata	Abetu kotsare	1700	AARSC/EIAR
Ak-1	Gamogofa	South Ethiopia	Genamer	Woyiki	1640	AARSC/EIAR
AK-5	Gamogofa	South Ethiopia	Genamer	Genamer	1600	AARSC/EIAR
AK-8	Gamogofa	South Ethiopia	Tolta	Tolta	1630	AARSC/EIAR
AK-14	Gamogofa	South Ethiopia	Metser	Zelet	1643	AARSC/EIAR
AK-42	Gamogofa	South Ethiopia	Alga	Wozader	1500	AARSC/EIAR
AK-43	Gamogofa	South Ethiopia	Koibe	Alkaido	1390	AARSC/EIAR
AK-58	Gamogofa	South Ethiopia	Banata	Meso	1400	AARSC/EIAR
AK-59	Gamogofa	South Ethiopia	Aykamer	Beso	1400	AARSC/EIAR

AK-61	Gamogofa	South Ethiopia	Aykamer	Beso	1400	AARSC/EIAR
AK63	Gamogofa	South Ethiopia	Aykamer	Beso	1450	TARC/EIAR
Aw-31	Sidama	Sidama	Dara	Aleme Kancha	1800	AARSC/EIAR
Aw-79	Sidama	Sidama	Bensa	Hedamo	1800	TARC/EIAR
AK-77	Sidama	Sidama	Dale	Loya	1750	ARC/EIAR
AK-43	Sidama	Sidama	Chicho	Bedecha	1830	AARSC/EIAR
AK-53	Sidama	Sidama	Debeka	Gerari	1770	MARSC
AK-14	Sidama	Sidama	Weto	Metero	1800	JARC/EIAR
AK-91	Sidama	Sidama	Mamena	Gona	1760	JARC/EIAR
AK-121	Sidama	Sidama	Bedelicho	Bedelicha	1980	JARC/EIAR
AK-123	Sidama	Sidama	Bedelicho	Bedelicha	1800	JARC/EIAR
AK-122	Sidama	Sidama	Foka	Kisho	1770	AARSC/EIAR
Koti			Improved Variety			AARSC/EIAR
74112			Improved Variety			JARC/EIAR
Angafa			Improved Variety			AARSC/EIAR
CH-1			Improved Variety			AARSC/EIAR
Rori			Improved Variety			AARSC/EIAR
Mechara-1			Improved Variety			MARSC
J-19			Improved Variety			TARC/EIAR
J-21			Improved Variety			TARC/EIAR
74140			Improved Variety			AARSC/EIAR
74110			Improved Variety			JARC/EIAR
Harussa			Improved Variety			JARC/EIAR
Dessu			Improved Variety			JARC/EIAR
Gesha			Improved Variety			JARC/EIAR
Mocha			Improved Variety			MARSC
75227			Improved Variety			JARC/EIAR
Odicha			Improved Variety			AARSC/EIAR

JARC =Jimma Agricultural Research Center; EIAR=Ethiopian Institute of Agricultural Research; AARSC=Awada Agricultural Research Center; TARC= Tepi Agricultural Research Center; MARSC= Mechara Agricultural Research Center

6.2.2 Sample collection

Young and healthy leaf samples were collected from growing tips of a single tree of each genotype using Eppendorf tube. Four discs leaf samples were directly placed in to an Eppendorf tube, and transported to a molecular laboratory of South Agricultural Research Institute, Hawassa, Ethiopia and stored under refrigerator until DNA extraction was done.

6.2.3 DNA Extraction

The genomic DNA extraction of coffee samples was performed using a modified cetyltrimethylammonium bromide (CTAB) protocol (Schenk *et al*., 2023). The leaf samples were crushed using a plastic micro pestle and 400ul of CTAB buffer added to the Eppendorf containing the crushed samples and vortex for 10 seconds before incubating under a water bath at 60°C for 30 minutes. The solution was incubated in a water bath at 60°C for 30 minutes. During the incubation process the solution was homogenized every 10 minutes. Following incubation, 60ul of chloroform-isoamyl alcohol was added to the mixture and centrifuged at 10,000 rpm for 5 minutes. The supernatant was transferred to a new Eppendorf tube and the chloroform isoamyl alcohol step was repeated and centrifuged again at 10,000 rpm for 5 minutes. Stages using chloroform isoamyl alcohol were performed three times to separate DNA from proteins, polysaccharides, and other impurity compounds.

The supernatant was transferred to a new 1.5 mLtube and cold isopropanol was added to 2/3 of the supernatant. The solution was homogenized and incubated at -25°C for 30 minutes. The solution was then centrifuged at 10,000 rpm for 5 minutes to form a pellet and supernatant. The pellet containing DNA was washed three times using 70% ethanol and centrifuged at 10,000 rpm for 5 minutes. The DNA pellet was re-suspended with TE-RNase and stored at -25°C. The DNA quantity and purity was measured by both a nano spectrophotometer (IMPLENNP80) and gel electrophoresis. The concentration (ng/µL) and purity of DNA was observed by nanophotometer at A260/A280. The quality of extracted DNA was observed on a 1% agarose gel. DNA was visualized under UV light using gel documentation (UVITEC).

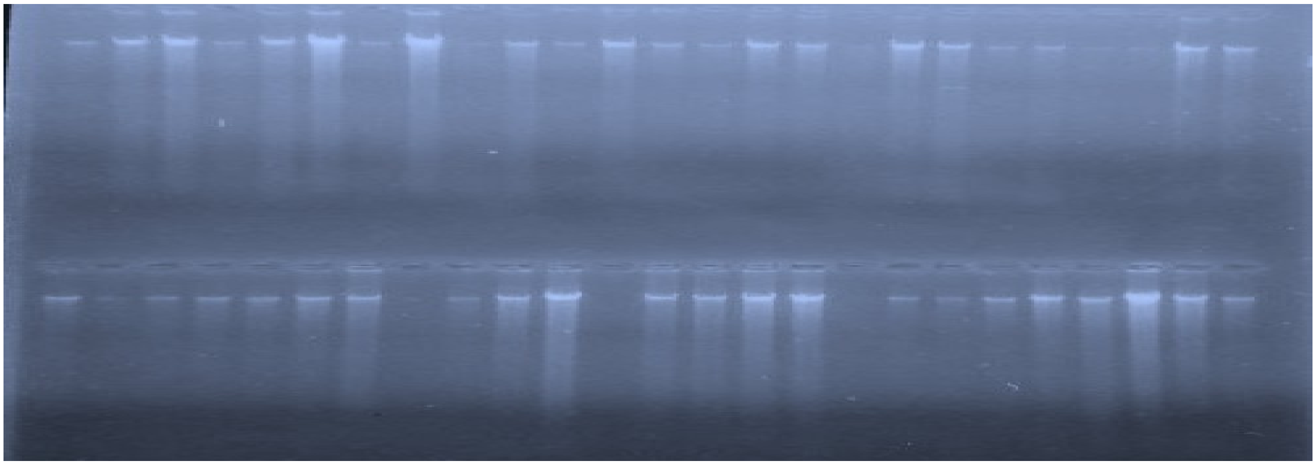

Figure 6. 1 Sample picture of visualized DNA quality using gel electrophoresis

6.2.4 PCR amplification with ISSR primers

The polymerase chain reactions (PCR) were performed to investigate the suitability of the extracted DNA for amplification. The PCR reaction was carried out using 1µg of primer, 1.5µg of template DNA, and 10.5µl of H_2O (grade), for a total volume of 13µl. The amplifications were carried out for 35 cycles of 96 °C for 3 minutes, 96 °C for 30 seconds, 49 °C for 30 seconds, 72°C for 30 seconds, followed by a final extension step of 72 °C for 5 min (Segarra-Moragues and Gleiser, 2009). The amplified DNA products were loaded in agarose gel. Gel electrophoresis was carried out on a 1.2% agarose gel in 100 ml of 1× TAE buffer at 80 volts for 90 minutes. Amplified products were visualized using gel documentation system. A total of 9 universal ISSR primers were used to determine the molecular diversity of the genotypes under examination; of which 4 primers showed high percentage of polymorphism (Table 6. 2).

Table 6. 2 List of ISSR primers used in this study

Sample No	**ISSR primers name**	**Primer Sequences**	**Length of (bp)**	**Annealing T (^{0}C)**
1	UBC825	(AC)8T	17	52
2	UBC826	(AC)8C	17	47
3	UBC842	(GA)8CG	18	52
4	UBC854	(TC)8RG	18	49
5	***UBC835***	(AG) 8 YC	18	48
6	UBC868	(GAA)6	18	55
7	UBC879	(CTTCA)3	15	51
8	UBC888	BDB(CA)7	17	52
9	UBC889	DBD(AC)7	17	48

The reproducible profiles with template DNA's are detailed below; B = (C, G, T), i.e., not A; D = (A, G, T), i.e., not C; R = (A, G), Y= (C, T); UBC = University of British Colombia, Canada

6.2.5 Agarose gel analysis and ISSR Data Scoring

The integrity of DNA was judged by agarose gel analysis using the following steps: 0.8% agarose gel was prepared in 100ml 1X TBE (Tris-borate EDTA) buffer containing 2.5µl of ethidium bromide. The gel was cast in the tray and allowed to solidify until the wells were properly formed. 20 µl of DNA samples along with 5µ| a DNA ladder were individually loaded into each well after mixing them with a drop of gel tracking dye. The gel was run at 80 V for 90 minutes. After the gel was run to about 3/4th of the tray, the gel was removed from the cast and visualized under UV light. The presence of a single compact band at the corresponding band of the DNA ladder indicates that the DNA isolated is of high molecular weight.

