DIVERSITY OF SELECTED SORGHUM GENOTYPES USING MORPHOLOGICAL, MOLECULAR AND BIOCHEMICAL MARKERS

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DECLARATION

Declaration by the student

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DEDICATION

I dedicate this thesis to my parents, Mr. and Mrs. Rutto, for their continual support and prayers, whose unwavering encouragement and sacrifices have shaped my academic journey and provided me with the motivation and resilience to achieve my goals. Their unwavering encouragement and sacrifices have been instrumental in shaping my academic journey, providing me with the motivation and resilience to achieve my goals. I thank God for them. Their immense support has been instrumental in my pursuit of excellence, as they have consistently served as exemplary role models in my life.

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ABSTRACT

Sorghum (*Sorghum bicolor* L. Moench) is a cereal ranked the fifth most vital cereal crop globally following maize, rice, wheat, and barley. It is versatile and is used in numerous culinary and feed products around the world. Sorghum is an economic staple crop and the genetic diversity in its germplasm is an invaluable aid for its improvement. Characterization of the available Kenyan germplasm of sorghum is important in comprehending the dynamics of the genetic material/pool and in improving and sustaining its productivity. The purpose of this study was to assess the genetic diversity among selected sorghum genotypes in Kenya. Thirteen Sorghum genotypes sourced from the University of Eldoret/Rongo university and three checks from Kenya seed Company were analysed using morphological traits, biochemical profiles and ISSR DNA Markers. The field experiments were conducted at Endebess (35°28'10" E longitude and 1°29'17" N latitude) and Sigor (34°51'24" E longitude and 1°4'26" N latitude), replicated three times and arranged in Randomized Complete Block Design. Biochemical and molecular analysis were carried out at the Chemistry and Biotechnoloy Laboratories respectively. Clustering was carried out using UPGMA, AMOVA and PCoA to assess their genetic relationships. PCA revealed that the 3 important PCs contributed 81.78%, 15.33% and 1.5% of the total variation. AMOVA revealed 97% and 3% genetic variation within and among populations respectively. Shannon Weiner Diversity Index (H=2.74) and Shannon-Weiner Evenness Index (J=0.988) revealed a moderate to high level of biochemical diversity and relatively uniform distribution. Genotypes E95A, E1 and T53B were high yielding, early, dual and nutrient dense and could be promoted for commercialization. These findings offer informed precision in selection and improvement for high yield performance drought resistance and nutritious sorghums in the breeding programs in Kenya and Similar Agro-ecologies.

Key Words: Genetic, diversity, traits, Agro-morphological, Biochemical, Markers, DNA Variability.

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LIST OF ABBREVIATIONS

AEZ-	Agro-Ecological Zones
AFLP-	Amplified Fragment Length Polymorphism
ANOVA-	Analysis of Variance
AOAC-	Association of Official Analytical Chemists
ASALs-	Arid and Semi-arid Lands
C4 Plants-	Plants exhibiting a c4 photosynthetic pathway
CABI-	Centre for Agriculture and Bioscience International
CTAB-	Cetyltrimethylammonium bromide
DNA-	Deoxyribonucleic acid
DUS-	Distinctiveness, uniformity and stability
ERA-	Economic Review of Agriculture
ESTs-	Express Sequence Tags
FAO-	Food and Agriculture Organization
FAOSTAT-	The Food and Agriculture Organization Corporate Statistical Database
G×E-	Genotype and Environment Interaction
IBPGR-	International Board for Plant Genetic Resources
ICRISAT-	International Crop Research Institute for Semi-Arid Tropics
ISSR-	Inter simple sequence repeat
KALRO-	Kenya Agricultural Livestock and Research Organization.
MoA-	Ministry of Agriculture, Kenya
NGO-	Non-Governmental Organization
NPGs-	National Plant Germplasm System
NPGS-	National Plant Germplasm System
PC-	Principal Component
PCA-	Principal Component Analysis
PCR-	Polymerase chain reaction
RAPD-	Randomly Amplified Polymorphic DNA
RCBD-	Randomized Complete Block Design
RFLP-	Restricted Fragment Length Polymorphisms
SAT-	Semi-Arid Tropical regions
SNIPs-	Single Nucleotide Polymorphims
SSR-	Simple Sequence Repeat
STS-	Sequence Tag Sites
UPGMA-	Unweighted Pair Group Method with Arithmetic Mean

CHAPTER ONE

INTRODUCTION

1.1 Background information

Sorghum (*Sorghum bicolor* (L.) Moench) is a cereal of immense global importance, particularly in arid and semi-arid regions (Kazungu et al., 2023). It is estimated to ave been domesticated approximately more than five thousand years ago in the north -East of Africa and has since spread to most parts globally (Burgarella et al., 2021). currently, after Mazie, wheat, rice and barley, Sorghum (*S. bicolor*) is ranked fifth in the world (Ngidi et al., 2024). Its genetic versatility has enabled sorghum to support millions of livelihoods, from smallholder farmers in Africa and Asia to commercial producers in the United States and Australia (L. Pereira & Hawkes, 2022).

Sorghum is vital for food security in many countries that are developing, especially where rainfall is low and soils are poor. It is a staple grain used by more than 500 million people, providing essential calories and nutrients (Berge et al., 2019; Abreha et al., 2021; Khalifa & Eltahir, 2023). In addition to its role as food, sorghum serves as fodder for livestock (Kagwiria et al., 2019; Julian et al., 2025) especially in mixed farming systems. Its adaptability also makes it suitable in ethanol production for industrial purposes, biodegradable packaging materials, and brewing industries (Qi et al., 2019; Xiao et al., 2021). Regionally, in Sub-Saharan Africa and parts of India, it is a cultural cornerstone, incorporated into traditional diets, rituals, and economies.

Sorghum is consumed as flour, whole or popped grain and fermented food products (Adebo, 2020). It is brewed into traditional alcoholic beverages in some cultures. Its use in feed provides highly digestible and the biomass exploited for biofuel production. Sorghum starch is utilized in making adhesives, paper and biodegradable plastics (de Carvalho et al., 2024).

sorghum is resilient under drought and heat stress as it exhibits multiple physiological traits that offer tolerance to harsh environments (Mwamahonje et al., 2024; Prasad et al., 2021). Despite sorghum's economic and ecological significance, there is still a lack of knowledge about and utilization of its genetic diversity (Charyulu et al., 2024). For diversity evaluation, agromorphological traits have always been used but are highly affected by the environment hence calling for integration with biochemical and molecular markers because they are not influenced by environmental factors and are reliable (Carcedo et al., 2022; Bidyananda et al., 2024; Mesfer et al., 2022).

Genetic diversity forms the foundation for crop improvement and agricultural sustainability. Diverse genetic resources enable breeders to develop varieties with enhanced performance, better resistance to pests and diseases, tolerance to abiotic stresses like drought and salinity, and improved nutritional quality. Traditional landraces of sorghum, shaped over centuries of farmer selection, represent invaluable sources of such traits, often harboring adaptations that are absent in modern varieties. Loss of genetic diversity restricts breeding options and increases vulnerability to emerging stresses, posing a threat to food security. Conservation and utilization of this diversity are important not only for immediate productivity agriculturally but also for the long-term resilience of the food system in the face of changing climatic and socio-economic conditions.

1.2 Statement of the Problem

Despite its crucial role in food security and climate resilience in arid and semi-arid regions, sorghum production in Kenya remains suboptimal, with yields consistently falling below the crop’s genetic potential (Njagi et al., 2019). This persistent yield stagnation is largely attributed to a narrow genetic base in the cultivated varieties, making them highly vulnerable to emerging pests, diseases, and increasing environmental stresses exacerbated by climate change (Upadhyaya & Vetriventhan, 2018); Allan et al., 2020) . There remains a considerable gap in utilization and full understanding of sorghums genetic diversity despite its economic and ecological importance majorly in areas where it is the main crop (Charyulu et al., 2024). Genetic erosion poses a threat to the future resilience and adaptability of sorghum (Khoury et al., 2022).

The fragmented approach also fails to capture the genetic architecture of sorghum, which restricts the capacity of breeders to make informed decisions for developing resilient and high-performing cultivars (Bitima et al., 2016; Mwamahonje et al., 2024).

1.3 Justification

Sorghum is a highly versatile crop that plays a crucial role in ensuring food and feed security, particularly in dryland farming systems. It is a staple food, animal feed, and industrial raw material for millions (Omoro 2013; Khoddami et al., 2023). Comprehensive knowledge of the diversity of sorghum germplasm genetically is an important prerequisite for sustainability of sorghum production (Salih et al., 2016). Understanding the genetic diversity is vital in planning cross combinations in breeding programs, determining the adaptation of varieties to diverse environments, helps in varietal identification during plant variety protection (DUS tests) (Salgotra & Chauhan, 2023). Crop genetic resources can only be improved by constant introductions contemporary breeding methods that necessitate diversity assessment (Sahu et al., 2023). In order to increase crop improvement for the desired features, it is extremely vital to quantify genetic diversity and its components by using morphological traits (Bouargalne et al., 2022).

Despite its potential, sorghum remains underutilized and under-improved, with current cultivars yielding well below their genetic potential, largely due to limited genetic diversity, environmental stresses, and emerging biotic threats. A detailed understanding of the genetic architecture and variation among varieties that are commercialized and breeding lines will not only support breeding for resilience and productivity but also ensure long-term sustainability of sorghum improvement efforts.

While some genetic diversity evaluations have been done using either morphological, biochemical, or molecular markers, the integration of all three approaches is rare, particularly within the Kenyan context. Morphological markers are low-cost and accessible but are often influenced by environmental conditions, while molecular markers offer precision, stability, and early-stage evaluation capacity (Beyene et al., 2005; Jonah et al., 2023). Among DNA markers, Inter Simple Sequence Repeats (ISSRs) are notable for being simple, cost-effectiveness, and high polymorphism detection, therefore good for resource-limited breeding programmes(Ansari et al., 2024; Petolescu et al., 2024).

There is therefore urgent need to identify and quantify genetic diversity among Kenyan sorghum breeding lines and varieties, unlock nutritional attributes within local germplasm, inform strategic parent selection and hybrid development, strengthen variety protection through distinctiveness (DUS) characterization and enhance the overall efficiency and impact of sorghum breeding programs through informed, data-driven selection. ISSR markers have been effective because of their ability to target and amplify microsatellite regions scattered across the genome (Ansari et al., 2024).They are cost effective, simple to analyse and are highly polymorphic detecting rates in them makes them appropriate for study of diversity (Petolescu et al., 2024).

1.4 Objectives of the study

1.4.1 Overall objective

To assess the extent of genetic variability among selected sorghum genotypes through integrated agro-morphological, biochemical, and molecular approaches.

1.4.2 Specific objectives

1. To assess the phenotypic diversity of selected sorghum genotypes using agro-morphological markers.
2. To determine the genetic variation among the sorghum genotypes using ISSR (Inter-Simple Sequence Repeat) markers.
3. To evaluate the biochemical diversity of the genotypes based on key metabolic and physiological indicators.

1.5 Hypothesis

1.5.1 Null hypothesis

1. There is no significant genetic diversity among the evaluated sorghum genotypes based on morphological analysis.
2. There is no significant genetic diversity among the evaluated sorghum genotypes based on biochemical analysis.
3. There is no significant genetic diversity among the evaluated sorghum genotypes based on ISSR molecular marker analysis

1.5.2 Alternative hypothesis

1. There is significant genetic diversity among the evaluated sorghum genotypes based on morphological analysis.

2. There is significant genetic diversity among the evaluated sorghum genotypes based on biochemical analysis.
3. There is significant genetic diversity among the evaluated sorghum genotypes based on ISSR molecular marker analysis

CHAPTER TWO

LITERATURE REVIEW

2.1 Sorghum botany and taxonomy

2.1.1 Botanical description

Sorghum (*Sorghum bicolor* (L.) Moench) is a genus of flowering plants and belongs in the grass family *Poaceae*, native primarily to tropical and the sub-tropical regions of Africa and Asia. It is a robust, annual or perennial grass, well-known for its drought resistance and adaptability to diverse agro-ecological environments (Dicko et al., 2006).Sorghum ranks fifth among the most vital cereals globally, after wheat, rice, maize, and barley (FAO, 2023).

The sorghum plant typically grows between 1.5 and 4 meters tall, although some cultivars may reach up to 5 meters under optimal conditions(Kumar, 2016) . It possesses a solid culm with nodes and internodes, and its root system is fibrous, often extending deep into the soil, enabling efficient water absorption (Hossain et al., 2022).The leaves are alternate, linear-lanceolate, and bear a prominent midrib, with sheaths that encircle the stem (House, 1985).

The inflorescence of sorghum is a terminal panicle, which varies in shape and density among cultivars, ranging from loose to compact heads. The flowers are perfect (bisexual), with three stamens and a superior ovary, and are usually self-pollinated, although a small amount of cross-pollination can occur due to wind (House, 1985; Nehru et al., 2017). Grain color, shape, and size vary widely and are influenced by genotype and environmental conditions. The grains are typically caryopses, encased by a glume, and may range in color from white and yellow to red and brown depending on pigmentation and tannin content.

Sorghum has a C4 photosynthetic pathway, contributing to its efficient carbon assimilation and high productivity under conditions of high light intensity, temperature, and water scarcity (Paterson et al., 2009; Kumari et al., 2025).This characteristic provides a physiological advantage in arid and semi-arid regions where sorghum is a staple crop.

2.1.2 Taxonomy and Classification

Taxonomically, sorghum is of family *Poaceae*, subfamily *Panicoideae,* tribe *Andropogoneae*, and genus *Sorghum* (Karimi & Saeidi, 2022).

The genus *Sorghum* comprises approximately 25 recognized species, which are grouped into five taxonomic subgenera: *Sorghum*, *Parasorghum*, *Chaetosorghum*, *Stiposorghum*, and *Heterosorghum* (Ananda et al., 2021; Cowan et al., 2022). Among these, *Sorghum bicolor* is the most crucial species economically, encompassing all cultivated types and many wild and weedy relatives.

According to (Harlan & de Wet, 1972) and (Adedugba et al., 2023) *Sorghum bicolor* is divided into three main races based on panicle and spikelet morphology: bicolor, guinea, and caudatum. Later, two additional races were added: durra and kafir. These races have been used as a basis for classification and breeding programs due to their distinct agro-morphological features and geographical distribution. Intermediate forms known as hybrid races (e.g., guinea-caudatum, durra-bicolor) also exist, resulting from natural and artificial hybridization.

The domestication of sorghum is thought to have happened over five thousand years ago in the northeastern quadrant of Africa, particularly in the Ethiopian-Sudanese region, which is considered the center of origin and diversity (Abdelhalim et al., 2021).Wild relatives of sorghum such as *Sorghum arundinaceum* and *Sorghum* *verticilliflorum* are thought to be progenitors of the cultivated species, contributing to the rich genetic base of modern cultivars.

2.1.3 Genetic and Cytological Aspects

Sorghum is diploid with a chromosome number of 2n = 2x = 20. It contains a relatively small genome of approximately 730 Mbp, which has been sequenced completely (Paterson et al., 2009; Galvez-Lopez et al., 2021). The availability of genomic information has significantly advanced the understanding of sorghum genetics, facilitating marker-assisted selection, gene mapping, and comparative genomics.

The genetic variability within *S. bicolor* is considerable, encompassing traits such as grain color, panicle type, plant height, and tolerance to biotic and abiotic stresses. This diversity is important for breeding programs whose objective improving yield, nutritional quality, and climate resilience.

2.2 Importance of sorghum

Sorghum has been regarded as a staple food crop to over seven hundred and fifty million people in the semi-arid tropics of Africa, Asia and Latin America where Africa records more than a third of the sorghum production globally (Rosentrater & Evers, 2018; Nkosi et al., 2024). Sorghum is produced on a large portion for human use in Africa and Asia but in USA it is planted mostly for animal feed, that is forage and grain, a bio-energy crop for production of biomass and also ethanol manufacture (Proietti et al., 2015). Sorghum grain is frequently used in the USA as a maize alternative for livestock feed due to their similar nutritional profiles. The fibers from the stems of sorghum can also be utilized to make biodegradable packaging. It is utilized in packaging materials for delicate electronic equipment since it does not build up static electricity.

