CHARACTERIZATION OF CASSAVA (*Manihot esculenta* Crantz) GERMPLASM IN KENYA

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DECLARATION

Declaration by the Student

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DEDICATION

I dedicate this work to my loving mother Joyce Gesare, my beloved wife, Janet Nyambeki, my daughter Given Bianca, my brothers; Paul Ateng'a, James Nyaega and Tom Onsare and sisters; Lydiah Bonareri and Edinah Mongeresa for their unwavering support and encouragement in writing this thesis work. God bless you all. In a special way, I also dedicate this work to my late father, Julius Nyamwamu (continue resting in eternal peace dad), having laid a solid foundation of discipline, hard work and determination in me.

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ABSTRACT

Cassava (*Manihot esculenta* Crantz) is a crucial income crop and staple food crop, providing substantial carbohydrates for millions globally and supporting small-scale farmers in Kenya. Due to incorrect characterization of the phenotypic and genetic materials available, the gene pool for cassava in Kenya are frequently overestimated or underestimated. Therefore, this study sought to characterize cassava germplasm in Kenya. Specific objectives were to; characterize and delimit cassava germplasm based on their phenotypic traits, investigate the genetic diversity among the cassava germplasm using molecular markers and also to determine the cyanide concentration levels in leaves, peels and pulp among cassava germplasm in Kenya. A total of 131 cassava accessions were collected from 7 major purposefully selected cassava growing Counties in Kenya. Each cassava accession was represented by a single plant. This plant was divided into 3-10 pieces and planted in single rows. Plants from this main plot were collected and planted in three sites. In each plot, three rows of five plants were planted. Data collection on phenotypic traits was done at 3, 6, 9 and 12 months. Twenty-one qualitative and four quantitative phenotypic data were collected and subjected to multivariate analysis. At a statistical significance of $p \leq 0.05$, the data was subjected to cluster analysis and dendogram construction. From the main plot, apical leaf samples from 40 proportionately sampled accessions were taken for evaluation of genetic diversity and population structure using start-codon-targeted (SCoT) molecular markers. Cluster analysis and dendogram development was done to establish the accessions' genetic variability. A total of 32 samples were processed for cyanide content analysis using picrate paper and spectrophotometric methods. In a field survey, 32 cassava sample accessions were randomly collected from various farms in Migori County for cyanide determination. Phenotypically, a dendrogram generated categorized these accessions into four clusters with Cluster 1, 2, 3 and 4 containing 72.5%, 16.0%, 3.1%, and 8.4% of the genotypes, respectively. Out of the 25 phenotypic traits assessed, 11 principal components accounted for 71.58% of the genetic variation. A total of 119 fragments were amplified, with 89.9% being polymorphic, indicating moderate genetic diversity. The dendrogram grouped the accessions into two clusters at a 0.35 genetic similarity coefficient. Moderate genetic variation among the accessions was revealed by SCoT markers. The study also revealed significant variations in cyanide levels across different cassava parts and accessions. The spectrophotometric method recorded average cyanide levels of 5.89 mg/L, 7.42 mg/L and 8.20 mg/L in leaves, peels and pulps respectively while the picrate paper method showed 3.13 mg/L, 5.44 mg/L, and 7.97 mg/L. The highest cyanide concentration was found in Nyarkadera leaves (26.93 mg/L), Kazanzwara peels (17.82 mg/L) and Nyatanga pulps (26.93 mg/L). The lowest levels were in Nyatanga-002, Nyakanyamkago, and Kasukali leaves (0.40 mg/L), Kasukali peels (1.19 mg/L) and Mzungu pulps (0.40 mg/L). Moderate positive correlations ($r=0.547$ and $r=0.570$) between cyanide concentrations in leaves and peels, and a strong positive correlation ($r=0.936$) in pulps was established. Thus, cassava germplasm in Kenya exhibits moderate diversity, with molecular data revealing clearer differentiation among this germplasm. In addition, the germplasm shows significant cyanide variation necessitating integrated genetic and biochemical breeding approaches. Thus, these results may assist breeders and farmers in optimizing cassava germplasm utilization, ultimately contributing to food security.