Data Analysis

6.2.6 Statistical Analysis

Among the tested nine ISSR markers data only four polymorphic molecular markers were considered and fragments were scored based on a binary scoring matrix for the presence (1) or absence (0) visually based on the presence and absence of bands. Number of alleles (Na), number of effective allele (Ne), observed (Ho) and expected (He) heterozygosity, Shannon's information index (I), Diversity (h), Unbiased Diversity (uh), Band Frequency (p), No. Bands, No. Bands Freq. >= 5%, No. Private Bands, No. LComm Bands (<=25%), and No. LComm Bands (<=50%) as well as analysis of molecular variance (AMOVA) were computed with GenAlExver.6.503 software (Peakall and Smouse, 2016).

Cluster analysis was carried out using DARwin software version 6.0.021 (Perrier and Jacquemoud-Collet, 2006). A dendrogram was generated based on the dissimilarity matrix as input data to visualize pattern of cluster within and among the genotypes. Principal coordinate analysis (PCoA) was computed by GenAlex version 6.503 software (Peakall and Smouse, 2016).

6.3 RESULTS AND DISCUSSIONS

6.3.1 ISSR Analysis

Among the nine ISSR primers included in this study, the four universal primers, UBC 826, UBC 835, UBC 842, and UBC 889, provided a clearly amplified product and conspicuous banding pattern whereas the remaining primers were considered unsuitable due to poor amplification. The four ISSR markers are efficient in estimating the genetic diversity of coffee Arabica genotypes. The ISSR markers have been also applied to characterize coffee germplasm in Ethiopia (Aga, 2005; Tesfaye, 2006; Belami, 2007; Tadele, 2012).

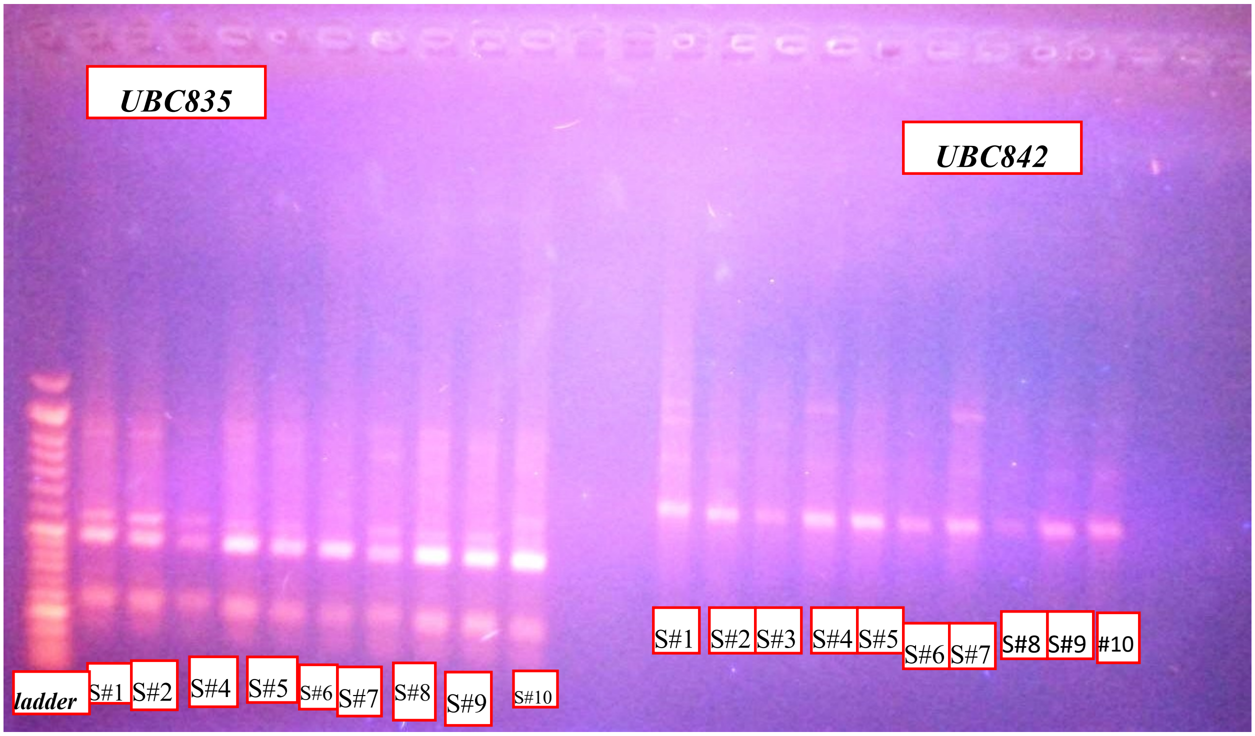

Figure 6. 2 Sample picture of banding pattern of UBC835 and UBC842 primers on improved varities.

6.3.2 Polymorphism of ISSR and Genetic diversity

The 4 ISSR markers used in this study detected 74 different bands with a frequency >= 5% for Gujji, Amaro, Jinka, Improved variety, and Sidama collections (Fig. 6. 3). The number of private bands (unique alleles) was higher for improved variety and Sidama collection 8 and 4 respectively. Private bands (unique alleles) are genetic variants found exclusively within a single population, not present in other populations being analyzed (Szpiech *et al.,* 2011). It can be crucial for a species' long-term survival and adaptation to changing environments. They might carry genes that confer resistance to new diseases, tolerance to drought, or other beneficial traits that are not found in the more common alleles (Govindaraj *et al*., 2015; Maruki *et al*., 2022). A high number of private bands among improved variety and Sidama collection suggests that the presence of many rare and unique alleles within the improved variety and Sidama coffee population being studied. Accordingly, improved variety and Sidama coffee populations with a high number of private alleles are considered particularly important for conservation efforts.

Improved variety and Sidama collection were found to show highest value of the lowest number of common bands (≤50%), 6 and 5 respectively, whereas the other populations showed the lowest value (1) (Fig 6.3). Common bands refer to alleles that are present at a high frequency across the entire sample set of populations being analyzed. They are alleles that are widespread within the population. The highest value of lowest number of common bands suggests a higher overall genetic diversity, presence of many rare alleles, reduced impact of selection bottlenecks, and heterogeneous population structure (Raychaudhuri, 2011; Kanaka *et al*., 2023). Accordingly, improved variety and Sidama collection having highest value of lowest number of common bands points to a more diverse and less homogenous coffee population, with a greater presence of rare or less frequent alleles.

Band frequency varied from 8.62% (Gujji) to 25.86% (Improved varieties) with average of 17.93% (Table 6.3). The observed high band frequency for improved varities indicates the presence of higher genetic diversity and a wider range of allele combinations among improved varities than the other populations. The detected polymorphic band in this investigation is relatively low as compared to Aga *et al*., (2003), Mishra *et al*., (2018) and Bidyananda *et al*., (2024) report; where as high as compered to Gichuru (2012), and Panaligan *et al*., (2020). Even though the genetic variability of Arabica coffee has been low due to the narrow genetic basis and self-fertile nature of the species, the existed genetic variation among the studied population is a promising result for coffee improvement program through selection of parental lines and hybridization.