Sorghum is generally used in Africa, and Kenya in particular, to make boiling porridge, as well as malted drinks like beer (Pereira & Hawkes, 2022). The stover is used for fodder, firewood, fencing, and thatching. Sorghum grain is an essential part of cattle and poultry feed. Currently, 12% of the US's grain sorghum production is used to make the fuel ethanol (Proietti et al., 2015).

In many regions of the world, grain is largely utilized to produce food (55%) and is used to make thick or thin porridges and flatbreads (Visarada & Aruna, 2019). According to Htet et al., 2022 the grain is also used in baking, brewing, and many other traditional recipes. For people who are allergic to wheat, sorghum grain provides an alternative because it is gluten-free and rich in antioxidants (Hegde et al., 2023; Hryhorenko et al., 2023). Furthermore, because sorghum grain has high levels of iron (>70 ppm) and zinc (> 50 ppm), it can be used globally to reduce micronutrient deficiencies.

About one billion people in the semi-arid tropical regions of the world rely mostly on sorghum for their protein and energy needs, and over 300 million people in developing nations include it in their staple diets as an important source of nutrients and energy (Proietti et al., 2015). Sorghum has a comparatively high content of nutrients when compared to other cereals and can be utilized as a supplement in places where other cereals are not cultivated or fail.

In addition, the crop residues from sorghum and green plants can be used as fuel, building materials, and animal feed, especially in the semi-arid tropical regions (SAT). The stalks are used to weave baskets and mats as well as for fire, and the leaves serve as food for farm animals (Proietti et al., 2015; Kazungu et al., 2023)

2.3 Sorghum production and distribution

Sorghum originated in Ethiopia and later spread to other regions of Africa, India, Asia, Australia, and the United States (Girard & Awika, 2018). After wheat, rice, maize, and barley, it is the world's fifth-most significant cereal crop (George et al., 2022). It is among the plants planted for the longest in many arid and semi-arid areas of sub-Saharan Africa and other places where other crops, such maize, would fail (Ngugi & Maswili, 2010). It is thought to be Africa's primary contributor to the world supply of food, along with fingermillet (Andiku et al., 2021)

Sorghum is cultivated in over one hundred countries globally with USA, Nigeria, China, Sudan, India, Ethiopia, Niger, Australia, Brazil, and Mexico being among the top ten countries. India, Nigeria, USA, Argentina, Mexico, Sudan, and China are major producers. In Mali, Gambia, Mauritania, Burkina Faso, Ghana, Niger, Somalia, and Yemen, the crop takes up at least 25% of arable land, while in Nigeria, Chad, Sudan, Tanzania, and Mozambique, it accounts for more than 10%. With average yields of 810 and 1150 kilograms per hectare, respectively, Africa and Asia account for 80% of the land used for sorghum production. The majority of African sorghum is produced in the savanna regions of east, west, and central Africa, where the crop's grain forms a staple of millions of people's daily diets. Nigeria is one of the twelve African nations that produce the most sorghum, accounting for 12 of the top 20 producers worldwide.

2.4 Genetic resources of sorghum

Sorghum bicolor is a diploid plant that has a basic chromosomal number of n=x=10 (2n=20). It is a crop that reproduces sexually and is heavily self-pollinating. It has a low cross-fertilization rate of only 6% (Sias et al., 2023).

With more than 42,000 accessions, sorghum constitutes one of the largest germplasm collections (Huang, 2004). An abundance of varied genotypes offers excellent prospects for enhancing plant adaptability and other agronomic features (Huang, 2004). The economic relevance of genetic materials has led to the development of international organizations to preserve the germplasm of different types of agriculturally significant crop species (Salgotra & Chauhan, 2023). The National Plant Germplasm System

(NPGS) of the United States maintains a sizable collection of sorghum germplasm and also The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has a large collection of sorghum landraces.

2.5 Germplasm characterization

Germplasm characterization and evaluation are fundamental processes in plant breeding and conservation. They help us understand the genetic diversity and potential of crop species, which helps us create strong, high-yielding cultivars. Characterization is the systematic recording of germplasm using standard descriptors. The goal is to find genetic variation based on traits that can be seen or measured. Evaluation looks at how well germplasm works for certain traits in certain environmental conditions. All of these things help find duplicates, sort accessions, and choose promising genotypes for crop improvement.

Recent studies have shown large and wide variability in sorghum germplasm collections worldwide. For instance, a study on Ethiopian sorghum accessions revealed significant genetic diversity and population structure, highlighting the potential of local germplasm in breeding for climate adaptation (Nemera et al., 2022). Research on sorghum landraces from both Saudi Arabia and Yemen utilizing Inter Simple Sequence Repeat (ISSR) markers demonstrated significant polymorphism, indicating considerable unexploited genetic resources (Basahi, 2015).

Biochemical characterization gives us an understanding of nutritional and functional qualities of sorghum. A study of Korean sorghum landraces showed that there were big differences in their antioxidant capacity and phenolic content giving a conclusion that selective breeding could be used to make sorghum more nutritious (Lee et al., 2022).

Comprehensive characterization and evaluation of sorghum germplasm are pivotal for uncovering the crop's genetic diversity. These processes support the identification of elite genotypes for targeted breeding and ensure the conservation of valuable genetic resources. With the ongoing challenges of climate change and food insecurity, the use of diverse sorghum germplasm strategically foresees an important promise for developing resilient and nutritionally enhanced cultivars

2.5.1 Agro-Morphological Diversity in Sorghum

According to (Takanashi, 2023) morphological markers include flower color, seed color and its shape, patterns of growth, texture, pigmentation, maturity, yield and pest and disease resistance. Understanding the similarities and differences across genotypes is made easier by the characterization of genotypes, which provides information that is descriptive about their features (IBPGR & ICRISAT, 1993). Plants are selected based on easily distinguishable morphological traits and shared ancestry with the desired attribute.

Morphological characters are affected by the environment and management practices (Carcedo et al., 2022), management practices are limited in number and influenced by developmental stage of the plant. Farmers choose and favor particular sorghum landraces based on their morphological characteristics. The majority of farmers cultivate sorghum landraces with desirable grain color, early maturation, high yield, tolerance to pests, diseases, and drought (Mwamahonje et al., 2021).

2.5.2 Biochemical Diversity in Sorghum

The biochemical composition of sorghum offers a significant crucial role in determining its nutritional value and suitability for food and industrial applications (Tanwar et al., 2023). Nutrients vary significantly among different genotypes. Various studies done sow landraces possessing superior nutritional qualities as compared to the modern cultivars pegging it on the wide genetic diversity. A clear understanding of the biochemical diversity of sorghum is critical for the development of nutrient-rich varieties and biofortification.

2.5.3 Molecular Diversity in Sorghum

Using genetic markers in diversity over phenotypic characterization is more advantageous because it is not influenced by environment and also allows identification of differences at the level of DNA which is not possible through phenotypic characterization (Jonah et al., 2023). The cost-effectiveness, simplicity, high throughput, number of samples, degree of relatedness between samples, research aims, and familiarity with the target genotype all play a role in the decision of which molecular markers to utilize (Hasan et al., 2021).

Inter Simple Sequence Repeats (ISSRs) are significant for characterization of sorghum and are relatively cheap. High levels of polymorphism, reproducibility power, highly discriminative, stability, and simplicity characterize ISSR markers. According to several studies the analysis only needs a small amount of genomic DNA, is amenable to automation and high-throughput platforms, and has been demonstrated to be very effective in revealing high diversity in sorghum landraces (Baggett et al., 2021).

2.5.4 Integration of Agro-Morphological, Biochemical, and Molecular Assessments

An integrated approach combining agro-morphological, biochemical, and molecular diversity assessments provides a comprehensive understanding of sorghum genetic resources. Such an approach enhances the accuracy of genotype selection, ensuring the development of high-yielding, nutrient-rich, and climate-resilient sorghum varieties. Previous studies have highlighted the advantages of integrating these methods to improve breeding efficiency and genetic conservation (Sejake et al., 2020; Andiku et al., 2022; Lee et al., 2022).

2.6 Challenges facing sorghum production in Kenya

Climate Variability is a huge challenge to the production of sorghum in Kenya (Njinju et al., 2022). Erratic rainfall patterns and droughts can significantly affect sorghum yields. Kenya's sorghum-growing regions are prone usually to weather conditions that is unpredictable, that has an impact planting schedules and crop development. Sorghum crops are susceptible to various pests and diseases, including aphids, stem borers, and fungal infections such as anthracnose and downy mildew (Beyene et al., 2024; Khaskheli et al., 2025). These can lead to reduced yields and quality if not properly managed. Small-scale farmers often struggle to access high-quality sorghum seeds adapted to local conditions (Muui et al., 2013) . Poor yields and less productivity is usually contributed by the use that is not certified seed and soils that lack or with low nutrients limiting good crop growth and its productivity among farmers. Application of fertilizer and conservation of soil are practices that should be adopted by farmers in order to improve their yields.

Poor access to markets, poor infrastructure and market information to sell produce at good prices also remains a challenge to the farmers in Kenya (Nyamamba et al., 2021). Unlike maize, Sorghum does not have a well-developed seed system hence hindering access to quality seed. Improper facilities for storage leads to post harvest losses and hence need to improve storage facilities in order to improve income to farmers. Lack of adequate research on sorghum and extension services remains to be a major challenge facing farmers in Kenya. (Kale & Mbataru, 2022). More research and extension services focused on sorghum production is needed. Providing farmers with access to up-to-date agronomic information, improved technologies, and training can enhance resilience and productivity to challenges. This challenges can be addressed by coordinated effort from government agencies, research institutions, NGOs, and other stakeholders to promote sustainable sorghum production and improve the incomes of sorghum farmers in Kenya.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Overview

This study consisted of three separate studies under field, greenhouse and laboratory conditions. The first study involved field screening of the sorghum genotypes selected in two sites (Elgon downs field in Endebess and Sigor farm in West Pokot) in the main season. The study's second phase assessed the genetic relatedness among the genotypes by evaluating biochemical diversity of the genotypes based on key metabolic and physiological indicators at the Chemistry Laboratory, University of Eldoret. Lastly, genetic diversity and similarities were established on all selected sorghum genotypes by use of ISSR Molecular Markers at the Biotechnology Laboratory by extraction of DNA from 2 weeks old plants grown at the greenhouse.

3.2 Assessment of Genetic Relatedness Among Selected Sorghum Genotypes Using Agro-Morphological Traits

3.2.1 Experimental materials/Plant Materials

The study comprised 16 sorghum genotypes, including 13 elite and stable lines sourced primarily from the University of Eldoret (UOE) and Rongo University, and 3 check varieties (Gadam, E-1291, and KARI Mtama 1) obtained from the Kenya Seed Company (KSC). Genotypes selection was informed by; representation of diverse genetic backgrounds e.g grain colour and adaptation potential to different agro-ecological zones. The inclusion of released varieties provided a benchmark for evaluating the performance of advanced lines, while the elite lines represented potential candidates for release or use as parental materials in breeding. The selected experimental materials provided a robust basis for evaluating morphological diversity in sorghum.

Table 1: Description of Sorghum genotypes used in the study

Entry	Sorghum genotype	Source	Description	Stage
1	Gadam (Check)	Kenya Seed Company	Gadam is a short variety with chalky white grain.	Released variety
2	E1291 (Check)	KSC	Grain is red in colour.	Released variety
3	KARI Mtama 1 (Check)	KSC	This is a tall variety with cream white grain.	Released variety
4	E117B	UOE/Rongo University	The grain is brown in colour	Elite line- stage 6
5	E118B	UOE/Rongo University	The grain is brown speckled in colour	Elite line- stage 6
6	E1	UOE/Rongo University	The grain is red in colour	Released variety
7	E5	UOE/Rongo University	The grain is red in colour	Elite line- stage 6
8	N57	UOE/Rongo University	The grain is brown in colour	Elite line- stage 6
9	N68	UOE/Rongo University	The grain is brown in colour	Elite line- stage 6
10	E95A	UOE/Rongo University	The grain is brown in colour	Released variety
11	T30B	UOE/Rongo University	The grain is brown in colour	Released variety
12	T53B	UOE/Rongo University	The grain is brown in colour	Elite line- stage 6
13	MUK-60	UOE/Rongo University	The grain is red in colour	Elite line- stage 6
14	C26	UOE/Rongo University	The grain is brown in colour	Elite line- stage 6
15	Kalatur	KSC	The grain is cream	Elite line- stage 6
16	foehn	KSC	The grain is Brown white	Elite line- stage 6

3.2.2 Experimental sites

The field screening of the selected sorghum genotypes was conducted in West Pokot County (Sigor) and Trans Nzoia County (Endebess), representing contrasting agro-ecological zones in Kenya (Table 2).

Table 2: Agro-Ecological features of the experimental location used in the study

Location	Altitude	Longitude	Latitude	Annual average rainfall (mm)	Soils	Temp.
West Pokot (Sigor)	1200-1600m ASL	35° 28' 10" E	1° 29' 17" N	450mm	Sandy loam reddish brown soils	25.02ºC
Trans-Nzoia (Endebess)	1600-1800m ASL	34° 51' 24E	1° 4' 26 N	1000mm	Black cotton soils	20.47ºC

(Jätzold, 2007)

3.2.3 Experimental design

The experiment was laid out by use of Randomized Complete Block Design (RCBD) and replicated three times. Each plot area of the experiment included of 4 rows of 2.5 m length with 0.6 m spacing between rows (inter-row spacing) and 0.20 m between plants (intra-row spacing). The total area of each plot had a size of 6 m^2. There was a 1m^2 distance between each plot pair. DAP Fertilizer was administered at a rate of 50 kg/acre according to the rate used by KALRO Seed Production Manual (Ayako et al., 2021). To avoid direct contact with the seed at the time of sowing, fertilizer was applied and thoroughly mixed with the soil. Urea was applied as a side dressing after 35 to 40 days of seedling emergence (knee height stage). To maintain plant spacing and balance plant density, thinning was done three weeks following planting.

3.2.4 Data collection

Data was collected on Qualitative and quantitative characters used to characterize sorghum genotypes according to descriptors for sorghum (*S. bicolor* (L.) Moench)(IBPGR & ICRISAT, 1993). For characterisation, the parameters used are described in Table 3.

Table 3: Description and Scoring of qualitative and quantitative traits of Sorghum

Trait type	*Trait Name*	*Stage*	*Description*	*Scoring / Measurement*
Qualitative	Seedling Vigour	15 days after emergence	Determined by seed longevity, germination speed, seedling growth, and early stress tolerance	3=low, 5=intermediate or 7=high
	Leaf Midrib Color	Middle stage	Color of the leaf midrib	1=white, 2=dull green, 3=yellow, 4=brown, 5=purple and 6=other.
	Panicle Compactness	Maturity	Structure of the panicle	1=very lax, 2=very loose erect, 3=very loose drooping,4= loose erect, 5=loose drooping, 6=semi-erect, 7=semi-loose drooping, 8=compact
	Glume Color	Maturity	Color of glumes enclosing the grains	1=white, 2=sienna, 3=mahogany, 4=red,5= purple, 6=black,7= grey or 8=others
	Grain Color	Maturity	Color of mature grains	1=White, 2=yellow, 3=red, 4=brown, 5=buff and 6=others
	Leaf Orientation	Maturity	Position of the leaves on the plant	1=Erect, 2=Drooping, 3=Horizontal
	Grain covering	Maturity	Amount of grain covered by glumes	1=very short (25%), 3=short (50%), 5=medium (75%) ,7=grain fully covered (100%), and 9=glumes longer than grain
	Awns	Maturity	Presence or absence of awns	1= Present 0=Absent
	Threshability	Harvest	Ability of the grain to be removed from the glumes	very difficult (less than 50%), difficult (60-69%), intermediate (80-84%), good (90-94%) and Excellent (95-100%).
Quantitative	Plant Height	Maturity	Distance from base to tip of panicle	Measured in cm
	Panicle Length	Maturity	Length of the panicle from base to tip	Measured in cm
	Panicle Width	Maturity	Width of the panicle at its widest point	Measured in cm
	Days to 50% Flowering	50% Flowered	Number of days from sowing to 50% flowering	Measured in days
	Days to Maturity	Maturity	Number of days from sowing to physiological maturity	Measured in days
	Leaf Length	Maturity	Length of the leaf blade	Measured in cm

Trait type	***Trait Name***	***Stage***	***Description***	***Scoring / Measurement***
	Leaf Width	Maturity	Width of the leaf blade	Measured in cm
	Number of Leaves	Maturity	Total number of leaves per plant	Counted per plant
	Seed Size	Harvest	Size of individual grains	Measured in mm or visually scored 1=Small, 2=Medium, 3=Large
	100-Grain Weight	12% Moisture content	Weight of 100 grains	Measured in grams (g)
	Grain Yield per Plant	Harvest	Grain weight per plant	Measured in grams (g)
	Plant Dry Weight	Harvest	Dry biomass weight of whole plant	Measured in grams (g)

3.1.5. Analysis of Data

Using GenStat statistical software Edition 14, data on quantitative and qualitative traits were analyzed at 5%. The significant differences among the sorghum genotypes were tested using analysis of variance (ANOVA). The average variations between the experimental genotypes and the relationships were tested using Fishers' test to ascertain whether the observed differences were significant. Results were presented in terms of Correlations and principal component (PCA). The distribution frequency of the traits on each genotype were also performed and ranked(Mofokeng, 2015).