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ACRONYMS AND ABBREVIATIONS

AFLP	Amplified FragmentLength Polymorphisms
AMOVA	Analysis of Molecular Variance
ANOVA	analysis of variance
CIAT	International Center for Tropical Agriculture
DArT	Diversity Arrays Technology
DNA	Deoxyribonucleic Acid
ESCA	Eastern, Southern, and Central Africa
IBPGR	International Board for Plant Genetic Resource
ICIPE	International Center of Insect Physiology and Ecology
IITA	International Institute of Tropical Agriculture
KALRO	Kenya Agricultural Livestock Research Organization
MAP	Months After Planting
MAS	Marker Assisted Selection
PCA	Principal Component Analysis
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
QTL	Quantitative Trait Locus
RAPD	Random Amplified Polymorphic DNAs
RFLP	Restriction Fragment Length Polymorphisms
SCAR	Sequence-Characterized Amplified Region
SCoT	Start Codon Targeted

SNP	Single Nucleotide Polymorphism
SSR	Single Sequence Repeat
TMS	Tropical Manioc Selection
UPGMA	Unweighted Pair Group Method with Arithmetic Means
UV	Ultra Violet

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

The name cassava denotes a tuber crop serving as a major source of carbohydrates and is a dietary staple in most tropical nations. This plant is also known as *yuca,* (in Latin America), *manioc* (in French and Brazil) and *mandioca* (in South America). While native to South America, cassava now is extensively cultivated as an annual crop in many tropical and subtropical regions of the world including Africa, India and Indonesia, with Africa being its largest center of production (FAOSTAT, 2022). As an important staple food, cassava (*Manihot esculenta* Crantz) is mostly grown in tropical countries, especially within sub-Saharan Africa region, South America area and Asian region (FAOSTAT, 2020). More than 800 million people are fed by the approximated 277 million tons of cassava that are produced per year on over 24.5 million hectares of land (FAO, 2020). Over sixty percent of the everyday calorific demands of people in tropical Africa and Central America are met by cassava crops (Leon-Pacheco *et al*. 2020). The cassava crop comes in fourth place behind rice, sugar cane and maize as an alternative source of carbohydrates in the tropics (FAOSTAT, 2020). Approximately ninety percent of cassava grown in Africa is utilized for human nourishment, with only 6% going to feed cattle (Adu *et al*., 2018). In Kenya, the necessity to possess a significant portion of the biological variety and cultural traditions linked with this plant can be linked to one of the difficulties in domesticating cassava (Masinde *et al*., 2018). Cassava is mainly cultivated to enhance household food security, particularly in regions where it is used either for direct consumption as fresh food or for processing into various food products.

Morphologically, cassava plants reach heights of up to 4 meters and display spirally arranged palmate leaves with 5 to 9 lobes, supported by long petioles of up to 30 cm (Allem, 2002). The flowers are petal-less and appear in racemes. Roots are cylindrical, tapered, and encased in a tough, brown rind; internally, they possess white or yellowish flesh, rich in starch but poor in protein and other micronutrients (Montagnac *et al*., 2009). The plant is believed to have originated in Latin America, where the native Indians discovered it more than 4000 years ago (Mohidin *et al*., 2023). It is described as a dicotyledonous plant belonging to a genus *Manihot* of the family Euphorbiaceae, and it is grown in areas between 30°N and 30°S from the equator, where it is distributed throughout Africa, Asia, Central and South America (Feyisa, 2021).

Cassava is a smart-crop which withstands hot regions with little rainfall, and its production is all year round in harsh conditions with low-nutrient marginal soils (Lebot, 2020). Cassava is now utilized as a staple crop globally for food security (Okogbenin *et al*., 2007). Its importance as a staple meal has transcended geographical and cultural borders, making it a genuinely global crop (Savoury, 2023). Cassava has a fascinating history spanning thousands of years, and its transformation from a wild plant to an essential food source for millions of people is nothing short of astounding.

The crop's ability to thrive in poor soil and resist tough growing conditions makes it desirable especially in areas with variable weather (Savoury, 2023). As cassava production spread over the world, new varieties were bred to meet the unique needs of each region. This diversity of cassava strains led to its durability and adaptability, allowing its continuing production and consumption all over the world (Savoury, 2023). The domestication and dissemination of cassava not only altered meals, but also had a

significant impact on socio-economic fabric of the communities who accepted it. It became an essential component of cultural practices, culinary traditions and agricultural systems, influencing how humans interacted with the environs besides each other (Savoury, 2023).