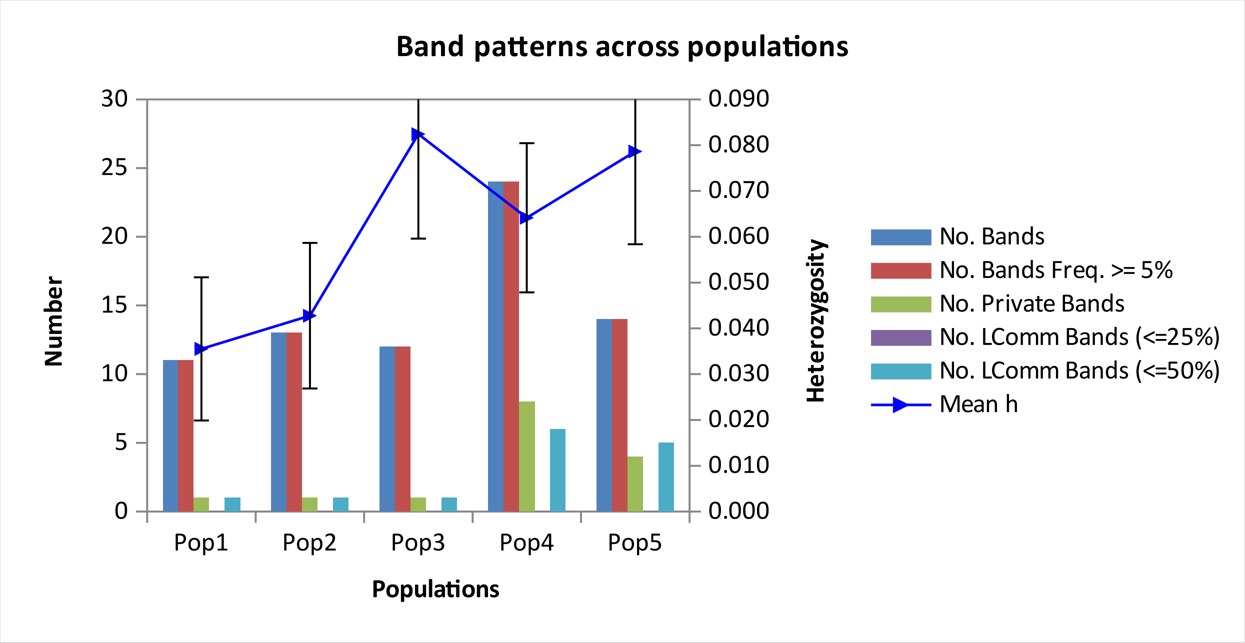

Figure 6. 3 Band patterns across populations

Where; No. Bands = No. of Different Bands; No. Bands Freq. >= 5% = No. of Different Bands with a Frequency >= 5%; No. Private Bands = No. of Bands Unique to a Single Population; No. LComm Bands (<=25%) = No. of Locally Common Bands (Freq. >= 5%) Found in 25% or Fewer Populations; No. LComm Bands (<=50%) = No. of Locally Common Bands (Freq. >= 5%) Found in 50% or Fewer Populations; h = Diversity = 1 - (p^2 + q^2)

The number of alleles per populations (Na) varied from 0.276 (Gujji) to 0.672 (improved varieties) with an average of 0.434 (Table 6.3). Number of effective alleles (Ne) varied from 1.063 (Gujji) to 1.149 (Jinka). Shannon's information index (I) varied from 0.052 (Gujji) to 0.12 (Sidama). Likewise, observed diversity (h) varied among populations from 0.036 (Gujji) to 0.082 (Jinka) with an average value of 0.061. On the other side, unbiased diversity (hu) ranged from 0.039 (Gujji) to 0.092 (Jinka) (Table 6.3). Based on Ne, I, h, and hu values, Jinka and Sidama population showed relatively high level of genetic diversity, whereas the results for the genetic diversity in Gujji and Amaro were convergent.

Table 6. 3 Genetic parameter estimates based on ISSR Marker among collection regions.

Population	**No. of genotypes**	**Na**	**Ne**	**I**	**h**	**uh**	%P
Gujji	10	0.276	1.063	0.052	0.036	0.039	8.62%
Amaro	10	0.345	1.07	0.065	0.043	0.048	12.07%
Jinka	10	0.397	1.149	0.118	0.082	0.092	18.97%
Improved variety	10	0.672	1.095	0.105	0.064	0.071	25.86%
Sidama	10	0.483	1.129	0.12	0.079	0.087	24.14%
Grand Mean	10	0.434	1.101	0.092	0.061	0.067	17.93%
Sum	50	2.173	5.506	0.46	0.304	0.337	89.66%

Where:- Na= number of allele; Ne=number of effective allele; I= Shannon's information index; h = Diversity = 1 - (p^2 + q^2); uh = Unbiased Diversity = (N / (N-1)) * h; p = Percent of polymorphic loci

6.3.3 Analysis of molecular variance

Analysis of molecular variance (AMOVA) partitioned the total genetic variation into among and within populations (Table 6.4). Of the total genetic variation, 67% of variation was attributed to the variability among populations and 33% accounted for variability within populations, indicating that the genetic variation among populations contributed more to genetic diversity (Table 6.4). The result is in agreement with previous works by Aga (2005) who reported that most of the variability was observed between populations than within populations. However, Tesfaye (2006), Belami (2007), and Geleta *et al.*, (2012) reported the higher proportion of genetic diversity was observed within the population rather than between populations.

Table 6. 4 **Analysis of molecular variance between population of Arabica coffee genotypes**

Sources of variation	Df	SS	MS	Estimated variance	Percentage of variation
Among populations	4	165.240	41.310	3.935**	67%
Within populations	45	88.000	1.956	1.956**	33%
Total	49	253.240		5.891	100%

df=degree of freedom; SS=sum of square; MS=mean square

6.3.4 Principal Component Analysis

The principal coordinate analysis (PCoA) generated by using genetic distance showed the presence of weak population structure and explained 42.96% of the cumulative variation (Figure 6.4). The first, second and third axis explained 19.45%, 14.73%, and 8.78% of the total genetic variation, respectively. As it is observed in two-dimensional plot of PCoA, genotypes collected from the same population often grouped together, and genotypes from different population differently (Figure 6.5). In both analyses, there was no definite clustering of populations, and showed consistent pattern of genetic relationship and differentiation among the accessions. This suggests gene flow or shared ancestry among populations, consistent with a weakly structured but genetically diverse population.

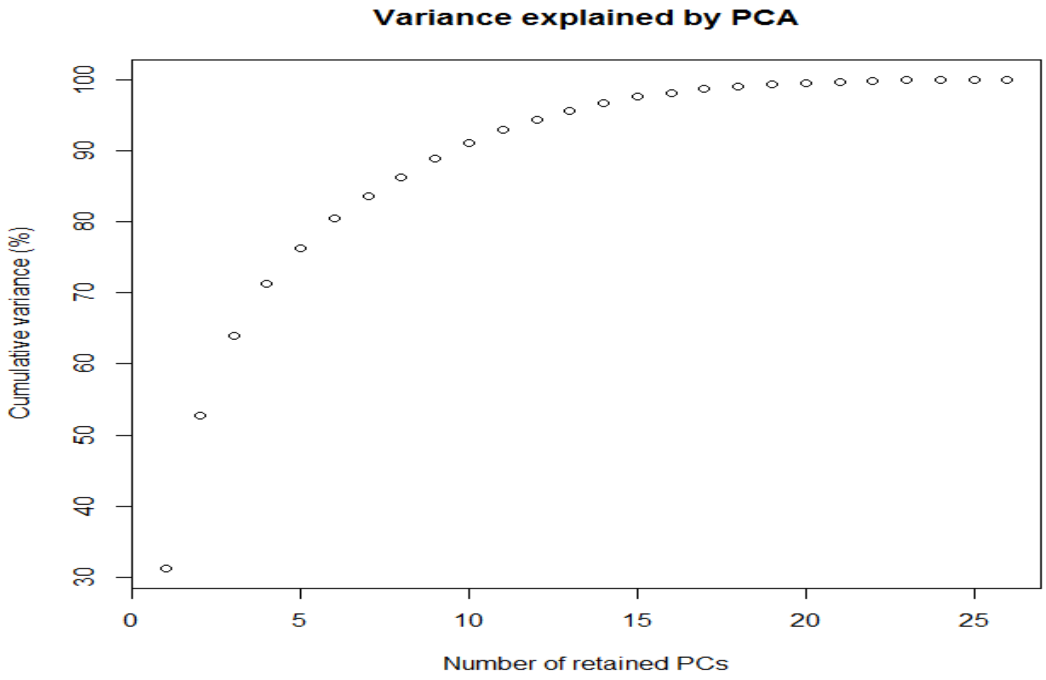

Figure 6. 4 Variance explained by principla component analysis

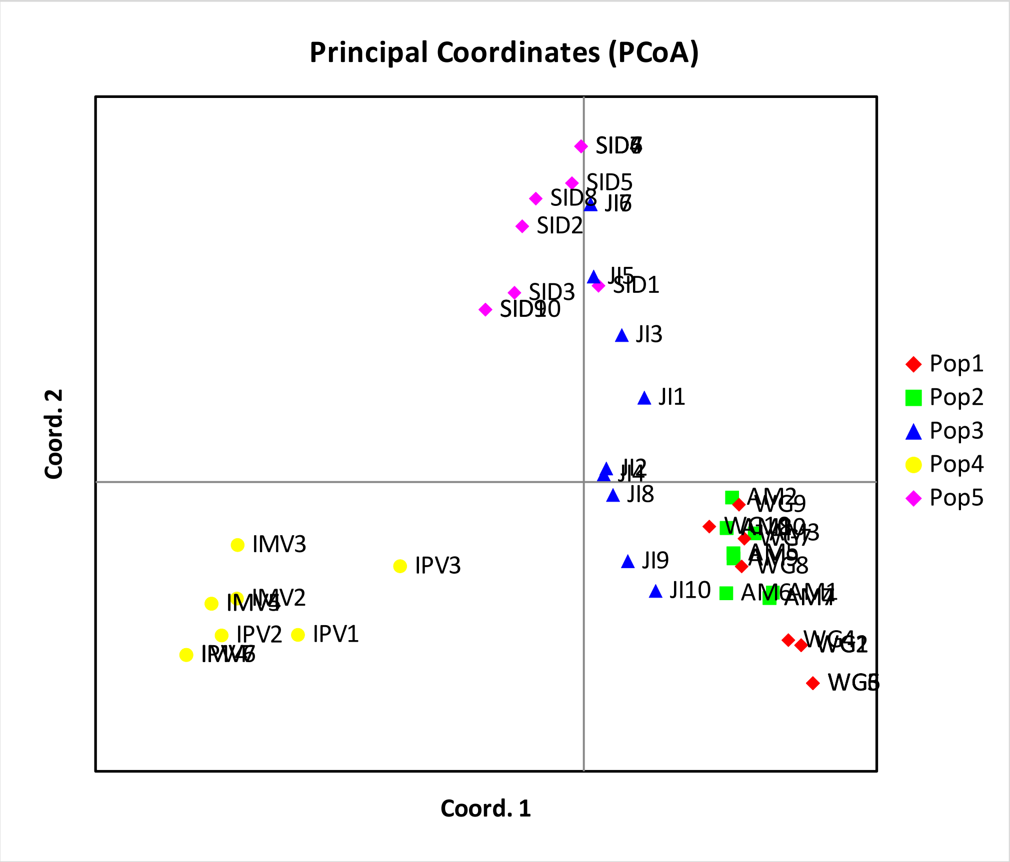

Figure 6. 5 Principal coordinates analysis (PCoA) bi-plot showing the clustering pattern of 50 Arabica coffee genotypes from five populations based on 4 ISSR marker.