3.3 Assessment of Genetic Relatedness Among Selected Sorghum Genotypes Using Biochemical Markers

3.3.1 Sample source and preparation

The listed genotypes in section 3.1 sub-section 3.1.1 were assessed for protein content, phosphorus, fats, zinc and iron at the University of Eldoret Chemistry Laboratory. The materials were cleaned and any foreign materials or debris removed from the samples. The sorghum seeds were ground to fine powder using a mill.

3.3.2 Determination of protein content

Crude protein (CP) was determined using the modified Kjeldahl method of Cope (1889) (Technical, 2009; Langyan et al., 2022). A blank control with all reaction mixtures without the sample was performed parallel to the sample. The nitrogen value was then deduced by subtracting the experimental sample value from the experimental blank value.

Calculation

The concentration of nitrogen in the sample material expressed in %N was calculated as follows:

$$N\% = \frac{(a-b) \times v \times 100}{1000 \times w \times al \times 1000}$$

Where;

a = concentration of N in the solution; b = concentration of N in the blank; v = total volume at the end of analysis procedure; w = weight of the dried sample; al = aliquot of the solution taken.

% N x 6.25 = Protein

3.3.3 Determination of Fat content

Total lipid contents in the sorghum grains was determined using Soxhlet Extraction method (Wang et al., 2005). Content of fat was determined according to AOAC 945:16; (Tasie & Gebreyes, 2020) with slight modification that included evaporating the major portion of the solvent inside a fume hood.

Fat content was calculated as follows:

$$\%Fat = \left[\frac{W2 - W1}{W}\right] 100$$

Where;

W2 = weight of the flask and fat deposit; W1 = weight of the empty flask only; W = weight of the sample taken for the test.

3.3.4 Determination of zinc and iron

To measure these micronutrients, grains were digested first in a mixture of salicylic acid, sulphuric acid, hydrogen peroxide and selenium powder. It was then aspirated into a flame from an air acetylene mixture and detected through an atomic absorption spectrophotometer (Okalebo et al., 1993).

Determination of Iron

Atomic absorption was used as it absorbs radiation from an element-specific hollow cathode lamp at a wavelength of 248.3nm. A diluted sample, blank digests and the standard series were aspirated into the atomic absorption spectrophotometer calibrated for iron measurement at wavelength 248.3 nm and the absorbance measured. A calibration curve from the standard series was plotted and the concentration of the unknown calculations determined.

Calculations:

Iron concentration in the dried sample expressed in Fe mg kg $^{-1}$ were calculated as follows;

$$\text{Fe (Mg Kg-1)} = \frac{(a-b) \times v \times f \times 1000}{1000 \times w}$$

Where;

a = concentration of Fe in the solution; b = concentration of Fe in the mean values of the blanks; v = final volume of the digestion process; w = weight of the sample taken; f = the dilution factor

Determination of Zinc

Zinc was measured by atomic absorption at a wavelength of 213.9 nm. A suitably diluted sample, blanks digests and the standard series were aspirated into the spectrophotometer calibrated for Zinc measurement at wavelength 213.9nm and the absorbance measured. A calibration curve from the readings of the series was plotted and the concentration of the unknown determined.

Calculations;

Zinc concentration in the dried sample expressed in Zn mg kg $^{-1}$ were done as:

Zn mgkg $^{-1} = \frac{(a-b) \times v \times 100}{1000 \times w \times al \times 1000}$

Where;

a = concentration of Zn in the solution; b = concentration of Zn in the mean values of the blanks; v = final volume of the digestion process; w = weight of the sample taken; f = the dilution factor.

3.3.5 Total Phosphorus without pH Adjustment Using Ascorbic Acid

Five (5) ml of the supernatant clear watershed digest solution was pipetted into a 50-ml volumetric flask. About 20-ml distilled water was added to each flask and then added 10 ml of the ascorbic acid reducing agent to each flask, beginning with the standards to 50 ml with water; stopper and shake well. It stood for 1 hour to permit full colour development. Standards and sample absorbances (blue colour) were measured at 880 nm wavelength setting in a suitable colorimeter.

Aliquots of 0, 1, 2, 3, 4, 5 and 6 ml of the 10 ppm P working solution were put into 50 ml volumetric flasks. 10 ml of the ascorbic acid reducing solution was added to each flask and let to stand for 1 hour and read absorbance exactly like the sample solutions above to make standard solutions of P. The standards contained 0, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ppm P, respectively.

A graph of absorbance was plotted against standard concentration and then determined solution concentrations for each unknown and the 2 blanks. The mean blank value was subtracted from the unknowns; this gives a value for corrected concentration (= c in subsequent calculations).

$$P \; in \; sample \; (\%) = \frac{c \times v \times f}{w}$$

3.3.6 Data collection and analysis

Statistical data for protein content, fat, zinc and iron content was subjected to Shannon-weiner diversity index. The Shannon Diversity Index (H') was computed to assess the biochemical diversity among the sorghum genotypes. The index was calculated using the following formula:

$$H' = -\sum pilnpi$$

Where p*i* represents the relative abundance of each genotype based on its biochemical traits. The proportion for each genotype was determined by summing the values of all biochemical traits (phosphorus, protein, iron, zinc, and fat) and calculating the contribution of each genotype to the total biochemical content. Analysis of Variance was computed and Hierarchical clustering using UPGMA in GenStat software 14th Edition was performed and data presented in Bar charts and a dendrogram. PCA biplot was also plotted to compare the degree of association between the traits analyzed.

3.4 Assessment of Genetic relatedness among Selected Sorghum Genotypes using ISSR Molecular Markers.

3.4.1 Plant materials and experimental site

Genotypes of sorghum collected from UOE/Rongo University and Kenya Seed Company (as detailed in Chapter 3, Sub-section 3.2.1, Table 1), were used in te experiment. The genotypes were sown in the greenhouse facilitate the collection of young leaves for DNA extraction. Five seeds of each genotype were sown in 40 cm diameter plastic pots filled with seedling mix, arranged in three replicates. Required practices were applied as required throughout the growth period.

3.4.2 DNA extraction

For each sorghum line, small young leaves of two-week-old seedlings were randomly picked and used for the genomic extraction using modified DNA CTAB protocol (Mace et al., 2004). Six milligrams of leaf sample were used from every individual to form a bulk sample. Preparation of plant samples were done by cryogenically grinding tissue in a mortar and pestle after chilling in liquid nitrogen. Frozen dried plants were ground at room temperature. Five hundred (500) µl of preheated extraction buffer were added to each tube and 10µl of RNase (100mg/ml) added and vortexed to mix well. It was incubated in water bath and maintained at 65°C for 30 minutes, vortexed to mix well and returned to water bath twice within 30 minutes then cooled down to room temperature (about 15 minutes in the fridge). Three hundred microliter (300µl) of 6M ammonium acetate was added and stored at 4°C and then mixed well by vortexing and put in the fridge at 4°C for about 15 minutes. It was centrifuged for 5 minutes at maximum speed (13,000 rpm) at room temperature. The supernatant was transferred to fresh microfuge tubes and 50µl CTAB (10%, in 0.7M NaCL) added to each tube and mixed gently. Seven hundred microliter (700µl) chloroform-isoamyl alcohol (24.1) was added and swirled and centrifuged for 5 minutes at maximum speed of 13,000 rpm. The top aqueous phase was transferred to a new Eppendorf tube. Two-thirds volume of ice-cold isopropanol (˜500µl) was added and swirled and left to stand on ice for 30 minutes. It was centrifuged for 20 minutes at maximum speed of pellet DNA on bottom of the tube. The liquid was drained carefully and the pellet kept at the bottom of the tube 70% 1000µl of ethanol added and left for 3 minutes and centrifuged for 10 minutes at 10,000 rpm.

Alcohol was then drained and 1000µl of 90% ethanol added and the pellet kept in the tube. The contents were centrifuged for 10 minutes at 10,000rpm. Alcohol was drained and the pellet remaining at the bottom of the centrifuge tube dried (e.g. in a flow bench for 15 minutes. The pellet was re-suspended in 100µl TE and left to dissolve at 4ºC in the refrigerator for at least 30 minutes.

3.4.3 DNA Dilution

This is to ensure uniformity in the amount of DNA samples used. Using agarose 0.8% gel stained with ethidium bromide (1%), the DNA samples were diluted to 5ng/ µl concentration. This was done by adding 40 µl double distilled water into 5 µl of DNA for each sample to make a 1:10 dilution. For each sample, 2 µl of the diluted DNA was mixed with 1 µl bromophenol blue dye and 3 µl double distilled water and then they were filled in the wells of submerged gels in an electrophoresis unit, containing 1 x TBE buffer. The outer wells at the left edges of the gel were loaded with 5 nanograms and 10 nanograms concentrations of lambda DNA standards for comparison. An electric current of 120V was applied for 30 minutes. Due to the ethidium bromide (1%), the DNA fragments emitted a luminous glow under UV light and were photographed. The concentration of the DNA fragments was estimated according to the thickness of the band in comparison with the Lambda DNA standards at the edge of the gel.

3.4.4 DNA Amplification

Solis BioDyne Firepol Mastermix was used to run the PCR reaction at a volume of 20 µl per reaction and constitutions of 4 µl Firepol Mastermix, 1 µl primer, 2 µl template DNA and 13 µl molecular water. PCR program was 95°Cinitial denaturation for 5 min, 95°C denaturation for 30 seconds with annealing temperature at 50 °C for 1 minute, 72 °C extension for 1 minute and final extension for 7 minutes. The PCR product was run for 2.45 hours at 100 Volts in a 2.5% gel stained with Greenstar nucleic acid stain followed by visualization in BioDoc IT gel documentation

3.4.5 DNA Quality Check and Quantification

This was done before proceeding to genetic diversity to ensure high quality results. DNA check for quality was done using agarose gel 0.8% electrophoresis stained with Gel red (5µl/100µl). The DNA quality that was good showed a clear band while a bad one showed a smear. The DNA quantity was checked using a nanodrop spectrophotometer.

Table 4: List of ISSR Primers used in the experiment

S.No	Primer code	Nucleotide sequences (5’-3’)	Bases
1	ISSR-1	"GAGAGAGAGAGAGAGAYC"	18
2	ISSR-2	"AGAGAGAGAGAGAGAGG"	17
3	ISSR-3	"HVHTGTGTGTGTGTG"	15
4	ISSR-4	"GAGAGAGAGAGAGAGAT"	17
5	ISSR-5	"ACACACACACACACACACT"	19
6	ISSR-6	"GAGAGAGAGAGAGAGAYG"	18

**Y(Pyrimidine) = C (Cytosine) or Thiamine); H (G)= A(Adenine), C(Cytosine), or T; V(T) = A, C, or G(Guanine).*

3.4.6 Data collection and analysis

Bands of Amplified ISSR were scored as either absent (0) or present (1) to generate a binary matrix. GENALEX software version 6.2 was used to analyze data and to calculate Polymorphic Information Content (PIC), expected and observed heterozygosity, and allele and genotype frequencies. Analysis of Molecular Variance (AMOVA) was performed to assess genetic variation within and among genotypes. Principal Coordinate Analysis (PCoA) was used to visualize genetic relationships, with the first few axes explaining the highest percentage of variation among genotypes. A dendrogram was generated using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on genetic similarity coefficients to reveal distinct clustering patterns among the genotypes using Genetic Analysis in Excel (GenAlEx) version6.2 software (Peakall, &Smouse,2006).

CHAPTER FOUR

RESULTS

4.1 Mean response of sorghum genotypes in Endebess and Sigor

Highly significant differences ($p \leq 0.001$) were noted in the sorghum genotypes in weight of seed, 50% days to flowering, Days to harvest, grain yield, Length and width of leaf, panicle width and length, number of leaves, Plant dry weight and Plant height.

In Endebess, Plant height ranged from 85.4 cm (E1) to 158.4 cm (T30B), with taller genotypes generally performing better at Sigor than at Endebess. N57 exhibited the highest yield of grain at (3.10 t/ha), whereas Kalatur demonstrated the lowest grain yield at (0.35 t/ha).

E1291 recorded the longest leaves (67.87 cm), whereas Kalatur exhibited the shortest (36.13 cm). Leaf width was greatest in E1 (8.33 cm) and narrowest in Kalatur (3.29 cm). The leaves number per plant varied greatly, with E95A producing the most (10.6 leaves) and Kalatur having the fewest (5.33 leaves).

Panicle traits among the evaluated genotypes varied significantly. The longest panicles were observed in E5 (25.93 cm), while N57 had the shortest (18.93 cm). Significant differences ($p < 0.001$) were also noted in Plant dry weight, where E1291 recorded the highest weight (1.93 kg), and Foehn the lowest (0.73 kg). Stem thickness varied significantly across genotypes, with T30B exhibiting the thickest stems (7.28 cm) and N68 the thinnest (5.06 cm).

Days to emergence, flowering, and maturity showed substantial variation ($p < 0.001$). E1291 and C26 emerged earliest (21 days after sowing), while E117B emerged latest (28 days).

At Sigor, genotype E118B exhibited the highest grain yield (3.31 t/ha), while Kalatur scored least yield (1.017 t/ha), demonstrating poor performance across both sites. N57 attained the greatest height (246.4 cm), whereas Foehn was the shortest (139.9 cm). Mean comparisons between the two environments showed that Sigor recorded higher yield mean (2.01 t ha^{-1}) compared to Elgon Downs (1.73 t ha^{-1}). Plants at Sigor were taller (190.3 cm) than those at Elgon Downs (120.7 cm), with larger leaf dimensions (LL: 69.27 cm vs. 55.22 cm; LW: 9.28 cm vs. 6.04 cm). Moreover, sorghum genotypes matured earlier at Sigor (105.96 days) compared to Elgon Downs (173.25 days). Sigor recorded the earliest emergence (DE: 8.85 vs. 23.75) respectively. Sigor site recorded the highest plant dry weight (1.28 vs 1.15) respectively. These findings point to both great genetic variability and pronounced environmental influence on sorghum trait.