1.1.1 World cassava production

Global cassava production has steadily increased, reaching 315 million tons (t) in 2021 from 286 million tons in 2017 (Table 1.1) (FAO, 2023). Africa is the largest cassava-producing region, accounting for approximately 204 million tons in 2021, up from 177 million tons in 2017. Herein, Oceania remains the lowest producer, with 266,362 tons in 2021.

Table 1.1: Global cassava production quantity ('000)

Region	Year				
	2017	**2018**	**2019**	**2020**	**2021**
World	286,718	301,077	296,321	303,751	314,807
Africa	177,012	195, 653	188,945	193,878	203,573
America	27,150	26,619	26,380	26,773	26,715
Asia	82,309	78,553	80,741	80,741	84,252
Caribbean	1,747	1,674	1,752	1,500	1,501
Oceania	247	251	255	251	267

Source: FAOSTAT, (2023).

Nigeria continues to be the world's largest producer (Table 1.2), accounting for 20% of global production in 2021 with 63 million tonnes (FAO, 2023). The Democratic Republic of Congo (DR Congo), Thailand, Ghana, and Brazil are among the other top producers (Table 1.2). The average yield in Nigeria, however, has drastically decreased, going from 11.2 tonnes per hectare in 2011 to 6.9 tonnes per hectare (t/ha) in 2021 (Otekunrin *et al*., 2019). In contrast to this drop, Cambodia has the greatest overall yield in 2021 at 27.4 t/ha, although ranking tenth in terms of production quantity (FAO, 2019; Otekunrin *et al.,* 2019).

Table 1.2: Top 10 cassava producers, production quantity in 2021 (‘000)

Rank	Country	Production (tons)	Percentage production
1	Nigeria	63,031	20.02
2	DR Congo	45,673	14.51
3	Thailand	30,108	9.56
4	Ghana	22,682	7.20
5	Brazil	18,098	5.75
6	Indonesia	17,749	5.64
7	Viet Nam	10,566	3.36
8	Angola	9,867	3.13
9	Cambodia	7,722	2.45
10	Côte d’Ivoire	6,961	2.21
	Rest of the world	82,348	26.16
	World Total	**314,807**	**100**

Source: FAOSTAT, (2023).

From 5.6 million hectares in 1961 to 21.6 million hectares in 2019, the area under cassava cultivation in Sub-Saharan Africa (SSA) increased by more than 280%. This resulted in an increase in output from 31.5 million tonnes in 1961 to 429.1 million tonnes in 2019 (FAOSTAT, 2021). Smallholder farmers produce most of the cassava used for subsistence (FAO, 2019). By contributing 63.3% of the world's cassava production in 2020, SSA surpassed a 2005 projection (IFAD, 2005), which was 60%. With 204 million tonnes, or 65% of global production, SSA is the world's largest producer of cassava, according to the most recent projection for 2021 (IAEA, 2023). There are still few non-food applications for cassava in SSA, despite this substantial production. According to reports, barely 3,000 tonnes were allocated to non-food uses in 2019 (FAO, 2019; Fathima *et al*., 2023).

1.1.2 Cassava production in Kenya

Grown all over Kenya, but primarily in the Western/Nyanza, Coastal, Central/Eastern and Rift valley regions (particularly in arid and semi-arid areas), cassava is ranked second root-crop after the Irish potatoes (Muinga *et al*., 2010; Githunguri *et al.,* 2017) (Table 1.3). Since cassava yields in East Africa and Kenya are lower than those reported in Asia and America, Kenyan farmers hardly ever realise 15 tonnes per hectare (Gichuki, 2021). According to FAOSTAT (2023), 60% of Kenya's cassava production is in the lake region (Table 1.3). Kenya’s total cassava production is estimated at 900,000 tonnes (Masinde *et al*. 2018).

Cassava is primarily grown in low-altitude locations, such as the coast, or in mid-altitude portions in the central aside from eastern, regions as well as Nyanza and western provinces in Kenya, according to Mware *et al.* (2009). About 49,000, 34,000 and 19,000

acres are farmed in the western, coastal and central regions, respectively. The yields in the western region average 9.0 t/ha, compared to an average of 4.0 t/ha in the coastal region (Sing'ombe *et al.* 2015).

Table 1.3: Percentage cassava growing regions in Kenya

Region/ zone	**Percentage production (tons)**
Nyanza + Western	60
Coastal	30
Central +Eastern	6
Rift valley	4

Source: Githunguri *et al.,* 2017; FAOSTAT, 2023).