To infer in the genetic structure of the populations, a discriminate analysis of principal component (DAPC) analysis was conducted using the 40 accessions and 10 improved varities. The DAPC analysis revealed five genetic clusters and four discriminant eigenvalues (Figure 6.6). Each of these clusters corresponded with genotypes derived from population collected from each collection regions. The DAPC identified five genetic clusters, with four discriminant eigenvalues capturing between-group variation. These clusters generally aligned with regional origins, providing further insight into the underlying genetic structure shaped by geographic and environmental factors.

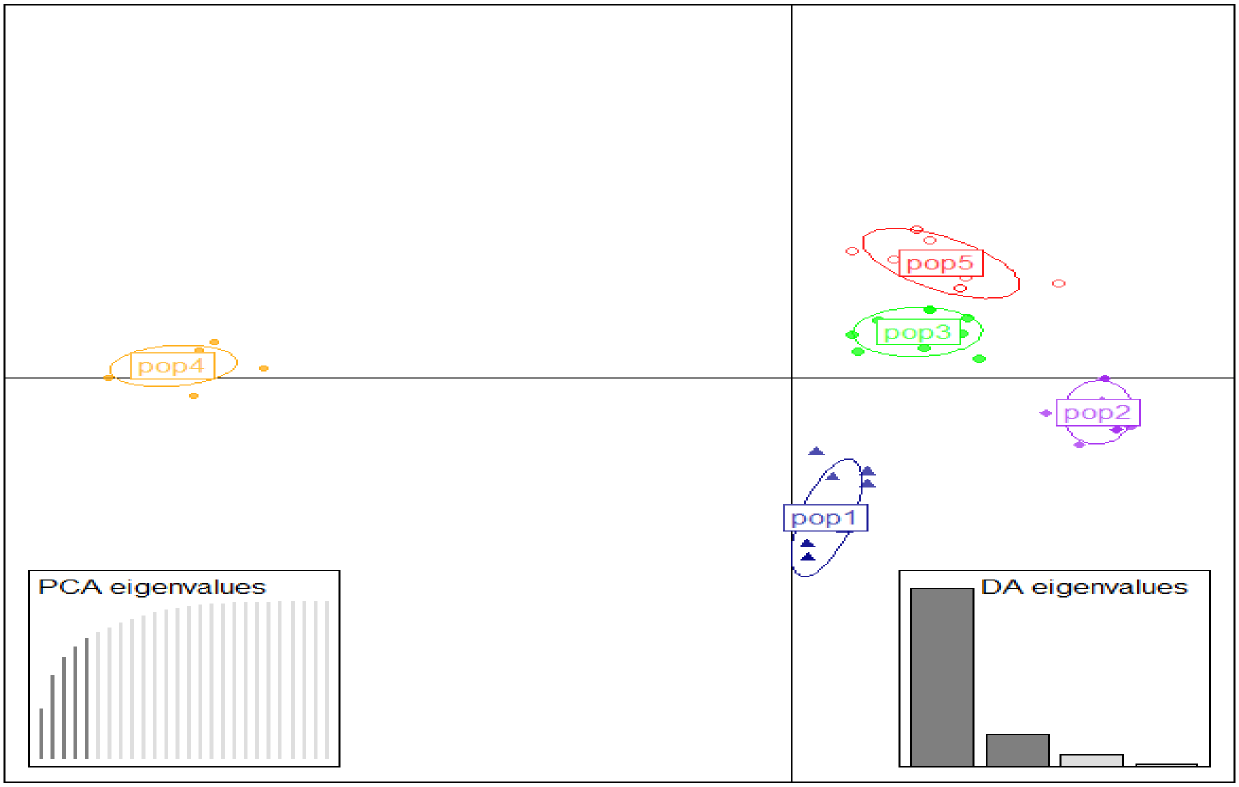

Figure 6. 6 Discriminant analysis of principal components (DAPC) for the 50 arabica coffee genotypes. Dots represent individuals whereas colours denoting sampling origin.

6.3.5 Cluster analysis

The cluster analysis based UPGMA method grouped the 40 Arabica coffee accessions and 10 improved varities into four major clusters, indicating the presence of significant genetic diversity among them (Figure 6.7). Cluster I consisted 10 genotypes (20%), suggesting a moderately distinct group with shared genetic characteristics. Cluster II comprised 14 genotypes (28%), representing the second-largest cluster, possibly reflecting a group with closer genetic similarity. Cluster III was the smallest with 6 genotypes (12%), implying a more genetically distinct or less common group of genotypes that might possess unique traits. Cluster IV, the largest group with 20 genotypes (40%), likely includes more genetically related genotypes. The formation of these distinct clusters and sub-groups suggests a wide range of genetic variation within the Arabica coffee genotypes studied.

The clustering of accessions and improved varieties into four distinct groups has important implications for breeding. It highlights the presence of significant genetic diversity, which can be exploited to select genetically distant parents for crossing, thereby enhancing hybrid vigor and trait improvement. The smaller and more distinct Cluster III may harbor unique alleles valuable for introducing novel traits. The grouping also suggests potential adaptation to specific environments, guiding breeders in developing varieties suited to diverse agro-ecologies. Moreover, understanding the genetic structure helps in conserving valuable germplasm and avoiding redundancy in breeding programs, ultimately supporting the development of improved, resilient, and high-performing coffee cultivars.

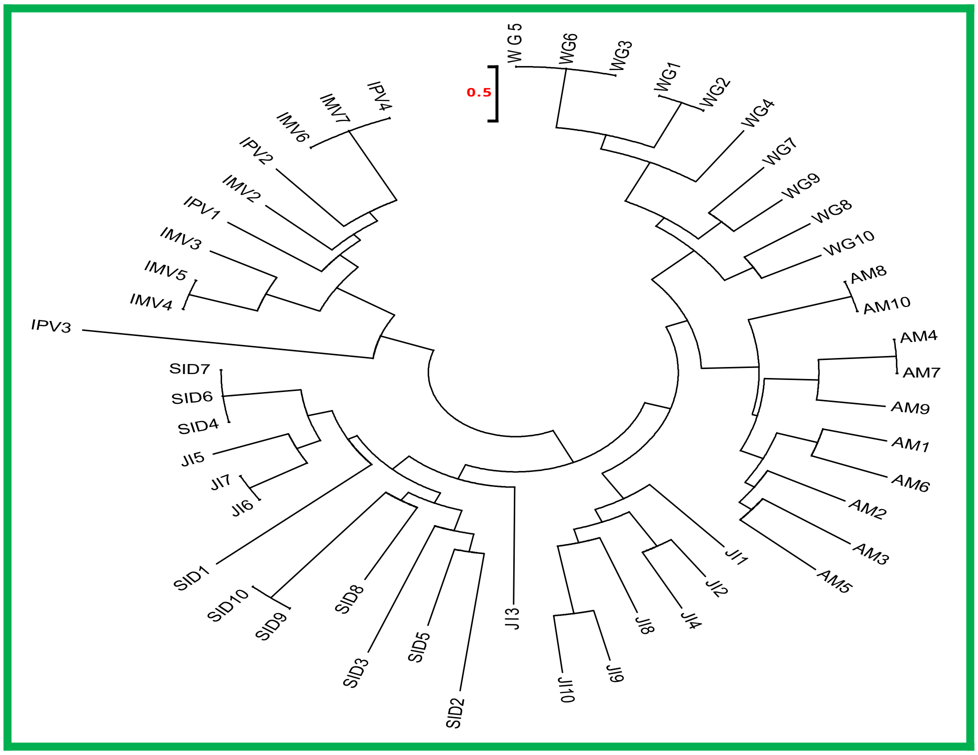

Figure 6. 7 UPGMA dendrogram representing the genetic relationships among the indicated Arabica coffee genotypes.