Table 5: Mean of thirteen quantitative traits of the sixteen selected sorghum genotypes evaluated at Endebess

Genotype	**100-SDW**	**DE**	**DF**	**DH**	**GYLD**	**LL**	**LW**	**NL**	**PAL**	**PAW**	**PDW**	**PH**	**STK**
E117B	0.65^{a}	28.00^{fg}	97.67^{h}	169.00^{ab}	1.17^{cd}	56.40^{cde}	7.30^{gh}	9.20^{efg}	24.60^{def}	7.67^{bcd}	1.43^{ef}	101.10^{b}	6.43^{de}
N68	0.67^{a}	24.33^{cd}	96.67^{fgh}	172.7^{de}	1.82$_{efg}$	58.87^{cde}	6.47^{fg}	8.93^{efg}	19.13^{ab}	5.47^{a}	0.97^{abcd}	144.80^{d}	5.06^{a}
T30B	0.70^{ab}	22.67^{abc}	93.33^{defg}	174.3^{efg}	2.17$_{gh}$	62.13^{ef}	5.33^{bc}	8.67^{def}	19.40^{abc}	8.27^{cde}	1.20^{cdef}	158.40^{ef}	7.28^{f}
E95A	0.73^{abc}	23.00^{bc}	92.67^{def}	174.00^{ef}	1.94^{fgh}	61.87^{def}	6.87^{fgh}	10.60^{h}	25.33^{ef}	8.40^{de}	1.37^{ef}	118.50^{c}	6.44^{de}
N57	0.73^{abc}	23.00bc	92.00^{de}	174.7^{fg}	3.10^{jk}	55.27^{cde}	6.40^{efg}	9.07^{efg}	18.93^{a}	7.07^{bcd}	0.87^{abc}	146.90^{de}	5.78^{abcd}
T53B	0.77^{abc}	22.67^{abc}	92.00^{de}	174.3^{efg}	1.41^{de}	57.07^{cde}	5.33^{bc}	7.80^{cd}	20.47^{abc}	9.27^{e}	1.17^{bcdef}	137.30^{d}	5.66^{abc}
E5	0.80^{abc}	22.67^{abc}	91.67^{cde}	174.3^{efg}	3.57^{k}	54.80^{cd}	6.27^{cdef}	8.53^{de}	25.93^{ef}	7.60^{bcd}	1.53^{f}	108.20^{bc}	5.98^{bcde}
E1291(check)	0.83^{abc}	21.00^{a}	85.00^{b}	176.00^{g}	2.36^{hi}	67.87^{f}	7.47^{hi}	9.93^{gh}	26.27^{f}	7.73^{bcd}	1.93^{g}	171.30^{f}	6.06^{bcde}
C26	0.90^{bc}	21.00^{a}	92.00^{de}	176.00^{g}	2.40^{hi}	66.78^{f}	5.47^{bcde}	9.13^{efg}	22.53^{bcde}	8.13^{cde}	1.27^{def}	117.40^{c}	6.40^{cde}
E1	0.90^{bc}	26.67^{ef}	95.00^{efgh}	170.30^{bc}	1.55^{def}	61.20^{def}	8.33^{i}	9.67^{fgh}	24.93^{def}	8.20^{cde}	0.93^{abcd}	85.40^{a}	6.60^{ef}
E118B	0.90^{bc}	28.67^{g}	97.67^{h}	168.30^{a}	0.22^{a}	55.04^{cde}	6.53^{fgh}	8.93^{efg}	22.88^{cdef}	6.87^{abc}	0.80^{ab}	110.80^{bc}	5.56^{ab}
MUK-60	0.93^{c}	22.67^{abc}	92.00^{de}	174.30^{efg}	2.67^{ij}	56.27^{cde}	6.33^{def}	9.33^{efg}	21.80^{abcd}	6.53^{ab}	1.37^{ef}	105.20^{bc}	6.39^{cde}
Kari Mtama 1(check)	1.33^{d}	23.33^{bc}	90.00^{cd}	173.70^{ef}	0.60^{ab}	53.47^{c}	5.31^{b}	7.27^{bc}	25.40^{ef}	7.60^{bcd}	1.13^{bcde}	116.10^{c}	5.89^{bcde}
Kalatur	1.43^{d}	25.33^{de}	87.67^{bc}	171.70^{cd}	0.35^{ab}	36.13^{a}	3.29^{a}	5.33^{a}	24.53^{def}	7.33^{bcd}	0.73^{a}	110.80^{bc}	5.94^{bcde}
Gadam(check)	1.47^{d}	22.67^{abc}	80.33^{a}	174.30^{efg}	0.75^{bc}	43.57^{b}	3.34^{a}	6.33^{ab}	19.40^{abc}	6.60^{ab}	0.99^{abcd}	112.10^{bc}	5.78^{abcd}
Foehn	1.50^{d}	23.00^{bc}	77.00^{a}	174.00^{ef}	1.54^{def}	36.87^{ab}	5.42^{bcd}	5.67^{a}	24.73^{def}	7.87^{bcde}	0.73^{a}	112.10^{bc}	6.50^{de}
Means	**0.95**	**23.75**	**90.77**	**173.25**	**1.73**	**55.22**	**6.04**	**8.40**	**22.89**	**7.54**	**1.15**	**120.70**	**6.11**
F pr.	**	**	**	**	**	**	**	**	**	**	**	**	**
L.S.D 5%	0.23	1.83	4.05	1.83	0.48	7.17	0.95	1.01	3.50	1.48	0.38	13.37	0.76
S.E	0.14	1.10	2.43	1.10	0.29	4.30	0.57	0.61	2.10	0.89	0.23	8.02	0.46
CV%	14.40	4.60	2.70	0.60	16.70	7.80	9.40	7.20	9.20	11.80	19.50	6.60	7.50

** Significant at 5% level ($p \leq 0.05$), ** = Highly significant ($p \leq 0.01$), NS = Not significant ($p \geq 0.05$ where; SDW- 100-seed weight (g), DEM- 50% days to emergence, DF- 50% days to flowering, DH- Number of days to harvest, GYLD-Grain yield (tha^{-1}), LL- Leaf length (cm), LW-Leaf width (cm), NLVS- Number of leaves, PAL- Panicle length (cm), PAW- Panicle width (cm), PDW- Plant dry weight (tha^{-1}), PHT- Plant height (cm), STM-T- Stem thickness (cm), LSD- Least Significant Difference, Fpr- F Probabilities, CV%- percentage of coefficient of variation.*

**Means with the same letter are not significantly different*

Table 6: Mean of thirteen quantitative traits of the sixteen selected sorghum genotypes evaluated at Sigor

Genotype	**SDW**	**DE**	**DF**	**DH**	**GYLD**	**LL**	**LW**	**NLVS**	**PAL**	**PAW**	**PDW**	**PH**	**STK**
N68	0.70^{a}	8.33^{ab}	74.00^{ab}	111.00^{ab}	1.77^{bcd}	65.00^{abc}	9.34^{bc}	10.67^{a}	23.67^{ab}	8.60^{bc}	1.40^{bc}	206.20^{de}	5.60^{ab}
E117B	0.73^{ab}	8.00^{ab}	85.00^{b}	108.00^{ab}	2.72^{fg}	70.75^{abcd}	9.78^{bcd}	12.44^{a}	23.08^{ab}	8.30^{bc}	1.47^{bc}	243.80^{fg}	6.61^{abc}
T53B	0.83^{abc}	9.00^{ab}	69.33^{ab}	98.00^{a}	1.02^{a}	65.67^{abc}	11.41^{d}	11.17^{a}	28.42^{b}	6.60^{ab}	1.67^{c}	184.10^{bc}	8.14^{c}
E1	0.83^{abc}	9.00^{ab}	72.33^{ab}	108.70^{ab}	1.34^{ab}	73.58^{cd}	9.28^{bc}	10.25^{a}	22.42^{ab}	6.53^{ab}	1.45^{bc}	195.60^{cd}	5.24^{a}
E95A	0.87^{abc}	10.00^{ab}	77.00^{ab}	101.00^{ab}	1.50^{abc}	73.50_{cd}	9.56^{bc}	13.22^{a}	19.42^{a}	7.73^{bc}	0.87^{a}	224.60^{ef}	6.46^{abc}
N57	0.87^{abc}	8.67^{ab}	80.67^{ab}	105.00^{ab}	2.59^{ef}	68.47^{abcd}	10.25^{bcd}	11.92^{a}	24.25^{ab}	8.13^{bc}	1.50^{bc}	246.40_{g}	6.73^{abc}
C26	0.97^{abcd}	8.67^{ab}	77.33^{ab}	98.30^{a}	2.32^{def}	73.78^{cd}	10.56^{cd}	11.94^{a}	19.42^{a}	8.23^{bc}	1.53^{c}	170.90^{b}	6.00^{ab}
E118B	0.97^{abcd}	7.67^{a}	75.33^{ab}	134.70^{c}	3.31^{g}	67.08^{abcd}	10.56^{cd}	11.75^{a}	19.92^{a}	7.47^{abc}	1.20^{b}	173.60^{b}	6.67^{abc}
GADAM(check)	0.97^{abcd}	7.67^{a}	69.33^{ab}	112.70^{ab}	2.11^{cdef}	67.58^{abcd}	5.73^{a}	11.89^{a}	23.87^{ab}	8.40^{bc}	1.40^{bc}	174.30^{b}	7.47^{bc}
E5	1.00^{bcd}	9.33^{ab}	69.33^{ab}	101.00^{ab}	2.38^{def}	71.75^{bcd}	9.17^{bc}	11.33^{a}	23.67^{ab}	6.53^{ab}	0.80^{a}	179.30^{bc}	6.40^{abc}
Kari Mtama 1(check)	1.00^{bcd}	7.67^{a}	77.67^{ab}	102.00^{ab}	2.03^{cde}	72.33^{bcd}	9.97^{bcg}	10.92^{a}	22.93^{ab}	7.70^{bc}	1.47_{bc}	182.40^{bc}	5.98^{ab}
Kalatur	1.07^{cd}	10.33^{ab}	75.67^{ab}	99.00^{a}	1.02^{a}	$71.58a^{bcd}$	5.27^{a}	12.17^{a}	25.92^{ab}	7.20^{abc}	1.70_{c}	183.60^{bc}	6.50^{abc}
Foehn	1.17^{d}	9.67^{ab}	60.33^{a}	93.00^{a}	1.87^{bcd}	65.64^{abc}	8.6^{b}	10.75^{a}	23.53^{ab}	5.53^{a}	0.87^{a}	139.90^{a}	5.42^{a}
T30B	1.17^{d}	10.67^{ab}	70.00^{ab}	106.70^{ab}	2.09^{cde}	63.75^{ab}	9.41^{bc}	11.00^{a}	24.17^{ab}	9.00^{c}	0.87^{a}	197.70_{cd}	5.52^{a}
E1291(check)	1.20^{d}	10.67^{ab}	69.67^{ab}	121.70^{bc}	1.51^{abc}	62.75^{a}	9.53^{bc}	10.56^{a}	27.13^{b}	6.87^{ab}	1.60^{c}	195.10^{cd}	6.27^{abc}
MUK-60	1.20^{d}	8.33^{ab}	70.33^{ab}	94.70^{a}	2.30^{def}	75.17^{d}	10.00^{bcd}	12.22^{a}	24.15^{ab}	7.13^{abc}	0.77^{a}	147.00^{a}	7.03^{abc}
Means	**0.97**	**8.85**	**73.3**	**106**	**1.993**	**69.27**	**9.28**	**11.51**	**23.5**	**7.5**	**1.28**	**190.3**	**6.38**
F pr.	*	NS	NS	NS	**	NS	**	NS	NS	NS	**	**	NS
L.S.D 5%	0.28	2.69	19.08	21.98	0.61	8.98	1.81	3.29	6.86	2.07	0.31	19.87	1.92
S.E	0.17	1.62	11.44	13.18	0.37	5.38	1.08	1.98	4.12	1.24	0.19	11.92	1.15
CV%	17.30	18.20	15.60	12.40	18.40	7.80	11.70	17.20	17.50	16.60	14.40	6.30	18.10

** Significant at 5% level ($p \leq 0.05$), ** = Highly significant ($p \leq 0.01$), NS = Not significant ($p > 0.05$) Where; SDW- 100-seed weight (g), DEM- 50% days to emergence, DF- 50% days to flowering, GYLD-Grain yield (tha^{-1}), LL- Leaf length (cm), LW-Leaf width (cm), NLVS- Number of leaves, PAL- Panicle length (cm), PAW- Panicle width (cm), PDW- Plant dry weight (tha^{-1}), PHT- Plant height (cm), STM-T- Stem thickness (cm), LSD- Least Significant Difference, Fpr- F Probabilities, CV%- percentage of coefficient of variation. *Means with the same letter are not significantly different*

Table 7: Mean response of the selected sorghum genotypes at the two sites

Genotype	100- SDW	DE	DF	DH	GYLD	LL	LW	NL	PAL	PAW	PDW	PH	STK
Foehn	1.33^{a}	16.33^{abc}	68.67^{d}	133.50^{c}	1.71^{ef}	51.25^{e}	7.01^{b}	8.21^{d}	24.13^{ab}	6.70^{b}	0.80^{e}	113.60^{g}	5.96^{ab}
Kalatur	1.25^{ab}	17.83^{ab}	81.67^{abc}	135.30^{c}	0.69^{h}	53.86^{e}	4.28^{c}	8.75^{cd}	25.23^{ab}	7.27^{ab}	1.21^{bcd}	147.20^{e}	6.22^{ab}
GADAM(check)	1.22^{ab}	15.17^{c}	74.83^{cd}	143.5^{abc}	1.42^{efg}	55.58^{de}	4.54^{c}	9.11^{bcd}	21.63^{b}	7.50^{ab}	1.19^{bcd}	143.20^{e}	6.62^{ab}
Kari Mtama 1(check)	1.17^{abc}	15.50^{bc}	83.83^{abc}	137.80^{bc}	1.35^{fg}	62.90^{bc}	7.64^{ab}	9.09^{bcd}	24.17^{ab}	7.65^{ab}	1.30^{bc}	149.20^{de}	5.93^{ab}
MUK-60	1.07^{bcd}	15.50^{bc}	81.17^{abc}	134.50^{c}	2.49^{b}	65.72^{abc}	8.17^{ab}	10.78^{abc}	22.98^{ab}	6.83$_{b}$	1.07^{cd}	126.10^{f}	6.71^{a}
E1291(check)	1.02^{cd}	14.83^{c}	77.33^{bcd}	148.80^{ab}	1.93^{cd}	65.31^{abc}	8.50^{a}	10.25^{abc}	26.70^{a}	7.30^{ab}	1.77^{a}	183.20^{b}	6.16^{ab}
C26	0.93^{de}	14.83^{c}	84.67^{abc}	137.20^{bc}	2.36^{b}	70.28^{a}	8.01^{ab}	10.54^{abc}	20.98^{b}	8.18^{ab}	1.40^{b}	144.20^{e}	6.20^{ab}
E118B	0.93^{de}	18.17^{a}	86.33^{ab}	151.50^{a}	1.79^{cde}	61.06^{bcd}	8.55^{a}	10.34^{abc}	21.40^{b}	7.17^{ab}	1.00^{de}	142.20^{e}	6.11^{ab}
T30B	0.93^{de}	16.67^{abc}	81.67^{abc}	140.50^{abc}	2.16^{bc}	62.94^{bc}	7.99^{ab}	9.83^{bcd}	21.78^{b}	8.63^{a}	1.03^{cde}	178.10^{b}	6.40^{ab}
E5	0.90^{de}	16.00^{abc}	80.50^{abc}	137.70^{bc}	2.98^{a}	64.41^{abc}	7.72^{ab}	9.93^{abcd}	24.80^{ab}	7.07^{b}	1.17^{bcd}	143.80^{e}	6.19^{ab}
E1	0.87^{def}	17.83^{ab}	83.67^{abc}	139.50^{abc}	1.47^{efg}	67.39^{ab}	8.81^{a}	9.96^{abcd}	23.68^{ab}	7.37^{ab}	1.18^{bcd}	140.50^{e}	5.92^{ab}
E95A	0.80^{ef}	16.50^{abc}	84.83^{abc}	137.50^{bc}	1.72^{cdef}	67.68^{ab}	8.21^{ab}	11.91^{a}	22.38^{ab}	8.07^{ab}	1.12^{cd}	171.50^{bc}	6.45^{ab}
N57	0.80^{ef}	15.50^{bc}	86.33^{ab}	139.80^{abc}	2.89^{a}	61.87^{bcd}	8.33^{a}	10.49^{abc}	21.59^{b}	7.60^{ab}	1.18^{bcd}	196.70^{a}	6.26^{ab}
T53B	0.80^{ef}	15.83^{abc}	80.67^{abc}	136.20^{bc}	1.21^{g}	60.23^{cd}	8.38^{a}	9.48^{bcd}	24.44^{ab}	7.93^{ab}	1.42^{b}	160.70^{cd}	6.90^{a}
E117B	0.69^{f}	18.00^{a}	91.33^{a}	138.50^{bc}	1.94^{cd}	63.58^{abc}	8.53^{a}	10.82^{ab}	23.84^{ab}	7.98^{ab}	1.45^{b}	172.40^{bc}	6.52^{ab}
N68	0.68^{f}	16.33^{abc}	85.33^{abc}	141.80^{abc}	1.79^{cde}	61.93^{bcd}	7.91^{ab}	9.80^{bcd}	21.40^{b}	7.03^{b}	1.18^{bcd}	175.50^{b}	5.33^{b}
Mean	**0.96**	**16.30**	**82.05**	**139.60**	**1.87**	**62.25**	**7.66**	**9.96**	**23.19**	**7.52**	**1.22**	**155.50**	**6.24**
Fpr	**	**	NS	**	**	**	**	*	*	**	**	**	*
L.S.D	0.25	2.85	13.28	15.04	0.55	8.31	1.48	2.4	5.34	1.83	0.34	16.54	1.60
S.E	0.15	1.75	8.14	9.21	0.34	5.09	0.91	1.47	3.27	1.12	0.21	10.13	0.98
CV%	15.80	10.70	9.90	6.60	18.00	8.20	11.90	14.80	14.10	14.90	17.20	6.50	15.70