1.1.3 Cultivation of cassava

Cassava comes in a variety of forms, ranging from tiny plants to branching bushes. Some of these types are suited to dry regions with alkaline soil, while others are suited to the banks of acid muck near rivers (Hershey, 2017). Cassava is taking the place of some other root crops, such as yams, which need more fertile soil to grow and are more susceptible to insect infestation, because it can thrive in non-humid climates. The cassava plant produces the most food energy per farmed area per day of any crop plant, except of sugarcane (Lebot, 2020). Different management techniques are needed at every stage of the cassava life cycle, from planting to harvest, with the goal of maximising productivity and enhancing root quality (CIAT, 2019). Healthy stem cuttings with five to seven nodes and a length of 20 to 30 cm are chosen for planting, either buried vertically or horizontally in ridges or furrows. To ensure the best root expansion, plants and rows

should be spaced 1 metre apart and planted 5 to 10 cm deep. Ecological factors including temperature and precipitation have an impact on this early stage, which typically lasts 15 to 30 days (CIAT, 2019).

For successful sprouting and early shoot development, the soil must be sufficiently moist (FAOSTAT, 2022). Thus, the cassava plant starts to store carbohydrates in its storage roots between the ages of one and six months. During this crucial stage of growth, potassium (K) application is essential. According to Agyemang *et al*., (2020), potassium promotes starch synthesis, increases disease resistance, and improves root bulking. To evade moisture deficiencies hindering root development, irrigation should be applied, especially during dry spells. Because cassava is vulnerable to threats like mealybugs and cassava mosaic virus at this stage, pest and disease management is especially crucial (FAOSTAT, 2022). Vegetative development decreases as energy is diverted to starch accumulation and root thickening during root maturation (Agyemang *et al.,* 2020). The roots usually reach their ideal size and weight for harvest six to nine months after planting (FAOSTAT, 2022). Although nutrient intake declines during this stage, residual fertility and sound soil structure are still essential. Cassava is harvested 8–12 months after planting for best quality based on the type and intended application (Howeler *et al*., 2013). If harvesting is delayed for more than a year, lignification may occur, which will negatively impact the texture and flavour of the roots (Agyemang *et al*., 2020).

1.1.4 Root composition

Numerous chemical components can be found in roots (Suresh *et al*., 2011). These constituents include tannins, scopoletin, and balanophonin, all of which have been shown

to have advantageous characteristics such as anti-inflammatory, anti-proliferative, and antioxidant qualities (Yuan *et al*., 2021). However, cassava roots can also contain harmful chemical substances called cyanogenic glycosides, lotaustralin and linamarin (Yuan *et al*., 2021). These substances are known to be harmful to humans as they can cause neurological disorders, especially when cassava is consumed raw or when the tubers are inadequately processed over time (Rivadeneyra-Domínguez and Rodríguez-Landa, 2020). Researchers and consumers have categorized cassava roots and leaves into two major types, namely; sweet and bitter cassava varieties (FAO, 2020).

The quantity of cyanide compounds produced by the two types of cassava is the only distinction between them (Lebot, 2020). The bitter variety yields more cyanide chemicals in the cassava tubers than the sweet variety, despite both being grown in a similar way (IITA, 2020). For example, cyanide compounds in cassava tubers may be as low as 10 parts per million (ppm) in sweet cassava types (FAO, 2020). It is less toxic because it needs little processing to be safe to consume. According to research, cyanide compounds in bitter cassava variants can reach 490 parts per million (ppm) (Lebot, 2020). Notably, amounts of cyanogens exceeding 50 parts per million is dangerous and unfit for human ingestion. If the cyanide compounds in cassava are not processed before eating, they pose a serious risk to health and may even be fatal (Mlingi *et al*., 2019). Only until the cyanide chemicals are eliminated from the cassava tubers can be declared safe to consume (Lebot, 2020). Several detoxifying techniques that may be used on high cyanide content varieties include drying and fermentation (Mohidin *et al*., 2023; Swamy, 2024).