6.4 Summery and Conclusion

Genetic diversity analysis of 50 arabica coffee genotypes using 4 SSR markers showed the presence of genetic vdiversity among genotypes. The different diversity indices obtained in this study showed that the ISSR markers used were efficient and informative for Ethiopian Arabica coffee diversity study. High level of genetic diversity among population of Jinka and Sidama was identified; suggesting that the germplasm has enormous opportunity in the improvement program through direct selection. The analysis of molecular variance (AMOVA) revealed the presence of significant genetic variability; with diversities mainly distributed among populations. The generated UPGMA dendrogram along with PCoA confirmed the result from AMOVA. The findings indicate a moderate level of genetic diversity within and among populations, highlighting the necessity for conservation measures to preserve unique genotypes that are crucial for sustainable coffee production. In these population clustering analyses, genotypes from the same populations were found to be clustered similarly and vise-versa.

Although ISSR markers utilized and proved useful genetic diversity information in this study, it is limited due to its dominant, lower reproducibility, and difficulty in pinpointing specific genomic locations. Therefore, it is strongly recommended that future studies adopt co-dominant marker systems such as SSR (Simple Sequence Repeats) or SNP (Single Nucleotide Polymorphisms), which are a co-dominant, highly reproducible, and can provide precise information about gene locations, enabling more effective association mapping and gene discovery for south Ethiopian coffee yield and quality traits in future research.

While this study provided useful information on genetic diversity, it lacks the resolution necessary to pinpoint specific genes influencing important agronomic traits to facilitate a more direct and efficient link between phenotypes and genotypes. Therefore, I strongly recommend a transition to association studies breeding strategies to link phenotypes with genotypes for yield and quality improvement of coffee in south Ethiopia. The association study will facilitate the identification of candidate genes underlying important agronomic traits.

6.5 Reference

Adem, A., Mohammed, H., & Ayana, A. (2020). Phenotypic Diversity in Arabica Coffee Genotypes from Eastern Ethiopia. 5(4), 42–47. https://doi.org/10.11648/j.ijee.20200504.11

Aga (2005). Molecular genetic diversity study of forest coffee tree (Coffea arabica L.) populations in Ethiopia: implications for conservation and breeding (Vol. 2005, No.79).

Aga E, Bryngelsson T, Bekele E, Salomon B (2003). Genetic diversity of forest arabica coffee (Coffea arabica L.) in Ethiopia as revealed by random amplified polymorphic DNA (RAPD) analysis. Hereditas. 138: 36-46.

Aga, E., Bekele, E., & Bryngelsson, T. (2005). Genetic diversity of forest Arabica coffee (Coffea arabica L.) populations in Ethiopia as revealed by RAPD analysis. Hereditas, 142(1), 47–52.

Anthony, F., Combes, M. C., Astorga, C., Bertrand, B., Graziosi, G., & Lashermes, P. (2002). The origin of cultivated Coffea arabica L. varieties revealed by AFLP and SSR markers. Theoretical and Applied Genetics, 104(5), 894–900. https://doi.org/10.1007/s00122-001-0798-8

Bayetta Belachew, (2015). ACRN activity report for the period 2013 – 2015. IACO Abidjan, 2015.

Beksisa, L. (2021). GGE Biplot Analysis of Genotype x Environment Interaction and Bean Yield Stability of Arabica Coffee (Coffee arabica L .) Genotypes in Southwestern Ethiopia. 9(3), 110–115. https://doi.org/10.11648/j.ajbio.20210903.16

Belami, S. (2007) Genetic diversity analysis of the wild Coffea arabica L. populations from Harennaforest, Bale Mountains of Ethiopia, using inter simple sequence repeats (ISSR) marker, AAU.

Benti, T. (2017). Progress in Arabica Coffee Breeding in Ethiopia: Achievements, Challenges and Prospects. International Journal of Sciences: Basic and Applied Research, 33(2), 15–25. http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Benti, T., Gebre, E., Tesfaye, K., Berecha, G., Kyallo, M., & Yao, N. K. (2021). Genetic diversity among commercial arabica coffee (Coffea arabica L .) varieties in Ethiopia using simple sequence repeat markers. Journal of Crop Improvement, 35(2), 147–168. https://doi.org/10.1080/15427528.2020.1803169

Bøhn, S. K., N. C. Ward, J. M. Hodgson, and K. D. Croft. 2012. Effects of tea and coffee on cardiovascular disease risk. Food & function 3(6):575–591.

Cristancho, MA, Chaparro, AP, Cortina, HA and Gaitàn, AL. 2004a. Genetic variability of Coffea arabica L. accessions from Ethiopia evaluated with RAPDs. ASIC 20th. Bangalore, India.

EIAR (Ethiopian Institute of Agricultural Research), 2017. National Coffee Commodity Research Strategy of Fifteen Years (2016 -2030). Addis Ababa, Ethiopia. 124: 213–221.

Fairtrade Foundation, (2022). Coffee farmers. https://www.fairtrade.org.uk/farmers-and-workers/coffee/

Gebreselassie, H., Tesfaye, B., Gedebo, A. (2024): Genetic diversity of Arabica coffee genotypes in south Ethiopia using quantitative agro-morphological traits. Genet Resour Crop Evol 71, 3485–3506 . https://doi.org/10.1007/s10722-023-01853-9

Geleta M., Isabel H,, Arnulfo M. and Tomas B., (2012). Genetic diversity of arabica coffee (Coffea arabica L.) in Nicaragua as estimated by simple sequence repeat markers. The Scientific World Journal.

Geremew, M., Satheesh, N. and Fanta, S.W. (2022). Rrole of coffee in ethiopian ethnic culture-a coffee festival 2nd international conference on coffee and cocoa. Available at: www.iksad.co.uk%7Ccontact@iksad.co.uk.

Geremew, M., Satheesh, N., & Fanta, S. W. (2022). role of coffee in ethiopian ethnic culture-a coffee festival 2nd international conference on coffee and cocoa role of coffee in ethiopian ethnic culture-a coffee festivaL. June 2024. www.iksad.co.uk %7Ccontact@iksad.co.uk

Gichuru, E. K. (2012). Genetic diversity among commercial coffee varieties , advanced selections and museum collections in Kenya using molecular markers. 4(February), 39–46. https://doi.org/10.5897/IJBC11.231

Gökcen, B. B., & Şanlier, N. (2019). Coffee consumption and disease correlations. Critical Reviews in Food Science and Nutrition, 59(2), 336–348. https://doi.org/10.1080/10408398.2017.1369391

Gökcen, B.B. and Şanlier, N. (2019) 'Coffee consumption and disease correlations', Critical Reviews in Food Science and Nutrition, 59(2), pp. 336–348. https://doi.org/10.1080/10408398.2017.1369391

Govindaraj M., Vetriventhan M., Srinivasan M., (2015). Importance of genetic diversity assessment in crop plants and its recent advances: an overview of its analytical perspectives. Genet Res Int. doi: 10.1155/2015/431487. Epub 2015 Mar 19. PMID: 25874132; PMCID: PMC4383386.

Henry R.J., 1997. Molekular markers in plant improvement. In: Practical application of plant molecular biology. Chapman and Hall, New York, pp 101-134.

Higdon, J. V., and B. Frei. 2006. Coffee and health: a review of recent human research. Critical reviews in food science and nutrition 46(2):101–123.

ICO (2016). Historical Data on the Global Coffee Trade; http://www.ico.org/ new- historical.asp, accessed on 20 July 2022.

Kanaka, K. K., Sukhija, N., Goli, R. C., Singh, S., Ganguly, I., Dixit, S. P., Dash, A., & Malik, A. A. (2023). On the concepts and measures of diversity in the genomics era. Current Plant Biology, 33(March), 100278. https://doi.org/10.1016/j.cpb.2023.100278

Labouisse, J. P., Bellachew, B., Kotecha, S., & Bertrand, B. (2008). Current status of coffee (Coffea arabica L.) genetic resources in Ethiopia: implications for conservation. Genetic Resources and Crop Evolution, 55, 1079–1093. https://doi.org/10.1007/s10722-008-9321-7

Lachenmeier, D. W. (2023). Identification of Coffee Species, Varieties, Origins, and Processing and Preparation Methods—A Status Report. 9. https://doi.org/10.3390/icc2023-14824

Lashermes P, Trouslot P., Anthony C. and Charrier A., 1996. Genetic diversity for RAPD markers between cultivated and wild accessions of Coffea arabica. Euphytica 87, 59-64

Lashermes, P., Combes, M. C., Robert, J., Trouslot, P., D'Hont, A., Anthony, F., & Charrier, A. (1999). Molecular characterisation and origin of the Coffea arabica L. genome. Molecular and General Genetics, 261(2), 259–266. https://doi.org/10.1007/s004380050956

Maluf M.P, Silvestrini M., Ruggiero L.C.M., Guerreiro Filho O., Colombo C.A., 2005. Genetic diversity of cultivated Coffea arabica inbred lines assessed by RAPD, AFLP and SSR marker systems. Sci. Agric. 62:366-373.

Maruki T, Ye Z, Lynch M., (2022). Evolutionary Genomics of a Subdivided Species. Mol Biol Evol. 3;39(8):msac152. doi: 10.1093/molbev/msac152. PMID: 35895848; PMCID: PMC9372567.

Masumbuko L.I and Bryngelsson T., 2006. Inter simple sequence repeat (ISSR) analysis of diploid coffee species and cultivated Coffea arabica L. from Tanzania. Gen. Res. Crop Evol., 53: 357-366.