*** Highly significant ($p \leq 0.01$), * = Significant ($0.01 < p \leq 0.05$), NS = Not significant ($p > 0.05$) Where; SDW- 100-seed weight (g), DEM- 50% days to emergence, DF- 50% days to flowering, GYLD- Grain yield (tha^{-1}), LL- Leaf length (cm), LW-Leaf width (cm), NLVS- Number of leaves, PAL- Panicle length (cm), PAW- Panicle width (cm), PDW- Plant dry weight (tha^{-1}), PHT- Plant height (cm), STM-T- Stem thickness (cm), LSD- Least Significant Difference, Fpr- F Probabilities, CV%- percentage of coefficient of variation. *Means with the same letter are not significantly different*

4.2 Principal component analysis

The data on quantitative traits obtained at the from Sigor and Endebess were subjected to principal component analysis (Table 8), Results revealed that the three most important PCs contributed PC1 (81.78%), PC2 (15.33%) and PC3 (1.5%) of the variation in total. Plant height (0.889) contributed the more in the first PC. Number of days to harvest (0.814) had the most contribution in the second PC, whereas leaf length (0.842) was the larger contributor in the third PC.

Table 8: A factor loading of the quantitative traits in sorghum genotypes evaluated across the two locations.

	Factor loadings		
Traits	**PC1**	**PC2**	**PC3**
%100_SDW_g	-0.001	-0.005	-0.015
DE	-0.103	0.119	0.055
DF	-0.064	0.346	0.491
DH	-0.426	0.814	-0.069
GYLD_t	0.005	0.002	0.031
LL	0.109	-0.090	0.842
LW	0.028	-0.027	0.106
NL	0.027	-0.005	0.116
PAL	-0.006	-0.044	-0.070
PAW	0.004	0.020	0.017
PDW	0.003	0.005	0.013
PH	0.889	0.440	-0.102
STK	0.002	-0.002	0.006
% variation	81.780	15.330	1.500
Latent roots	388506.000	72840.000	7114.000

SDW-seed weight, DE- 50% days to emergence, DH- Days to harvest, DF- 50% days to flowering, GYLD- Grain yield, LL- leaf length, LW- Leaf width, NL- Number of leaves, PAL- Panicle length, PAW- Panicle width, PDW- Plant dry weight, PH- Plant height, STK-Stem thickness.

4.3 Combined Spearman's rank correlation analysis of qualitative and quantitative traits

Thirteen important qualitative and fourteen quantitative traits were done using Spearman's rank correlation coefficients (table 9). The days to 50% flowering strongly negatively correlated to 100-seed weight (r=-0.5) and strongly positively correlated to number of days to harvest (r=0.7) at P <0.01. Number of days to emergence positively correlated to the number of days to 50% flowering and maturity and negatively correlated to leaf lent plant height and number of leaves per plant. Number of days to harvest negatively correlated to leaf length, width, number of leaves in a plant and plant height. Disease resistance positively correlated to pest resistance and negatively correlated to seedling colour. Ear head shape and compactness of the sorghum genotypes was strongly negatively correlated to orientation of the leaves (r=-0.6) and colour of the seeds (r=-0.6) while it correlated positively to the ability to thresh (r=0.6) at *p*<0.01. The number of leaves also showed a positive correlation to plant dry weight and height of plant. Plant Dry Weight showed a strong positive correlation to plant height (r=0.5)

Table 9: Spearman’s rank correlation analysis of qualitative and quantitative traits

Trait	_SDW	DE	DF	DH	DR	DRR	EHSC	GCL	GCV	GY	LL	LOR	LP	LW	MDC	NL	PAL	PAW	PDW	PH	PR	SC	SS	STK	SV	TSH
SW	-																									
DE	0.0	-																								
DF	-0.5***	0.7***	-																							
DH	-0.1	0.9***	0.7***	-																						
DR	0.2	0.0	-0.1	0.0	-																					
DRR	-0.4	0.0	0.1	0.0	-0.1	-																				
EHSC	0.3	0.0	-0.1	0.0	0.0	-0.6***	-																			
GCL	-0.1	0.1	0.1	0.0	0.2	0.3	-0.1	-																		
GCV	-0.3	0.0	0.1	0.0	-0.1	0.4	-0.1	0.2	-																	
GY	-0.2	-0.3	0.0	-0.1	0.0	0.0	0.0	-0.1	0.1	-																
LL	-0.3	-0.6***	-0.3	-0.6***	-0.2	0.0	0.0	-0.1	0.0	0.3	-															
LOR	-0.1	0.0	0.1	0.0	-0.2	0.3	-0.5***	0.1	-0.2	-0.1	0.0	-														
LP	0.0	0.0	0.1	0.0	-0.4	0.1	0.3	-0.3	-0.2	0.0	0.1	0.2	-													
LW	-0.3	-0.7***	-0.4	-0.8***	-0.1	0.0	0.0	0.0	0.1	0.3	0.8***	0.0	0.0	-												
MDC	0.3	0.0	-0.1	0.0	0.1	0.0	-0.2	0.2	-0.3	-0.2	-0.2	0.4	-0.1	-0.1	-											
NL	-0.4	-0.6***	-0.1	-0.6***	-0.1	0.1	0.0	0.1	0.1	0.3	0.8***	0.0	0.1	0.8***	-0.3	-										
PAL	0.2	0.0	-0.3	-0.1	0.0	-0.1	0.2	-0.1	0.0	-0.3	-0.1	-0.3	0.0	0.0	-0.1	-0.1	-									

Trait	_SDW	DE	DF	DH	DR	DRR	EHSC	GCL	GCV	GY	LL	LOR	LP	LW	MDC	NL	PAL	PAW	PDW	PH	PR	SC	SS	STK	SV	TSH
PAW	-0.3	0.0	0.4	0.1	-0.1	0.1	0.0	0.1	0.0	0.2	0.0	0.1	0.1	0.1	0.0	0.3	-0.1	-								
PDW	-0.4	-0.2	0.2	-0.1	0.0	0.1	0.0	-0.1	0.1	0.2	0.4	-0.1	0.1	0.4	-0.2	0.5***	0.1	0.4	-							
PH	-0.3	-0.7***	-0.2	-0.6***	0.0	0.2	-0.1	0.0	0.0	0.2	0.6***	0.1	0.0	0.6***	-0.1	0.7***	-0.2	0.3	0.5***	-						
PR	0.0	0.0	0.0	0.1	0.5***	0.1	0.1	0.3	0.2	0.0	-0.1	0.3	-0.2	0.0	0.1	0.0	-0.1	0.0	0.0	0.0	-					
SC	-0.4	0.0	0.1	0.0	-0.5***	0.3	-0.6***	0.1	0.2	0.0	0.2	0.3	-0.2	0.1	-0.2	0.2	-0.1	0.0	-0.1	0.0	-0.4	-				
SS	0.3	0.1	0.0	-0.1	0.2	0.0	-0.1	0.3	-0.2	-0.1	-0.3	0.3	0.0	-0.2	0.5***	-0.1	-0.1	0.0	-0.2	-0.1	0.0	-0.1	-			
STK	-0.1	-0.2	-0.1	-0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.3	-0.1	0.1	0.1	0.3	0.1	0.1	0.1	0.0	0.0	-		
SV	0.0	-0.1	-0.1	0.0	-0.2	-0.3	0.0	-0.5***	-0.2	0.2	0.2	-0.3	-0.1	0.0	-0.2	0.1	0.0	-0.1	0.0	0.0	-0.4	0.1	-0.5***	-0.1	-	
TSH	0.1	0.0	-0.1	-0.1	-0.1	-0.4	0.6***	-0.2	0.2	0.2	0.0	-0.6***	0.1	0.0	-0.4	0.0	0.3	-0.1	0.1	-0.1	-0.1	-0.3	-0.1	0.1	0.3	-

****SDW-seed weight, DE- 50% days to emergence, DF- 50% days to flowering, DR- disease resistance, DRR- Drought resistance, EHSC- Ear head shape and compactness, GCL- Glume colour, GCV- Glume covering, GYLD- Grain yield, LL- leaf length, LOR- Leaf orientation, LP- Leaf pigmentation, LW- Leaf width, MDC- Midrib colour, NL- Number of leaves, PAL- Panicle length, PAW- Panicle width, PDW- Plant dry weight, PH- Plant height, PR- Pest resistance, SC- Seed colour, SS- Seed size, STK- Stem thickness, SV- Seedling vigour, TSH- Threshability.***

4.4 Frequency distribution of qualitative traits

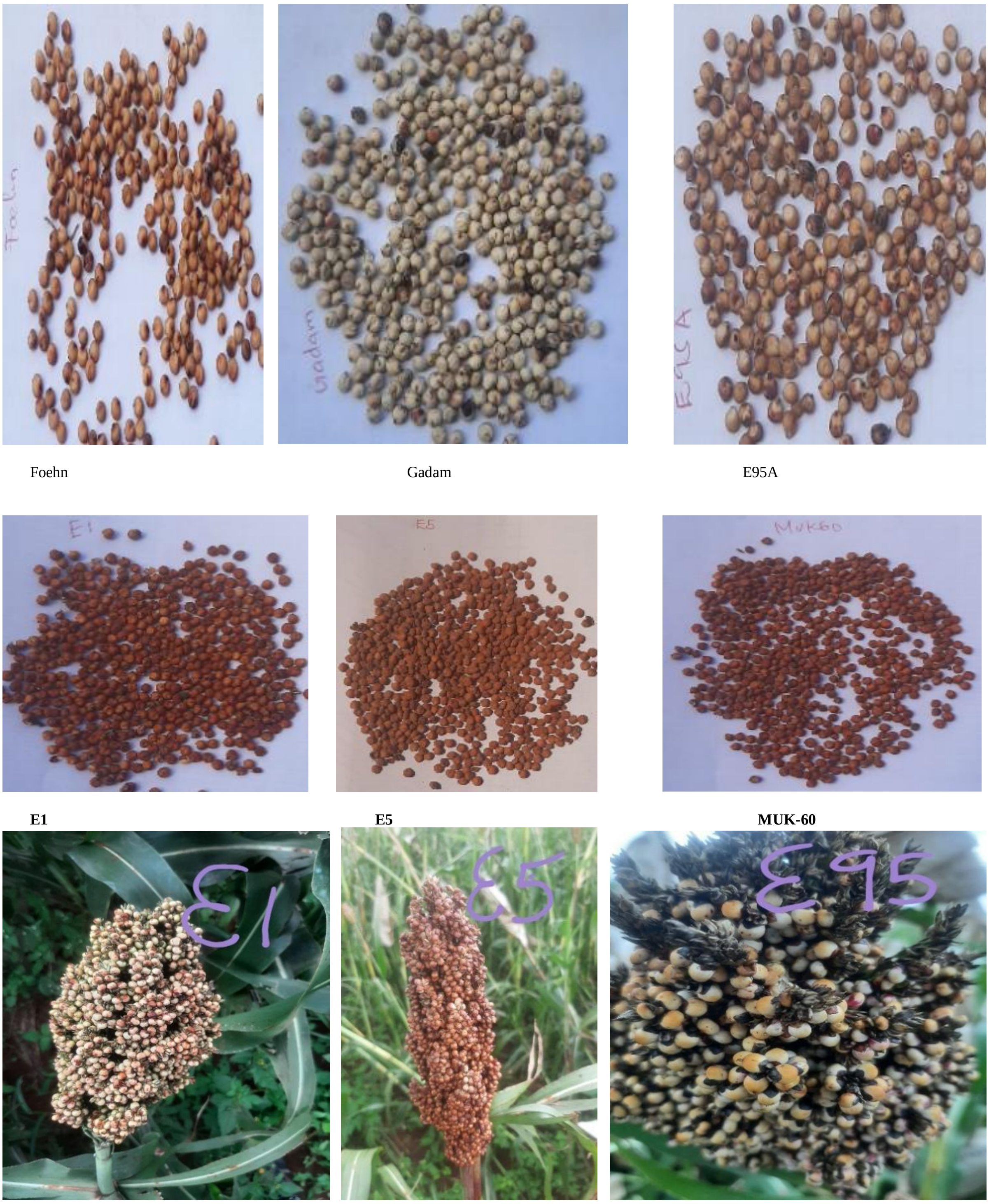
The frequency distributions of the sorghum accessions for the qualitative traits are presented in Table 10. Fifteen (93.75%) selected sorghum genotypes were pigmented while one (6.25%) were tan. Six (37.5%) were erect and ten (62.5%) had horizontal in terms of their leaf orientation. Ear head compactness and shape (EHCS) showed variation with five (31.25%) accessions being compact elliptic, two (12.5%) compact erect, three (18.75%) semi-compact elliptic, five (31.25%) semi-compact erect and one (6.25%) being semi-compact drooping. None of the genotypes had awns at maturity. There was also variation in the midrib colour among the accessions, in that five (31.25%) accessions had dull green midribs followed by eleven (68.75) accessions with white midribs. In terms of the glume colour, two (12.5%) genotypes had black glumes, one (6,25%) brown, one (6.25%) grey, six (37.5%) purple, four (25%) red, and two (12.5%) siena. eight (50%) of the genotypes had 25% glume covering and the rest eight (50%) had 50% glume covering. There was also variation in the seed sizes with one (6.25%) large, seven (43.75%) medium and eight (50%) small. With seed colour; eleven (68.75%) had brown colour, one (6.25%) chalky white, one (6.25%) cream, two (12.5%) red and one (6.25%) speckled white. For threshability, one (6.25%) had very good threshability, nine (56.25%) good, four (25%) medium and two (12.5%) with poor threshability.