1.1.5 Nutritive value of cassava roots and leaves

Cassava is a source of resistant starch, which scientists suggest can boost a person's gut health by helping nurture beneficial gut bacteria. Resistant starches remain relatively unchanged as they pass through the digestive tract. According to the U.S. Department of Agriculture (USDA). (2019), the nutritional profile of 1 cup (160 grams) of cooked cassava is as follows:

- calories: 306
- protein: 2.27 grams (g)
- carbohydrate: 63.4 g
- fiber: 3.04 g
- calcium: 27.2 milligrams (mg)
- magnesium: 35.2 mg
- potassium: 451 mg
- vitamin C: 29.1 mg
- thiamine: 0.131 mg
- riboflavin: 0.077 mg
- niacin: 1.35 mg

Cassava contains only small amounts of proteins and fats. As a result, people who use cassava as a primary dietary staple may need to eat extra protein or take protein supplements to avoid malnutrition (Montagnac *et al.,* 2009). In contrast, the leaves offer higher protein content but are deficient in methionine (Léotard *et al.,* 2009).

1.2 Statement of the problem

The diversity of morphological, genetic, and cyanogenic glycoside content in cassava varieties contributes to part of their adaptability to different agro-ecological zones and utilization. Farmers differentiate, manage and conserve the types they cultivate on farms

in part using morphological traits and cyanide contents in roots and leaves. On-farm selection and conservation are aided by morphological descriptors, though they appear inaccurate in differentiating between regionally tailored local varieties. Linguistic variations are barriers to the accurate identification of cultivars. Every location or farming community possess typical set of names for various or similar cultivars, each with a distinct significance. Since cassava was introduced to Kenya, farmers have given acquired and cultivated cultivars names based on the origin and source of the planting material, distinctive phenotypic traits, or cyanide content. Smallholder farmers are significant in protecting crop genetic diversity outside of origin regions. Vernacular names have historically been used by farming communities to preserve awareness of these genetic and phenotypic variations. Given that the same cultivar can go by multiple names in different groups and areas, this informal naming of variations can result in overestimations or underestimates of crop diversity. In western Kenya, farmers name varieties of cassava in accordance with the country of origin (e.g., Nyatanga, from Tanzania), the name of the farmer from whom they were obtained (e.g., NyarkaSamuel), the developmental characteristics of the stem (e.g., Adhiambo lera, "clean lady," "beautiful," or "Karembo," respectively), the cyanide content of the tuber (e.g., "Kasukali, sweet,", or Ongogo Mbidhi – bitter”. This cultivar naming was compounded when the Kenya Agricultural Livestock Research Organization (KALRO) and Non-Governmental Organizations (NGOs) distributed improved cultivars of cassava known as Tropical Manioc Selection (TMS) with coded numbers like MM96/4466, MH95/0183, SS4 and KME-1 that were high yielding, early maturing, short stature and resistant to CMD. These varieties were sourced from the International Institute of Tropical

Agriculture (IITA). Following tests on agronomic adaptation, KALRO released these varieties for commercial cultivation using their coded or breeding names. Farmers called all these types "*Agriculture*" because they found it difficult to recall these coded names. Farmers and breeders are unable to distinguish between the high and low cyanide varieties in the field. Thus, Kenyan cassava germplasm is frequently misinterpreted due to inaccurate characterization. Every year, farmers and breeders in Kenya lose cassava, thus exposing the crop to genetic drift. This research proposed to characterize the popular cassava germplasm in Kenya through their phenotypic (morphological), genetic and cyanogenic glycosides in leaves and tubers.

1.3 Justification of the study

Since they are quick and simple methods for determining the extent of cassava diversity, phenotypic characters are often utilized by farmers in the initial assessment of cassava germplasm in the field. This is due to the fact that these features show the genuine variation as the farmers view it. Farmers characterize phenotypes, particularly canopy, stem, tuber shape, and colour after peeling, so that hey can decide whether they like or opt for a certain cassava germplasm. Additionally, most researchers and policymakers have persuaded farmers to grow a few common cassava varieties, which has resulted in obsession of a small number of plants with a specific genomic foundation and possibly the loss of some priceless genetic material. It is unknown how much genetic variations exists within the Kenyan cassava germplasm. Additionally, Kenyan *ex-situ* field gene banks in the central ecologically cassava-production regions of Kakamega, Katumani, and Mtwapa are now preserving the genetic resources for cassava. It is still unnown how much genetic or phenotypic diversity these accessions possess.