Merga, D., Mohammed, H., & Ayano, A. (2021). Estimation of Genetic Variability , Heritability and Genetic Advance of Some Wollega Coffee (Coffea arabica L .) Landrace in Western Ethiopia Using Quantitative Traits. 9(4), 182–191. https://doi.org/10.11648/j.jps.20210904.18

Merga, W. (2022). Bean yield stability analysis of coffee (Coffea arabica L) genotypes in south western Ehiopia. Ternational Journal of Recent Scientific Research, 13(ISSN: 0976-3031), 1–8. https://doi.org/10.24327/IJRSR

Meyer R.S., Purugganan M.D. (2013). Evolution of crop species: genetics of domestication and diversification. Nat Rev Genet. 14(12):840-52. https://doi.10.1038/nrg3605.

Mishra M.K., Tornincasa P., De Nardi B., Asquini E., Dreos R., Del Terra L., Rathinavelu R., Rovelli P., Pallavicini A. and Graziosi G., (2011). Genome organization in coffee as revealed by EST PCR-RFLP, SNP and SSR analysis. J. Crop Sci. Biotech. 14: 17-24.

Mishra, M. K., Nishani, S., Gowda, M., Padmajyothi, D., Suresh, N., Sreenath, H., & Raghuramulu, Y. (2018). Genetic Diversity Among Ethiopian Coffee (Coffea Arabica L.) Collections Available In Indian Gene Bank Using Sequence Related Amplified Polymorphism Markers. Plant Breeding and Seed Science, 70(1), 29–40. https://doi.org/10.1515/plass-2015-0011

Muhie, S. H. (2022). Strategies to improve the quantity and quality of export coffee in Ethiopia, a look at multiple opportunities. Journal of Agriculture and Food Research, 10(April), 100372. https://doi.org/10.1016/j.jafr.2022.100372

Mulatu Geleta, Isabel Herrera, ArnulfoMonz, and T. B. (2012). Genetic Diversity of Arabica Coffee (Coffea arabica L .) in Nicaragua as Estimated by Simple Sequence Repeat Markers. 2012. https://doi.org/10.1100/2012/939820

Ni S., Yaho M., Chen L., Zhao L. and Wang X.C., (2008). Germplasm and breeding research of tea plant based on DNA marker approaches. Front. Agric. China. 2, 200-2007.

Nkondjock, A. 2009. Coffee consumption and the risk of cancer: an overview. Cancer letters 277(2):121–125.

Ogutu, C., Fang, T., Yan, L., Wang, L., Huang, L., Wang, X., … Han, Y. (2016). Characterization and utilization of microsatellites in the Coffea canephora genome to assess genetic association between wild species in Kenya and cultivated coffee. Tree Genetics & Genomes, 12(3), 54.

Oktavioni, M., Mada, U. G., Irawati, I., Andalas, U., Syukriani, L., Andalas, U., Setiawan, M. A., & Andalas, U. (2020). Analysis of genetic diversity of Arabica coffee [Coffea arabica L .] in Solok Regency by SRAP molecular markers

Panaligan, A. C., Baltazar, M. D., & Alejandro, G. J. D. (2020). Short communication: Genetic polymorphism of registered and popularly cultivated coffee (coffea spp.) in the philippines using inter-simple sequence repeats markers. Biodiversitas, 21(9), 4228–4233. https://doi.org/10.13057/biodiv/d210938

Peakall, R. and P.E. Smouse. 2016. GENALEX6: Genetic Analysis in Excel. Population genetic software for teaching and research. Molecular Ecology. 6: 288-295.

Perrier, X. and J.P. Jacquemoud-Collet. 2006. DARwin software. http://darwin.cirad.fr/Darwin

Raychaudhuri S., (2011). Mapping rare and common causal alleles for complex human diseases. 147(1):57-69. doi: 10.1016/j.cell.2011.09.011. PMID: 21962507; PMCID: PMC3198013.

Reddy, M. P., Sarla, N., & Siddiq, E. A. (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica, 128, 9–17. https://doi.org/10.1023/A:1020691618797

Scalabrin, S., Toniutti, L., Di Gaspero, G., Scaglione, D., Magris, G., Vidotto, M., Pinosio, S., Cattonaro, F., Magni, F., Jurman, I., Cerutti, M., Suggi Liverani, F., Navarini, L., Del Terra, L., Pellegrino, G., Ruosi, M. R., Vitulo, N., Valle, G., Pallavicini,.. A., Bertrand, B. (2020). A single polyploidization event at the origin of the tetraploid genome of Coffea arabica is responsible for the extremely low genetic variation in wild and cultivated germplasm. Scientific Reports, 10(1), 1–13. https://doi.org/10.1038/s41598-020-61216-7

Schenk, J. J., Becklund, L. E., Carey, S. J., & Fabre, P. P. (2023). What is the "modified" CTAB protocol? Characterizing modifications to the CTAB DNA extraction protocol. Applications in Plant Sciences, 11(3), 1–11. https://doi.org/10.1002/aps3.11517

Segarra-Moragués J.G. and G. Gleiser. 2009. Isolation and characterization of di and tri nucleotide microsatellite loci in Rosmarinus officinalis (Lamiaceae), using enriched genomic libraries. Conservation Genetics 10: 571–575.

Setotaw TA, Caixeta ET., Pereira AA. *et al*., 2013. Coefficient of parentage in Coffea Arabica L. cultivars grown in Brazil. Crop Sci 53:1237–1247. https://doi.org/10.2135/cropsci2012.09.0541

Silvestrini M., Junqueira M.G., Favarin A.C., Guerreiro-Filho O., Maluf M.P., Silvarolla M.B. and Colombo C.A., 2007. Genetic diversity and structure of Ethiopian, Yemen and Brazilian Coffea arabica L. accessions using microsatellite markers. Genet Resour Crop Evol 54:1367-1379.

Sousa, T. V., Caixeta, E. T., Alkimim, E. R., de Oliveira, A. C. B., Pereira, A. A., Zambolim, L., & Sakiyama, N. S. (2017). Molecular markers useful to discriminate Coffea arabica cultivars with high genetic similarity. Euphytica, 213(3). https://doi.org/10.1007/s10681-017-1865-9

Tadele, S., 2012. Genetic diversity of coffee (*Coffea arabica* L.) landraces from major coffee growing areas of southern Ethiopia as revealed by inter simple sequence repeat marker. An M. Sc. Thesis submitted to the school of graduate studies of Haramaya University, 10-45PP

Tesfaye K., 2006. Genetic Diversity of Wild Coffea arabica Populations in Ethiopia as a contribution to conservation and use planning. PhD thesis, Bonn University, Germany. Pp 142.

Vavilov, N.I., 1951. The origin, variation, immunity and breeding of cultivated plants. (Translated by S. K. Chestitee). Chronica Botonica, 13, 1-366

Weising, K, Nybom, H, Wolff, K and Kahl, G. 2005. DNA Fingerprinting in Plants: Principles, Methods, and Applications. 2nd ed. CRC Press, Taylor and Francis Group. http://dx.doi.org/10.1201/9781420040043

Weldemichael, G. (2023). Review on Coffee (Coffea arabica L .) Genetic Diversity Studies Using Molecular Markers. 11(5), 164–170. https://doi.org/10.11648/j.jps.20231105.14

Woyesa, T., & Kumar, S. (2021). Potential of coffee tourism for rural development in Ethiopia: a sustainable livelihood approach. Environment, Development and Sustainability, 23(1), 815–832. https://doi.org/10.1007/s10668-020-00610-7

Yan W, Hunt LA, Sheng Q, Szlavnics Z (2000). Cultivar evaluation and mega-environment investigation based on the GGE biplot. J Crop Sci 40(3):597–605

Yilma Yemane Teferaa, 2017. A guide to coffee production in Ethiopia. Addis Ababa Ethiopia. P 1-280.

Zietkiewicz, E., Rafalski, A., & Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics, 20(2), 176–183. https://doi.org/10.1006/geno.1994.1151

7. General Summery, Conclusion and Recommendations

7.1 Summery and Conclusion

Coffee is a well-known pillar of the Ethiopian economy, covering an average of 32% of the total government's export revenue, 35% of agricultural export share, and provides livelihoods to about 25 million smallholder farmers and other actors directly or indirectly. In addition to economic importance, it is also the defining feature and sphere of Ethiopian culture, politics, social life, and identity. Despite the importance of coffee in Ethiopia's economy, the presence of huge genetic resources, a favorable climate, and soil types for coffee production and productivity, the crop yield is by far very low as compared to the yield recorded in Vietnam (2.4 ton/ha), Brazil (1.4 ton/ha), Colombia (0.9 ton/ha), and the world average (0.8 ton/ha). Of the many reasons, lack of yield compitative improved variety is one of the major challenges for low prodctivity. On the other side, the little information was generated for wider adaptability and stability, morphological, biochemical, and molecular diversity among South Ethiopian genotypes. Thus, these studies were conducted to investigate the morphological, biochemical, and molecular diversity and their association with the yield and quality of coffee genotypes in the Ethiopian growing environment. The objectives of the study were to characterize and evaluate south Ethiopian coffee genotypes based on qualitative and quantitative agro-morphology traits, to determine the GEI on coffee bean yield and identify the stable genotypes that exhibit wide adaptation across the diverse agroecologies of south Ethiopia, to investigate biochemical and cup quality variability among south Ethiopian coffee genotype, and to analyze the genetic diversity and population structure of South Ethiopian coffee genotypes using ISSR markers.