Table 10: Frequency distribution of the qualitative traits of the sorghum genotypes observed at Elgon downs farm and Sigor

Trait	Descriptor	Genotypes out of 16 selected	Frequency %
Leaf pigmentation	Tan	1	6.25
	Pigmented	15	93.75
Leaf orientation	Erect	6	37.5
	Horizontal	10	62.5
Midrib colour	Dull green	5	31.25
	white	11	68.75
Glume colour	Black	2	12.5
	Brown	1	6.25
	Grey	1	6.25
	Purple	6	37.5
	Red	4	25
	Siena	2	12.5
Glume covering	25	8	50
	50	8	50
awns	absent	16	100
seed size	large	1	6.25
	Medium	7	43.75
	small	8	50
seed colour	Brown	11	68.75
	Chalky white	1	6.25
	Cream	1	6.25
	Red	2	12.5
	Speckled white	1	6.25
Threshability	Good	9	56.25
	Very good	1	6.25
	Medium	4	25
	Poor	2	12.5
EHCS	Compact erect	2	12.5
	Compact elliptic	5	31.25
	Semi -compact elliptic	3	18.75
	Semi -compact erect	5	31.25
	Semi -compact drooping	1	6.25

4.4.1 Morphological Variability Based on qualitative traits

Field evaluation of the 16 sorghum genotypes revealed notable phenotypic diversity in traits related to grain and panicle morphology. Clear differences were observed in seed color, ranging from white and cream to reddish-brown. Seed sizes also varied among the genotypes, with some producing large, bold grains and others yielding smaller, compact seeds. In terms of panicle shape, the genotypes displayed a spectrum ranging from compact and semi-compact to lose and open panicles. Compact panicles, as observed in genotypes *E5* and *E1 (Fig.1)*, are associated generally with higher grain density and reduced susceptibility to grain mold in humid areas.



Foehn Gadam E95A

E1 **E5** **MUK-60**

E1 E5 E95A

Figure 1: Sorghum genotypes indicating variability in seed size, colour and panicle shapes.

4.2 Diversity based on nutritional composition of tested nutrients in the selected sorghum genotypes

Fat content

The tested sorghum varieties differed greatly in terms of fat content (0.001). While E117B (2.6) variety had a lower fat level than other types, some, such E95A (15.27), MUK-60 (14.45) and KARI-Mtama (13.68), showed noticeably higher fat content. This implies that depending on their fat content, various sorghum types may be better suited for particular uses (Table 10).

Iron content

iron nutrient composition displayed a significant variation among the genotypes. E118B (0.26), E1291 (0.15) and E5 (0.12) were found to have a higher percentage of iron while E95A and N68 (0.06) occupied the lower end of the spectrum.

Phosphorous content

A significant variation (p<0.001) was noted in terms of phosphorus accumulations by the sorghum genotypes. This was shown by the different levels of percentage phosphorus in each sorghum variety tested. Varieties such as C26 (0.26), T30B (0.24) and Foehn (0.22) showed significantly higher levels of phosphorus while N68 (0.076) and Gadam (0.16) had the lowest levels of phosphorus. The rest of the varieties contained relatively moderate levels of phosphorus.

Protein composition

This revealed highly significant differences among genotypes. T53B (11.19), Foehn (6.81), C26 (6.8) varieties recorded a relatively higher level of protein content as compared to E1291 (2.85) and N68 (3.11) varieties that exhibited lower levels of proteins.

Zinc composition

The Fpr value (*p*<0.001) indicates that genotypes were statistically significant at the 5% level. Significant differences were observed within the chosen genotypes in their zinc composition. E118B and N57 recorded the highest zinc levels while E95A and E117B Recorded the lowest zinc levels (Table 10).

Table 10: Means of the nutritional composition of the selected Sorghum genotypes

Genotype	Zinc	Proteins	Phosphorous	Iron	Fat
T53B	0.043cde	11.19a	0.1892cde	0.0645hi	11.97d
E118B	0.047e	3.688def	0.2283b	0.2598a	8.85g
N57	0.047e	4.71cde	0.1698ef	0.0703fghi	12.42d
C26	0.045e	6.801b	0.2567a	0.1166c	7.83h
MUK-60	0.0445de	5.687bc	0.2283b	0.0872defgh	14.45b
KALATUR	0.04375cde	5.113bcd	0.2123bc	0.0792efghi	11e
E5	0.04215bcde	4.619cdef	0.1783def	0.1175c	5.75i
T30B	0.0392abcd	3.699def	0.2308b	0.0917def	9.72f
FOEHN	0.0388abc	6.813b	0.2223b	0.0912defg	10.58e
KARI MTAMA(check)	0.03875abc	3.527def	0.2077bc	0.0934de	13.67c
E1291(check)	0.0386abc	2.849f	0.134g	0.1517b	9.7f
GADAM (check)	0.03765ab	3.941cdef	0.1623f	0.1041cd	9.7f
E1	0.0358a	4.251cdef	0.1802def	0.0879defg	9.6f
N68	0.03565a	3.114ef	0.0762h	0.0687ghi	9.65f
E95A	0.0353a	3.803def	0.1971cd	0.0576i	15.27a
E117B	0.0342a	3.803def	0.1698ef	0.0815defgh	2.6j
Means	**0.04047**	**4.85**	**0.1902**	**0.1014**	**10.173**
Fpr	<.001	<.001	<.001	<.001	<.001
L.S.D	0.006	1.7753	0.02369	0.02286	0.5409
S.E	0.003	0.8329	0.01111	0.01073	0.2538
CV%	6.5	17.2	5.8	10.6	2.5

**** LSD (5%)-least Significant Difference, S.E- Sum of errors, C.V%- Percentage of coefficient variations***

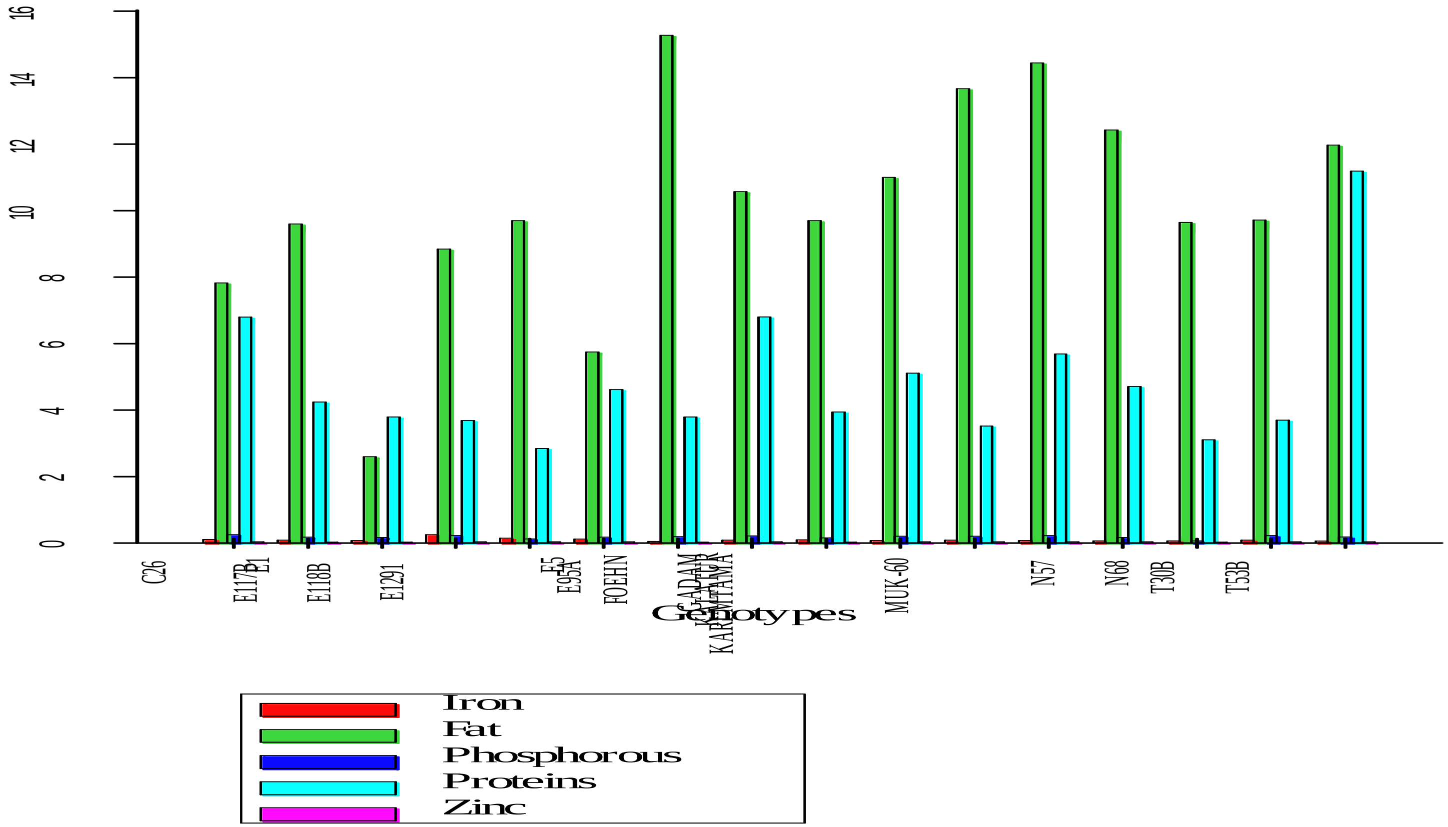


Figure 2: Nutritional composition of the selected sorghum genotypes

4.3 Genetic Relationships between Sorghum Genotypes Based on Dendrogram representation

The UPGMA dendrogram based on nutrient composition revealed distinct patterns of relatedness among the sorghum genotypes (Figure 3). A closely related cluster was observed among Gadam (check), E95A, E117B, T30B, and KARI Mtama1 (check), indicating a high degree of similarity in their nutritional profiles. Similarly, E1 and E118B formed a separate but tightly linked group. Another cluster comprising N57, E5, Kalatur, and MUK-60 showed moderate similarity in nutritional traits. N68 and E1291 (check) were positioned between the main cluster and the outlier genotypes, indicating intermediate nutrient relationships. In contrast, T53B emerged as the most divergent genotype. Additionally, Foehn and C26 formed a unique cluster, relatively distant from the primary groupings.

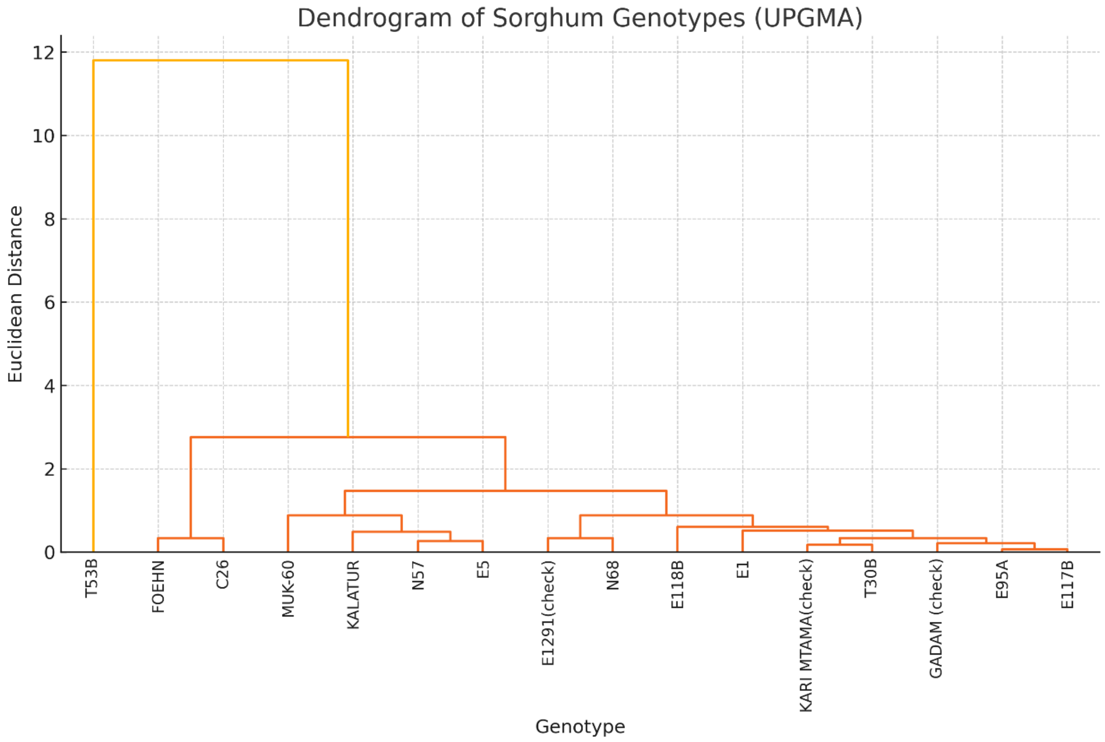


Figure 3: Dendrogram of 16 sorghum genotypes revealed by UPGMA cluster analysis based on chemical composition

4.2.3 Genotype variance and distribution

Principal component PC1 (57.2%) captured most of the biochemical diversity in the genotypes. PC2 (21.5%) revealed additional variation but to a lesser extent. The two components explained about 78.7% of the total variance suggesting a great variability. Genotypes that were positioned closely in the PCA plot have similar biochemical profiles. T53B appeared far from the others, confirming its distinct nutrient composition (fig. 4). Check varieties GADAM, KARI MTAMA, E1291 clustered together, indicating consistency in their biochemical traits.

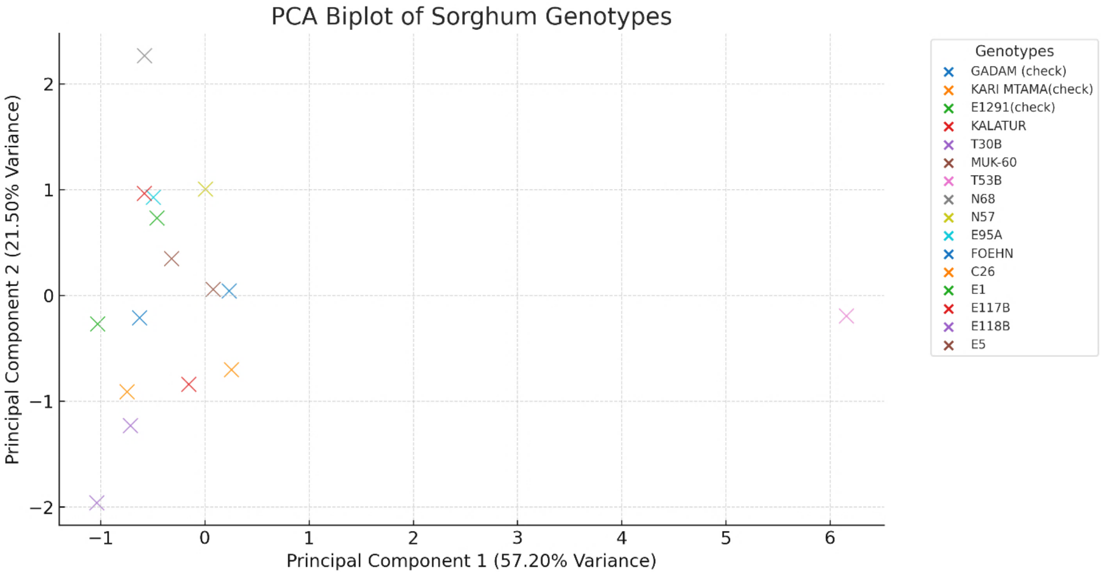


Figure 4: PCA Biplot Depicting Diversity within sorghum genotypes

4.2.4 Shannon-Weiner's Diversity Index

The Shannon-Weiner Diversity Index (H') for the biochemical traits of sorghum genotypes yielded a total value of 2.739. This indicated that there is a moderate to a high diversity among the selected sorghum genotypes. Zinc (H' = 2.767) exhibited the highest diversity, followed closely by Phosphorous (H' = 2.745) and Fat (H' = 2.720). Protein (H' = 2.702) and Iron (H' = 2.688) showed the lowest diversity.

The Shannon-Weiner Evenness Index (J'= 0.988) suggests an even distribution among the selected genotypes. Zinc and Phosphorus displayed the most evenness, with Iron recording the lowest evenness, implying that some of the genotypes contribute more significantly to the traits than others.

Table 11: Shannon-Weiner Diversity Index (H') and Evenness Index (J') for different biochemical traits

Diversity Index	Fat	Iron	Phosphorus	Protein	Zinc	Total
Shannon-Weiner H	2.720	2.688	2.745	2.702	2.767	2.739
Shannon-Weiner J	0.981	0.969	0.990	0.974	0.998	0.988

4.3 Evaluation for diversity among selected sorghum genotypes using ISSR Molecular markers

4.3.1 Analysis of Molecular Variance among selected sorghum genotypes

The variance components of sorghum population under the study showed differences among and within populations using significance tests based on 1,000 permutations. The Analysis of Molecular Variation (AMOVA) revealed that in the variability of the sorghum population there was 3% and 97% among and within populations, respectively (table 12). The estimation of the variance components among and within populations using AMOVA was significant ($P<0.01$).