Farmers would be wise to practise sustainable farming by increasing the diversity of their farms geared towards safeguarding their businesses from co-evolving diseases besides pest concerns. Molecular markers are the most effective way to measure genetic variation. A re-known marker arrangement utilized for numerous genomic uses particularly the evaluation of genomic inconsistency in germplasm assemblages and pedigree establishment (Mogga *et al*., 2018), is the Start Codon Targeted (SCoT) markers. To prevent losses and ensure the safety of these inherent resources, actions that aim to maximise the conservation and utilisation of cassava germplasm are required (Mithra *et al*., 2018). Farm-grown cassava developed according to cassava characterisation studies might be managed more easily and used more frequently in breeding programmes. Therefore, the characterization of the existing germplasm serves as a basis in informing cassava breeding programme. Since DNA is not immediately impacted by the environment, molecular characterization using markers centred on DNA amplification is greatly advised (Fukuda *et al*., 2010; Fasahat *et al.,* 2016). Start Codon Targeted (SCoT) molecular markers have become essential in genetic diversity studies due to their efficiency, simplicity, and ability to reveal polymorphisms. Targeting the conserved region around the start codon (ATG), SCoT markers focus on functional regions of the genome related to important agricultural traits. This specificity enables researchers to study genetic diversity across various plants devoid of preceding sequence information. Moreover, SCoT markers have proven effective in differentiating crop genotypes, as seen in studies involving yams, rice, mangoes, wheat, and grapes, which aid breeding programs by highlighting genetic variation (Collard & Mackill, 2009; Habiba *et al*., 2021). Besides these these markers exhibiting reproducibility, they are also

highly versatile, applicable across a wide range of crops, including non-model species. Their high polymorphism and ease of use make them a valuable tool for genetic studies, particularly in crops with limited genomic resources. SCoT markers have been utilized to authenticate *Physalis* (Solanaceae) species, further enhancing breeding efforts (Feng *et al*., 2018). Their ability to target gene-related sequences provides valuable insights into crop improvement and conservation (Habiba *et al*., 2021).

Cassava's wide genetic variation offers advantageous traits for *in-situ* conservation and research on genomic assortment besides development. Specifically, the cultivars of grown plants represent a type of genetic material that should be conserved and kept in order to convey qualitative traits (Mwamba *et al*., 2021). The knowledge gained from this study about the various cyanide accumulation levels in cassava cultivars will be essential for raising users' awareness through campaigns. Furthermore, since they will be able to recognise and distinguish amongst sweet and bitter cassava types in the field, such knowledge will be of tremendous importance to farmers who grow cassava. The results will also be crucial for agricultural officers who provide extension services aimed at educating farmers and consumers about varieties of cassava that contain high levels of cyanide toxicity and how to identify and handle them before production and consumption. These findings should be of major significance to other regulatory authorities (biosafety regulatory bodies), given that they will develop strategies to address the concentrations of cyanide toxicity in cassava varieties before approving them for human use. Thus, it is anticipated that this study will contribute to the characterization of the morphological and genotypic features and the computation of the magnitude of cyanide in Kenyan cassava varieties.

1.4 Objectives of the study

1.4.1 Broad objective

To characterize selected cassava (*Manihot esculenta* Crantz) germplasm in Kenya.

1.4.2 Specific objectives

1). To characterize and delimit cassava germplasm based on their phenotypic (morphological) traits.

2). To investigate the genetic diversity among the collected cassava germplasm using molecular markers.

3). To determine the cyanide concentration levels in leaves, peels and pulp among cassava germplasm in Kenya.

1.5 Hypotheses

H_{O1}: Cassava germplasm do not have distinguishing phenotypic traits.

H_{O2}: There are no genetic differences between the collected cassava germplasm.

H_{O3}: There is no variation in cyanide concentration levels among the cassava germplasm in Kenya.

1.6. Scope

The study focused on selected 7 major cassava growing regions (Counties) in Kenya which included Coastal (Kilifi), Eastern/Central (Makueni and Nakuru) and Western (Busia, Kisumu, Homa bay, Migori). The study was confined to the characterization of the phenotypic and genotypic traits of cassava varieties in Kenya. The study also determined cyanide concentration levels among the collected cassava germplasm.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

According to Wikipedia (2024), cassava is a member of the family Euphorbiaceae, sub-family Crotonoideae, tribe Manihoteae, genus *Manihot*, species *Manihot esculenta* Crantz). The genus is divided into two sections namely; the Arborae, which includes tree species and the Fructicosae, which includes slow-growing shrubs that are suited to desert or savannah grasslands (Amelework and Bairu, 2022).