Five studies were conducted to investigate morphological, biochemical and genetic diversity and their associations with yield and quality of coffee genotypes. The first two studies were designed to characterized 17 coffee genotypes consisting fourteen promising selection and three released varieties for qualitative and quantitative agro-morphological traits in Southwest Ethiopia using RCBD with three replications. In the third study, 17 coffee genotypes were evaluated in twelve environments in south Ethiopia to analyze the extent of genotype by environment interaction (GEI) effect on Arabica coffee yield and to identify the stable yielding genotypes. In the fourth experiment, variability in physical and cup quality as well as biochemical composition of 30 coffee genotypes (the 17 used for characterization and 13 additional promising selections from Awada subcenter) was analyzed. The fivth activity was carried out to analyze genetic diversity of the 50 coffee genotypes collected from Sidama, Gujji, Amaro, and Jinka areas of Southern Ethiopia using 4 Inter Simple Sequence Repeat Markers at Hawassa Biotechnology Research molecular laboratory.

The characterization of Arabica coffee genotypes for qualitative traits displayed a wide range of phenotypic variation, with the Shannon-Weaver diversity index ranging from 0.22 (least polymorphic) to 1.12 (most polymorphic) and an overall mean of 0.67. The chi-square test revealed significant differences for the tarits of branching habit, stipule shape, young leaf colour, leaf shape, leaf apex shape, leaf petiole colour, young shoot colour, overall appearance, canopy diameter, fruit colour, fruit shape, pulp thickness, seed shape, suggesting dominant phenotypic variation among the evaluated traits. Path coefficient analysis showed a positive direct effect of angle of insertion of primary branches, canopy diameter, overall appearance, and leaf apex shape on branching habit. From the PCA, important variables in coffee genotype with respect to qualitative morphological characters were leaf shape, leaf apex shape, canopy diameter, overall appearance, angle of insertion of primary branches, plant height, branching habit, pulp thickness, and young leaf color.

The genotypes were classified into five groups based on the results of the qualitative phenotypic cluster analysis. Cluster-V had the maximum number of genotypes (41.18%), followed by cluster-III (35.29%), and cluster-IV (11.76%). 74112 is the only variety found in cluster I and this genotype had a chance to develop hybrid vigor through crossing diverged parents found in different clusters. The pairwise generalized squared distance among the clusters showed significant divergence between the clusters. Accordingly, the maximum genetic recombination and higher heterotic F1 offspring are expected from crosses involving parents selected from clusters 1 genotype 74112 and clusters 5 genotype AW1777, Feyate, AW7494, AW105, AW9628, Angafa, AW9662.

The analysis of variance for quantitative morphological characters revealed significant variation among genotypes, environments, and GEI for the traits of number of primary-branches, fruit length, fruit width, fruit thickness, empty fruit rate, fruit filling coefficient, seed length, seed width, seed thickness, and yield. Mean performance analysis confirmed the presence of superior selection over the released check varieties for bean yield. The presence of variability among the genotypes reveals that there is a good chance of improving south Ethiopian coffee through selection and hybridization. The combined analysis of variance showed significant variation among genotypes and locations for most of the studied quantitative traits and GxE interaction for bean yield, fruit length, number of primary-branches, fruit thickness, fruit width, empty fruit rate, fruit filling coefficient, seed width, seed length, and seed thickness. The presence of significant GxE interaction indicated the inconsistency in performance of the genotypes for those traits across environments.

The estimation of genotypic, phenotypic, and environmental coefficients of variance showed a wider range of variation for the studied quantitative traits. The phenotypic variance was higher than the genotypic variance for the characters studied, suggesting a substantial influence of the environment besides the genetic variation on the expression of these traits. High-to-moderate GCV and PCV values were observed for the traits of fruit filling coefficient, height up to the first primary, bean yield, plant height, canopy diameter, number of secondary-branches, length of the longest primary, and leaf petiole length, and these traits can provide a high chance for effective selection. Moderately high to very high heritability, along with moderate to high expected genetic advance as a percent of the mean, was observed for bean yield, length of longest primary branch, stem girth, number of secondary branches, canopy diameter, leaf length, number of primary branches, leaf petiole length, leaf width, single berry weight, height up to the first primary, inflorescence stalk length, stamen stalk length, and seed width. Therefore, selection in the next generation based on these characters would be effective.

The correlation coefficient analysis revealed positive associations for most of the studied agro-morphological characters among themselves and with yield in ton ha^{-1} showing the possibility of indirect selection in the yield improvement program. Genotypic path coefficient analysis showed a positive direct effect of 11 characters (number of secondary branches, number of primary branches, leaf length, length of longest primary branch, leaf width, inter node length on longest primary branch, canopy diameter, seed length, single berry weight, stem girth, and stamen stalk length) on yield, indicating the significance of these traits for direct selection to increase coffee yield ton ha^{-1}. The number of primary branches exerted the highest positive direct effect on bean yield and a positive indirect effect on all significantly associated traits except internode length, main-stem leaf width, and single berry weight. Therefore, direct selection using the number of primary branches would be advisable for the improvement of coffee yield. Cluster analysis grouped genotypes into two main clusters and four sub-clusters. From the principal component analysis result, the most important traits contributing more to the variation were yield, number of primary branches, inter-node length on the main stem, number of secondary branches, length of the longest primary, inter-node length on the longest primary, leaf width, leaf length, fruit length, fruit width, single berry weight, and seed thickness. As a result, the aforementioned traits must be considered for effective parent selection in the coffee genotype. The result confirmed the existence of sufficient diversity among quantitative agro-morphological traits in south Ethiopian coffee genotypes, which could make it possible to increase the yield potential of coffee genotypes under south Ethiopian specialty growing conditions.

The study of genotype by environment interaction and stability analysis using AMMI and GGE-Biplot models revealed the existence of highly significant variation among coffee genotypes, environments, and GEI for coffee bean yield performance tested across different environments and seasons. The existence of significant variation confirmed the presence of variability in the inherent genetic make-up among the tested coffee genotypes, differences in climatic and edaphic conditions at different testing environments, and the differential response of tested genotypes across environments for the bean yield trait. The main proportion of the total difference in bean yield was explained by environmental factors (71.74%), followed by GEI (17.52%), indicating that the environment is the most important factor influencing the coffee bean yield performance in Ethiopia. As a result, testing genotypes in various environments prior to deciding on a variety to use in a larger agroecology is an important task for coffee breeders.

The multivariate analysis methods used in this study to explore GEI effects were analysis of variance, AMMI, and GGE-biplots analysis. Each method provided essential information on interaction structuring in a different manner but was complementary, thereby making them all useful. Furthermore, using multiple models is helpful to increase the reliability of genotypes' stability potential across different environments.

According to the AMMI biplot, E7, E1, E5, E3, and E11 were identified as high-yielding and favorable environments, whereas E12, E6, E10, E9, E4, E2, and E8 were the lower-yielding and unfavorable environments. From the high-yielding environments, E3, E11, and E7 tended to have the highest contributions to GEI. Likewise, the high-yielding genotypes that produce above-average means are G7, G5, G14, G15, G16, G12, G11, and G13, which are suited to the right side of the abscissa, whereas the low-yielding genotypes are G4, G3, G10, G8, G9, and G6. Among the high-yielding genotypes, G14, G5, and G16 were found to be more stable, whereas G13 and G7 were highly unstable.

The which-won-where polygon view of the GGE-biplot was formed by connecting seven vertex genotypes, viz., G13, G12, G5, G7, G4, G10, and G9. It partitions the twelve environments into five different coffee-growing mega-environments with different winning genotypes. According to the GGE-biplot model, E1 and E5 environments were the most representative and discriminative of the genotypes, whereas E6, E12, E10, E9, and E8 environments provided little information about the genotypes differences. In this model, G5 and G16 were both high-yielding, stable genotypes and therefore considered for further breeding programs. In general, this study showed that it could be possible to increase the yield potential of coffee genotypes under its growing conditions either by using more widely adaptable coffee types or location-specific high-yielding genotypes under favorable environmental conditions.

The analysis of variance for physical characteristics, cup quality, and biochemical content characters revealed the existence of statistically significant variation among genotype, location, and GEI effects for all studied traits. Therefore, the studied genetic resources should be properly utilized in the quality improvement program through selection and hybridization for the emerging specialty coffee markets, and the coffee quality improvement program should give due attention to incorporating genetic and environmental influences by using a multi-locational selection strategy.

The overall coffee quality score for tested genotypes in three locations were above 80% for most of the studied genotypes. Therefore, in terms of quality, most of the tested genotypes can be used to produce specialty coffee in the study areas. Out of all tested genotypes in this study, AW9648 achieved the highest score in green bean physical attributes and cup quality parameters at all three-tested locations. Therefore, AW9648 could be used as the best selection and best parent for hybridization for South Ethiopian coffee quality improvement.