Table 12: Molecular Variance of Sorghum population

Source	df	SS	MS	Est. Var.	%	P	PhiPT (ΦPT)
Among Populations	2	3.021	1.510	0.040	3%	0.010	0.153
Within Populations	13	17.667	1.359	1.359	97%		
Total	15	20.688		1.399	100%		

Key: PhiPT(ΦPT) **-** the estimate of population genetic differentiation based on permutation across the full data set; df - degree of freedom; TSS - total sum of squares; MSS - Mean sum of squares.

4.3.2 Principal co-ordinate analysis (PCOA)

The genetic relationships among the 3-sorghum population based on ISSR markers using principal co-ordinate (PCoA) analysis (Figure 7). The first three major axis of differentiation (PC1, PC2, PC3) explained cumulative percentage of 33.64%, 24.67% and 17.3% of variation.

Table 13: Percentage of variation explained by the first 3 axes

Percentage of variation explained by the first 3 axes			
Axis	**1**	**2**	**3**
%	33.64	24.67	17.49

The PCoA classified the 3 sorghum populations with some distinct major groups on as shown in figure 5. The pattern of clustering was almost distinct for few genotypes of check, UOE and KSC. Significant dispersion was observed on UOE genotypes. The first two principal coordinates accounted for a substantial proportion of the molecular variation in total. In the PCoA biplot (Figure 5). Genotypes such as MUK60, T30B, and N68 were positioned on the left of the plot. On the right side, FOEHN, T53B, and

E118B clustered closely. CHECK lines like E1291 and E95A grouped together near the center-top quadrant. Genotypes from UOE and KSC were more scattered.

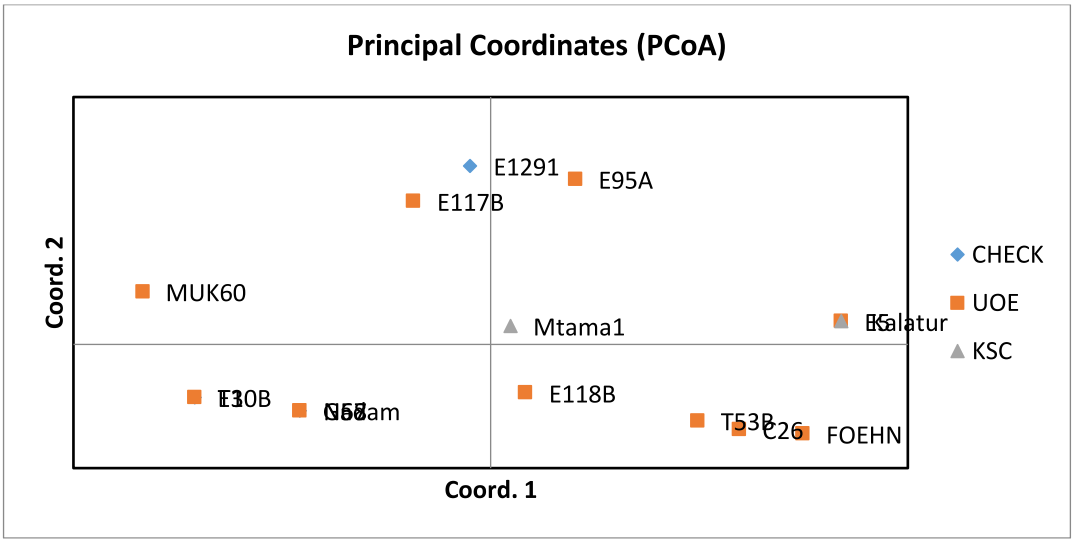


Figure 5: Principal coordinates of the sorghum genotypes

4.3.3 Genetic distance between populations of sorghum

Pairwise comparison of Nei ’s unbiased genetic identity among the 3 populations ranged from a low of 0.000 between check and UoE populations to a high of 0.239 between check and KSC populations (Table 15). Other populations that showed low genetic distances were UOE and KSC with a Nei’s genetic distance of 0.150. In addition, populations that had a high unbiased Nei ’s genetic distance were check and UOE that had genetic identity of 1.001 and between UOE collection from KSC of 0.861 and those collected from KSC and Check with a Nei’s genetic distance of 0.787 (Table 14).

Table 14: Pairwise Population Matrix of Nei Unbiased Genetic Distance

CHECK	UOE	KSC	
1.000			CHECK
1.001	1.000		UOE
0.787	0.861	1.000	KSC

Table 15: Pairwise Population Matrix of Nei Unbiased Genetic Identity

CHECK	UOE	KSC	
0.000			CHECK
0.000	0.000		UOE
0.239	0.150	0.000	KSC

4.3.4 Band Patterns across Populations

The banding pattern analysis across three populations (CHECK, UOE, and KSC) revealed notable variation in genetic diversity figure 6. The number of bands in total was highest in the UOE population (13), followed closely by CHECK, and while KSC had the lowest number of bands (9). The bands with a frequency ≥5% showed a similar trend, with UOE and CHECK populations having higher numbers compared to KSC. Notably, UOE was the only population that exhibited private bands. Heterozygosity was also highest in the UOE population (0.30), followed by KSC, and while CHECK exhibited the lowest heterozygosity. The presence of low common bands (≤25% and ≤50%) was slightly higher in UOE.

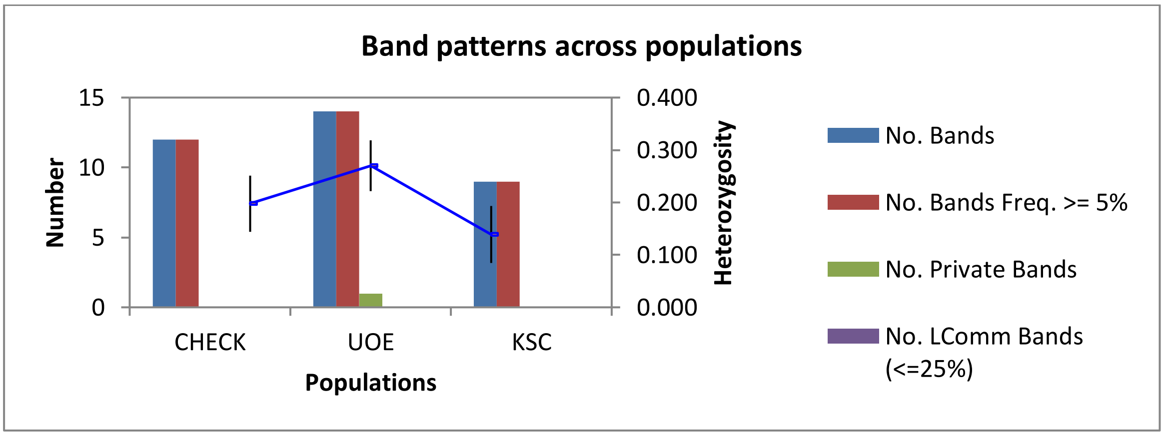


Figure 6: Band Patterns for Binary (Haploid) Data in Molecular Analysis

4.3.5 Band, allele frequencies, and estimated diversity in sorghum population

The diversity estimates across three sorghum populations (CHECK, UOE, and KSC) revealed significant differences in genetic variability as shown in the table 16 below. The University of Eldoret (UOE) population exhibited the highest values across most diversity indices. It had the highest number of individuals (N = 11.00), effective number of alleles (Ne = 1.75), Shannon's information index (I = 0.62), gene diversity (h = 0.42), and unbiased gene diversity (uh = 0.47).

The CHECK population had moderate diversity estimates with Na (1.50), Ne (1.53), I (0.42), h (0.29), and uh (0.44). In contrast, the KSC population exhibited the lowest genetic diversity, with Na (1.17), Ne (1.33), I (0.23), h (0.17), and uh (0.33). The grand mean across all loci and populations was Na (1.56), Ne (1.54), I (0.42), h (0.29), and uh (0.42), with standard errors ranging between 0.05 and 0.08.

Table 16: Diversity Estimates Band, Allele Frequencies, and Estimated Diversity in Sorghum Population

Pop	Mean/SE	N	Na	Ne	I	h	uh
CHECK	**Mean**	3.000	1.500	1.533	0.424	0.296	0.444
	SE	0.000	0.342	0.169	0.134	0.094	0.141
UOE	**Mean**	11.000	2.000	1.746	0.615	0.424	0.467
	SE	0.000	0.000	0.058	0.019	0.018	0.020
KSC	**Mean**	2.000	1.167	1.333	0.231	0.167	0.333
	SE	0.000	0.307	0.211	0.146	0.105	0.211
Grand Mean and SE over Loci and Pops	**mean**	5.333	1.556	1.538	0.423	0.296	0.415
	SE	0.977	0.166	0.096	0.073	0.051	0.081

N- Number of Individuals, Na-Number of Different Alleles, Ne-Number of Effective Alleles, I-Shannon Information Index, h- Nei's Gene Diversity (Expected Heterozygosity), uh- Unbiased Expected Heterozygosity.

The percentage of polymorphic bands among the three sorghum populations varied considerably table 17. The UoE population recorded the highest polymorphism at 100.00%. The CHECK population had 66.67% polymorphism. In contrast, the KSC population had the lowest percentage of polymorphic bands at 33.33%. The overall mean percentage of polymorphism across all populations was 66.67%

Table 17:Percentage of Polymorphic Loci

Population	%P
CHECK	66.67%
UOE	100.00%
KSC	33.33%
Mean	66.67%
SE	19.25%

4.3.6 Cluster structure

The dendrogram showed three major cluster: Cluster I comprised genotypes from all three groups: Foehn (KSC), T53B (UoE), C26 (UOE), Kalatur (KSC), E5 (UoE) and Mtama 1(Check). These genotypes exhibited close genetic relationships despite their different sources, forming a mixed cluster. Cluster II was more diverse and subdivided into several sub-clusters: One sub-cluster grouped T30B (UoE) with E1 (UoE) and MUK60 (UoE), indicating moderate similarity among these genotypes. Another sub-cluster consisted of N57 (UoE) and Gadam (check), showing a genetic closeness between a check variety and a UoE line. N68 (UoE) and E118B (UoE) also formed a distinct group. The most distant cluster III group included E95A (UoE), E1291 (check), and E117B (UoE). These genotypes showed the highest genetic divergence from the rest.

4.3.7 Genetic Diversity Trends

The UoE population showed wide dispersion throughout the dendrogram, clustering with both KSC and check varieties across different branches. The check varieties, though fewer, were scattered and did not form a tight cluster. The KSC genotypes clustered closely with a few UoE genotypes, particularly in Cluster I.

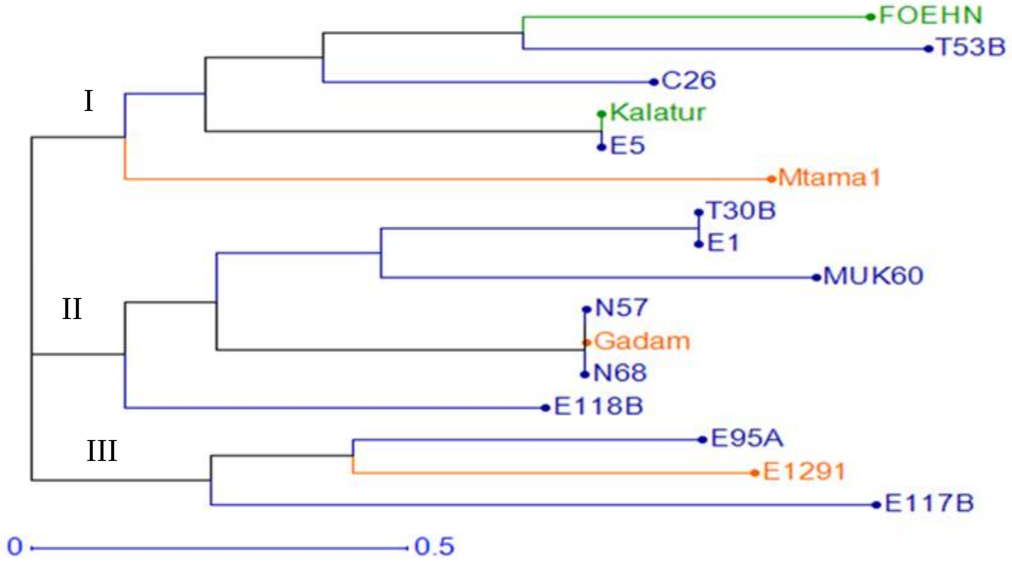


Figure 7: Genetic relationship among three populations of sorghum using dendrogram based on Darwin's Genetic Identity Distance

CHAPTER FIVE

DISCUSSION

5.1 Evaluation for diversity of selected sorghum genotypes using agro-morphological markers

The use of morphological markers is effective and only reliable if they give a variation range that is wide between accessions (Karugu, 2009). Sixteen sorghum accessions were described and assessed for agro-morphological traits where qualitative and quantitative features showed a huge variation. Crop germplasm has been subjected to phenotypic study using both qualitative and quantitative parameters, (Ngugi & Maswili, 2010; Kisilu & Ngugi, 2021) reported using phenotypic data to access diversity in 148 sorghum accessions, (Apunyo et al., 2022), also reported phenotypic diversity of sorghum accessions on farmers' fields in Northern and Eastern Uganda.

Other authors that have reported related studies using qualitative and quantitative traits in sorghums are (Mofokeng, 2015; Sejake et al., 2020; Andiku et al., 2022; Bouargalne et al., 2022). Traits such as mass of seed, yield, plant height and earliness are mostly used by farmers to determine desirable traits for future cropping. Understanding the significant correlation among the characters is crucial for many breeding programs since it allows for the simultaneous selection of desired genotypes and desirable phenotypes (Mofokeng, 2015) .

5.1.1 Mean response of the selected sorghum genotypes across the environments

Long panicles and increase plant dry usually go in and with yield of sorghum grain. Similar results were reported by (Santhiya et al., 2021; Vinoth et al., 2021) on good positive relation between yield, length of panicle and dry weight. This reinforces the need of selecting genotypes that good yielders with vital morpho-physiological traits.

Varieties that flower early are better suited to regions that are prone to drought because they can successfully complete their cycle before beginning of stress and also enable double cropping (El Mannai et al., 2011). Therefore, the genotypes that flowered early and reached maturity early are beneficial to areas with drought and short rains. Genotypes that showed delayed flowering are advantageous to areas with longer growing periods, these allows them to fully utilize available resources throughout the season and enrich biomass production improved uptake of nutrients and root development hence high yields (Agele et al., 2008; Craufurd & Wheeler, 2009; Carillo, 2025). Delayed flowering is vital in breeding programs seeking highland or tropical environments where early varieties might not fully exploit its potential (Ceccarelli, 2015; Tuberosa, 2012).

For plants to reach their photosynthetic capacity, the leaf size and number of leaves ave a direct impact on their potential. Sorghums wit longer and broader leaves accumulate more biomass because of the surface area. Similar results were reported (Santhiya et al., 2021). It also sustains the plants growth contributing positively to high yields. The number of grains per panicle and yield are also influenced by the size of the panicle in terms of its length and width as wider panicles have more florets hence success in reproduction. This was also reported by (Hmon et al., 2013). Biomass production and resistance to lodging is influenced by plant height and thickness of the stem as taller plants are well suited for fodder production. However, tall genotypes are susceptible to lodging while thicker ones reduce lodging as they offer firmness (Ezeaku & Mohammed, 2006). Combining the two traits should be a target in breeding programs.

5.1.2 Principal component Analysis

Principal Component Analysis (PCA) is a technique used to reduce data dimensionality, but it involves subjective decisions at various stages. These include choosing which variables to analyze, how to standardize the data, and how many components to retain. Such decisions can influence both the interpretation and the outcome of the analysis, making transparency essential (Kavithamani et al., 2019). In PC1, Plant height is the most significant trait influencing diversity, playing a core role in genotype differentiation. Mofokeng et al., (2017) similarly reported that plant height, panicle weight and grain yield significantly influence sorghum diversity. Number of Days to harvest and flowering contributed majorly in PC2 and 3 respectively which contribute to timing vital for adaptation and breeding. Kavithamani et al. (2019) conducted PCA on 100 sorghum germplasm accessions and found that 100-seed weight, plant height, leaf blade length, and leaf blade width were significant contributors to genetic diversity. Similarly, (Sejake et al., 2020) assessed 100 accessions of sorghum and reported that plant height, panicle length, and weight of grain per panicle played crucial roles in genetic variation. In another study, (Ngugi & Maswili, 2011) evaluated 148 Kenyan sorghum landraces and observed that panicle branches, panicle length, and grain weight were significant in determining phenotypic diversity. A study on Moroccan sorghum ecotypes revealed that height, leaf length, and length of panicle were among the primary traits contributing to agro-morphological variation (Bouargalne et al., 2022). These studies affirm that PCA is an efficient tool used to identify key traits that influence genetic variation in sorghum, aiding in the selection of genotypes tat are superior for breeding and conservation programs.

5.1.3 Pearson’s Correlation Analysis

This revealed several statistically significant relationships among key plant traits, which provide insights that are valuable for plant breeding strategies. These correlations can help breeders identify traits which can improved or indicate potential trade-offs.

To enhance plant structure or crop output, selection needs to be based on the association of relevant characteristics, which measures the associations or correlations between various plant attributes and identifies key traits that may be used as the basis for breeding to increase crop seed yield (Aklilu et al., 2020; Rahman et al., 2023; Tilahun et al., 2024). To select ecotypes that are promising which combine different agronomically important characters, analysis of correlation is an important indicator (Bouargalne et al., 2022).

A positive correlation between days to flowering and days to harvest suggests that plants that flower later also mature later. Similar results were exhibited in wheat, grain yield per plant showed a significant positive correlation with biological yield per plant, plant height, and thousand-grain weight, indicating that traits influencing plant development stages are interrelated (Devesh, 2021). Similar to these results are those of Mallu. (2015) who evaluated chick pea genotypes for yield and selection of agronomic traits in Kenya and found out that days to 75% maturity highly significantly correlated to days to 50% flowering.

Negative correlation showed that the late flowering plants had better seed weight as compared to the early flowering genotypes. Similarly, (Ouma & Akuja 2013) showed that days to flowering was significantly positively correlated with height and yield except for 100- seed weight. Those that matured early had lower number of seed and yield as compared to the medium and late- maturing genotypes, but greater seed size.

A negative correlation between the leaf number and days to flowering/harvest indicates that plants with more leaves mature earlier. Similar results by Dhurai. (2014) indicated that in rice, yield was significantly associated with harvest index and number of grains per panicle, suggesting that traits contributing to yield are interconnected.

A positive correlation between pest and drought resistance suggests that plants tolerant to drought may also exhibit pest resilience(Ndiso et al., 2007; Marenya et al., 2022) . Drought can weaken plants increasing pest vulnerability. Diseases like anthracnose, smut, blight, ergot are more likely to strike plants with pest infestations. Plants that have been harmed by pests are more vulnerable to infection by pathogens. Aphids, caterpillars, fall army worm, stem borer and other pests can harm leaves, increasing the likelihood that disease-causing organisms will infect them. Aphids cover leaves with honeydew, a sugary fluid that draws insects and dangerous fungus (Nazarov et al., 2020).

A significant correlation between seed size and panicle length indicates that larger seeds are associated with longer panicles. Similarly, in maize, plant height, ear length, and number of kernels per row recorded significantly positive genetic correlations with grain yield per plant, emphasizing the relationship between structural traits and yield components (Mahesh et al., 2022). A positive correlation between plant dry weight and panicle width suggests that plants with greater biomass tend to have wider panicles. In wheat, grain yield per plant showed significant positive correlations with biological yield per plant and plant height, indicating that biomass-related traits are crucial for yield improvement (Devesh et al., 2021).

5.1.4 Qualitative traits distribution

The diverse grain colour observed in this study is similar to that of Sejake et al. (2020), who reported that 46% of 100 landraces exhibited brown grain sorghum. In the present study, brown grain colour was higher which is important because the trait means high content of tannin and does not attract birds (Sejake et al., 2020). High tannin content means more nutritious therefore makes it suitable for beer production, feed for animal and food in terms of porridge and ugali in Kenya (Kazungu et al., 2023). Large percentage of 25% glume coverage and lack of awns also means excellent threshability. Similar results were reported on sorghums by (Ayana & Bekele, 2004; Kudadjie, 2006; Verma et al., 2017). The lack of awns also reduces evapotranspiration a trait that is important in the dry lowlands. Resistance to grain mould is usually good with glumes that are darker in sorghum (Verma et al., 2017; Alade & Obilana, 2022). In this study, the distribution of glume colors among the genotypes was as follows: black (12.5%), brown (6.25%), gray (6.25%), purple (37.5%), red (25%), and sienna (12.5%). The variation in glume color could serve as a useful trait for selecting sorghum genotypes for grain mold resistance. Variations in panicle shape have also been documented in other previous studies (Ringo et al., 2014; Raj et al., 2018)

The present study also found that accessions with compact panicles tended to have higher grain yields compared to other panicle types. This finding aligns with previous research, which suggests that compact panicles contain more seeds than open panicles, contributing to their higher yield potential. Additionally, genotypes with loosely branched panicles may be better suited for humid regions with high rainfall, as they are less susceptible to diseases such as ergot and grain mold (Ringo et al., 2014)

Leaf midrib color is an important characteristic that farmers use to differentiate between sweet sorghum and grain sorghum (Sejake et al., 2020). The color of the leaf midrib serves as an indicator of the stem's internal properties. Green midribs are typically associated with more succulent stems containing higher moisture content, indicating juiciness. In contrast, white midribs are associated with drier, pithier stems that contain more air pockets and less moisture (Boukrouh et al., 2023). This pithy nature of white-midrib stems affects their palatability and digestibility, making them less suitable as livestock feed. Given its significance in distinguishing sorghum varieties, leaf midrib color remains a crucial visual characteristic for farmers in sorghum selection.

5.2 Evaluation of diversity of sorghum using biochemical markers

The high variability in protein content observed is similar to the study by (Tasie & Gebreyes, 2020) who evaluated a total of 35 Ethiopian sorghum genotypes and observed protein contents ranging from 8.20% to 16.48% and fat contents between 2.48% and 4.60%. High levels of protein, zinc and fat in T53B is important in enhancing nutritious sorghums within areas with prevalent malnutrition. For enzymatic processes and immune function, Zinc is crucial and also makes it a good candidate for biofortification processes to address deficiency among people. A study by (Qureshi et al., 2021) showed that agronomic biofortification with zinc enhanced the zinc content in different sorghum accessions. Iron is important in metabolic processes and transport of oxygen and phosphorus is essential for metabolism and bone health. The same has been found by (Tasie & Gebreyes, 2020) who reported concentrations of iron ranging from 2.262 to 14.08 mg/100g across different genotypes.

The diversity observed in the Shannon-Weiner Index is crucial for improving the nutritional quality among the selected sorghum genotypes and adaptability across environments (Gebre et al., 2025). The lower diversity seen in protein and iron suggests targeted efforts to breed and enhance their distribution. Zinc is essential for human nutrition, and previous studies have emphasized the need for improving its bioavailability in staple crops like sorghum (Abdu et al., 2022; Stanton et al., 2022). The analysis further reinforces the existence of biochemically distinct genotype groups, which can be strategically utilized in hybridization programs to combine beneficial traits.

The findings in this study align with previous research indicating that genotypic diversity in biochemical traits enhances crop resilience, stress tolerance, and nutritional quality(Benitez-Alfonso et al., 2023; Tanwar et al., 2023) .

5.3 Evaluation of genetic relatedness among selected sorghum genotypes using ISSR Molecular Markers

The assessment of genetic diversity using inter-simple sequence repeats (ISSRs) molecular markers is a powerful approach for understanding population structure and variation within and among sorghum populations. The variations within and among populations indicates that most of the genetic variability exists at the individual level rather than between the defined population groups. Similar results have been reported by (Agrama & Tuinstra, 2003; Lee et al., 2025; Xu et al., 2011) on sorghum indicating high levels of intra-popolation variability. The computed PhiPT (similar to FST in AMOVA) showed that the differences between populations are statistically significant, although not very strong. Wright's rules say that PhiPT values between 0.05 and 0.15 show moderate genetic differentiation (Luong et al., 2021; Wright, 1978). Therefore, while the populations are genetically close, there is still a statistically significant structure, possibly due to adaptation to local environments or breeding selections. Wright's rules say that PhiPT values between 0.05 and 0.15 show moderate genetic differentiation

The low variation between populations and the high PhiPT value suggest that there is gene flow or shared ancestry between the groups, potentially because breeding programs are sharing germplasm. It might also show that there are similar selective factors in Kenya's agro-ecological zones. The findings underscore the necessity to expand the genetic foundation of sorghum breeding by integrating a greater variety of genotypes from other geographical areas or gene banks

ISSR markers have been proven effective in detecting polymorphisms and assessing diversity in sorghum (Turki et al., 2011; Bidyananda et al., 2024). Their reproducibility and ability to target multiple genomic loci make them suitable for evaluating genetic relationships, especially in crops like sorghum with high genetic variation. The relatively high within-population variance shown in this study further supports the effectiveness of ISSR markers in capturing subtle genetic differences at the intra-population level.

The findings in this study are vital in conservation of germplasm and breeding. They should give priority to intra-population selection and mixed populations to exploit diversity among genotypes. The observed moderate differentiation among populations can be used to combine traits that are desirable through inter-population hybridization. Results from Principal Coordinate analysis confirm wide variability among the selected sorghum populations wit KSC genotypes suggesting a narrower genetic base due to intensive selection. Similar results were reported by (Adugna, 2014; Nemera et al., 2022). The presence of a large variability found among the genotypes from UOE sows that they were selected from a larger pool. The same as been reported by (Abady et al., 2021). Some of checks had similar traits with those of UOE/Rongo University and that may indicate shared ancestry. Polymorphism of 100% from the UOE material is useful for targeting yield, quality and stress resilience. Similar results were found in analysis of 225 guar accessions which demonstrated a robust population structure that split the guar germplasm into three primary sub clusters and a modest level of genetic diversity(Malani et al., 2024).

On the other hand, the CHECK population's 60% polymorphism implies a moderate genetic base, possibly reflecting its use as a reference or standard with relatively fixed traits. The KSC population, with the lowest polymorphism at 53.33%, may consist of closely related lines or cultivars developed through selection and stabilization, potentially limiting its adaptability and utility in hybridization programs.

The overall mean of 71.11% indicates that while some populations are highly variable, others may require broadening of the genetic base. This information is crucial for identifying parental lines in hybrid development, germplasm conservation, and improving sorghum adaptability to diverse agro-ecologies.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

The PCA and AMOVA results confirmed considerable genetic variability among the sixteen sorghum genotypes, particularly between lines E117B and T53B, indicating potential for use in further breeding for drought. Genotypes such as E1, E95A and T30B demonstrated superior yield potential, early maturity, and dual-purpose use, making them promising candidates for breeding programs. Overall, this study identified two promising ideotypes: (i) early-maturing, drought-adapted genotypes such as E1 and (ii) nutrient-dense but moderate-yielding genotypes such as T53B. Crosses between these groups could generate dual-purpose cultivars combining resilience with improved nutrition. However, since the study only used 16 genotypes across 2 sites in 1 season, future research should include more genotypes, multi-location, multi-season trials and more advanced marker systems.

6.2 Recommendation

Future studies should integrate agro-morphological screening with biochemical profiling and ISSR molecular markers to identify climate-resilient and nutritionally enhanced sorghum lines suitable for Kenyan agro-ecological zones. This is to enhance selection precision and to achieve robust diversity assessment and efficient breeding.

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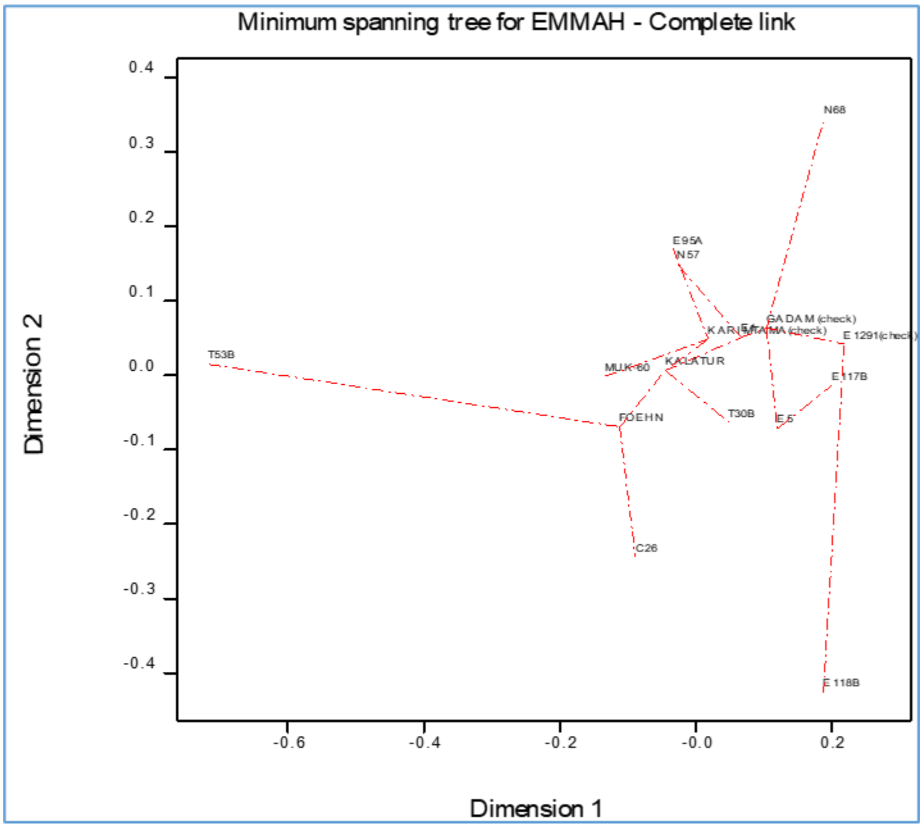
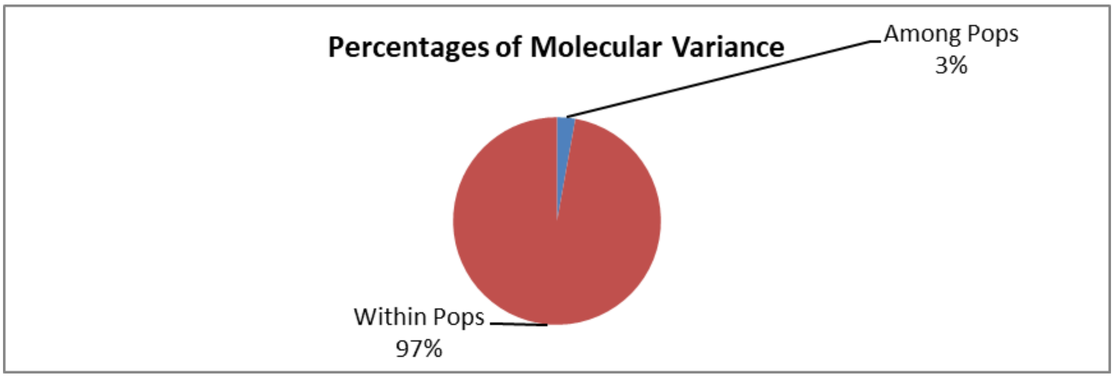
APPENDICES

Appendix I: Minimum spanning tree representing the sorghum genotypes in a compete link based on their chemical composition



Appendix II: Percentage of Molecular Variance



Appendix III: Similarity Report

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Certificate of Plagiarism Check for Thesis

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Similarity	10%
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