From the PCA, it may be concluded that important variables in the coffee genotype with respect to bean physical, cup quality, and biochemical traits were caffeine, chlorogenic acid, aromatic quality, trigonelline, acidity, astringency, flavor, and screen size. These variables might be taken into consideration for the effective selection of parents.

Genetic diversity analysis of 50 coffee genotypes using 4 SSR markers showed the presence of genetic variability among genotypes. The different diversity indices obtained in this study showed that the ISSR markers used were efficient and informative for Ethiopian Arabica coffee diversity study. High level of genetic diversity among population Jinka and Sidama was identified; suggesting that the germplasm has enormous opportunity in the improvement program through direct selection. The analysis of molecular variance revealed the presence of significant genetic variability, with the highest value of genetic variation found among populations (67%), whereas a low value of genetic variance was observed within populations (33%). The generated UPGMA dendrogram along with PCoA confirmed the result from AMOVA. The findings indicate a moderate level of genetic diversity within and among populations, highlighting the necessity for conservation measures to preserve unique genotypes that are crucial for sustainable coffee production. In these population clustering analyses, genotypes from the same populations were found to be clustered similarly and vise-versa.

Generally, the result from morphological, biochemical, and molecular diversity analyses showed the existence of genetic diversity among the south Ethiopian coffee genotypes. The analysis of variance for quantitative agro-morphological, physical characteristics, cup quality, and biochemical content characters revealed the existence of statistically significant variation among genotype, location, and GEI effects for all studied traits indicating that the environment, genotype, and GEI is the most important factor influencing the coffee bean yield and quality in Ethiopia. The highest value of genetic variation found among populations than within populations.

7.2 Recomendation

The agro-morphological, biochemical, and molecular diversity analyses of current investigateion revealed the existence of genetic variability among the studied south Ethiopian coffee genotypes. Therefore, the studied genetic resources should be properly conserved for the future yield and quality improvement program.

Hence, the genotype, environment, and GEI explained a large amount of variation on coffee bean yield and quality, testing genotypes across diverse environments prior to deciding on any variety to use under wider agro-ecology is recommended for coffee yield and quality improvement program by using a multi-locational selection strategy. More GEI study is recommended to investigate the extent and patern of GEI. Investigation of different management options after the high-yielding season is also advised to minimize high-seasonal yield oscillation.

Among all sthe studied genotypes, the highest overall three location and four harvesting season average bean yield in tons per hectare was recorded for genotypes and AW105. Therefore, these two selections were recomended for the development of high yielding variety and could be included in the national variety-testing program with a view to releasing a new high yielding variety for coffee growing areas in South Ethiopia and other similar agro-ecologies elsewhere.

Out of all tested genotypes in this study, AW9648 achieved the highest score in green bean physical attributes and cup quality parameters at all three-tested locations. Therefore, AW9648 could be used as the best selection for development of highest qulity variety and best parent for hybridization for South Ethiopian coffee quality improvement in South Ethiopia and other similar agro-ecologies elsewhere. Additionally, genotype AW5994 recorded the lowest caffeine content (0.64% dry weight basis), suggesting its unique value as a parent in breeding programs aimed at developing low-caffeine coffee varieties, which are increasingly sought after by health-conscious consumers.

Although ISSR markers utilized and proved useful genetic diversity information in this study, it is limited due to its dominant, lower reproducibility, and difficulty in pinpointing specific genomic locations. Therefore, it is strongly recommended that future studies adopt co-dominant marker systems such as SSR (Simple Sequence Repeats) or SNP (Single Nucleotide Polymorphisms), which are a co-dominant, highly reproducible, and can provide precise information about gene locations, enabling more effective association mapping and gene discovery for south Ethiopian coffee yield and quality traits in future research.

While this study provided useful information on genetic diversity, it lacks the resolution necessary to pinpoint specific genes influencing important agronomic traits to facilitate a more direct and efficient link between phenotypes and genotypes. Therefore, I strongly recommend a transition to association studies breeding strategies to link phenotypes with genotypes for yield and quality improvement of coffee in south Ethiopia. The association study will facilitate the identification of candidate genes underlying important agronomic traits.

Apendix

Apendix Table 1 Trait descriptions and codes used for the study

No.	Traits	Trait Description	Code	No.	Traits	Trait Description	Code
1	PH	Very short	1	9	YSC	Green	1
		Short	3			Dark brown	2
		Tall	7			Other	3
		very tall	9	10	OAA	Elongated conical	1
2	BH	Very few branches (primary)	1			Pyramidal	2
		Many branches (primary) with few secondary	2			Bushy	3
		Many branches (primary)	3	11	CD	Compact	1
		Many branches (primary) with many secondary and tertiary	4			Intermediate	2
3	AIPB	Drooping	1			Open	3
		Horizontal	2	12	FC	Yellow	1
		Semi erect	3			Yellow orange	2
4	SS	Round	1			Orange	3
		Ovate	2			Orange-red	4
		Triangular	3			Red	5
		Deltate (equilaterally triangular)	4			Red – purple	6
		Trapeziform,	5			Purple	7
		Other	6			Purple-violet	8
5	YLC	Greenish	1			Violet	9
		Green	2			Black	10
		Brownish	3			Others	11
		Reddish	4	13	FSH	Roundish	1
		Bronze	5			Obovate	2
		Others if any	6			Ovate	3
6	LSH	Obovate	1			Elliptic	4
		Ovate	2			Oblong	5
		Elliptic	3			Others	6
		Lanceolate	4	14	PTH	Thin	3
		Others if any	5			Intermediate	5
7	LASH	Round	1			Thick	7
		Obtuse	2	15	SSH	Round	1
		Acute	3			Obovate	2
		Acuminate	4			Ovate	3
		Apiculate, spatulate	5			Elliptic	4
		Others	6			Oblong	5
8	LPC	Green	1			Others if any	6
		Dark brown	2				
		Other	3				

Where: PH=Plant height; BH=Branching habit; AIPB=Angle of insertion of primary branch; SS=Stipule shape; YLC=Young leaf colour; LSH= Leaf shape; LASH=Leaf apex shape; LPC=Leaf petiole colour; YSC=Young shoot colour; OAA=Overall appearance; CD=Canopy Diameter; FC=Fruit colour; FSH=Fruit shape; PT=Pulp thickness; SSH=Seed shape.

Apendix Table 2 Scale of Coffee Raw Quality Attributes

Raw Value (40)					
Shape & Make (15)		Color (15)		Odor (10)	
Quality	Point	Quality	Point	Quality	Point
Very good	15	Bluish	15	Clean	10
Good	12	Greyish	12	Fair clean	8
Faire	10	Greenish	10	Trace	6
Average	8	Coated	8	Light	4
Mixed	6	Faded	6	Light moderate	2
Small	4	White	4	strong	0

Apendix Table 3 Form Completed by sensory evaluator during row quality evaluation

Scale	**Row Quality Evaluation**			
	Shape & Make		Color	Odor
1	**Quality**		Quality	Quality
2	**Very good**		Bluish	Clean
3	**Good**		Greyish	Fair clean
4	**Faire**		Greenish	Trace
5	**Average**		Coated	Light
6	**Mixed**		Faded	Light moderate
7	**Small**		White	strong

Apendix Table 4 Scale of cup quality attributes usied in this study

Cup Value (60)															
Aromatic Quality (5)		Aromatic Intensity (5)		Acidity (10)		Astringency (5)		Bitterness (5)		Body (10)		Flavor (10)		Overall cup Quality (10)	
Quality	Point	Quality	Point	Quality	Point	Quality	Point	Quality	Point	Quality	Point	Quality	Point	Quality	Point
Excellent	5	Very strong	5	Pointed	10	Nil	5	Nil	5	Full	10	Very good	10	Excellent	10
Very good	4	Strong	4	Medium pointed	8	Very light	4	Very light	4	Medium full	8	Good	8	Very good	8
Good	3	Medium	3	Medium	6	Light	3	Light	3	Medium	6	Average	6	Good	6

Regular	2	Light	2	Light	4	Medium	2	Medium	2	Light	4	Fair	4	Regular	4
Bad	1	Very light	1	Lacking	2	Strong	1	Strong	1	Very Light	2	Bad	2	Bad	2
Nil	0	Nil	0	Nil	0	Very strong	0	Very strong	0	Nil	0	Nil	0	Unacceptable	0

Apendix Table 5 Form Completed by sensory evaluator during cup quality evaluation

Scale	**Cup quality**							
	Aromatic Quality	Aromatic Intensity	Acidity	Astringency	Bitterness	Body	Flavor	Overall cup Quality
1	Quality	Quality	Quality	Quality	Quality	Quality	Quality	Quality
2	Excellent	Very strong	Pointed	Nil	Nil	Full	Very good	Excellent
3	Very good	Strong	Medium pointed	Very light	Very light	Medium full	Good	Very good
4	Good	Medium	Medium	Light	Light	Medium	Average	Good
5	Regular	Light	Light	Medium	Medium	Light	Fair	Regular
6	Bad	Very light	Lacking	Strong	Strong	Very Light	Bad	Bad
7	Nil	Nil	Nil	Very strong	Very strong	Nil	Nil	Unacceptable