There are about 8000 species in the genus *Manihot*, which is part of a vast family of flowering plants with 300 genera. *Manihot esculenta* Crantz) is the most economically significant of the numerous species in the genus *Manihot* and is extensively grown for both industrial and food purposes (Amelework and Bairu, 2022). Known by various names around the world, including *tapioca* (in India**,** Sri Lanka**,** Malaysia**,** Singapore and Indonesia), *manioc* (in French speaking countries and Brazil), *mandioca* (in South America) and *yuca* (in Latin America), cassava is a dicotyledonous perennial shrub (FAOSTAT 2023).

Cassava's height ranges between 1 - 4 meters. Its tuberous storage roots, which are collected for industrial use, animal feed, or direct human consumption, are high in starch (20–40%) (Fei *et al.,* 2023). The Guarani (*Tupi*) term *mandioca* or *manioca* is the source of both the generic name *Manihot* and the common name "*manioc*" for the plant. The Latin word for "edible" is *esculenta*. Originally from *Taíno caçabi*, the word "cassava" was borrowed from the French or Portuguese in the 16th century. Most likely, Taíno

people also gave the common term "*yuca*" or "*yucca*" to the Spanish word "*yuca*" or "*playa*" (Ferguson *et al*., 2019).

2.1.1 Origin of cassava

There is still uncertainty about the evolutionary and geographic origins of cassava (McCallum *et al*. 2017). The Cerrado region of South America, which is now Brazil, is where cassava originated from (Hillocks *et al*. 2002; Aloys and Hui Ming, 2006; Okogbenin *et al*. 2007). It is widely believed that cassava was originally domesticated about 10,000 years ago, in West-Central Brazil (CONAB, 2020). In a phylogeographic analysis based on the single-copy nuclear gene glyceraldehyde 3-phosphate dehydrogenase (G3pdh), Olsen and Schaal (1999) examined the domestication of this crop. These authors looked at 424 alleles from 212 cassava plants and found 28 haplotypes. These findings provided several crucial insights into the evolutionary origins of cassava and are among the first applications of a single-copy nuclear gene in a plant phylogeographic study. The study concluded that wild populations of *M. esculenta* along the southern Amazon basin border are probably where cassava was domesticated, the crop does not appear to be descended from multiple progenitor species as previously suggested and cassava does not share haplotypes with *Manihot pruinosa*, a closely related species that may hybridise.

Cassava and its wild relatives exhibit substantial amounts of non-coding sequence variation due to the G3pdh gene. The origin of cassava is now more clearly defined thanks to these studies. Both within *M. esculenta* and at the interspecific level, correlations between the G3pdh haplotypes are inconsistent with taxonomic boundaries when viewed in a genealogical context; this discrepancy is most likely due to lineage

sorting among these recently diverged species. According to evidence gathered from molecular markers, the perennial woody shrub *M. esculenta* ssp. *flabellifolia* (Pohl) Ciferri is the closest wild relative of cassava (Nasar and Ortiz, 2007). Since 1979, more than 43,000 cassava germplasm samples have been sent to 84 countries by the International Centre for Tropical Agriculture (CIAT), which maintains the most varied collection of cassava germplasm (Mohidin *et al*. 2023; Swamy, 2024).

2.1.2 Taxonomy of cassava

Cassava is a member of the Euphorbiaceae family, which has around 7200 species and is distinguished by the development of lactiferous capillaries composed of secretory cells known as lacticifers (Ceballos and de la Cruz, 2024). These generate the milky secretion, or "latex," that distinguishes the plants in this family. Plant architecture varies greatly within this family, ranging from arboreal varieties like rubber (*Hevea brasiliensis*) to shrubs with commercial relevance like the castor-oil plant (*Ricinus communis*). Numerous weeds, decorative plants, and therapeutic plants also belong to this family. The genus *Manihot*, to which cassava belongs, is one of this family's most important members (Ceballos and de la Cruz, 2024).

More than 100 species in the genus *Manihot* are found naturally between 33°N (Southwest USA) and 33°S (Argentina) (Fei *et al*. 2023; Swamy, 2024). The wild relatives *M. catingae* Ule, *M. dichotama* Ule, *M. glaziovii* Müll. Arg., *M. melanobasis* Müll. Arg., and *M. saxicola* Pohl have all been used in interspecific hybridisation. The only one of these that has significantly contributed to the development of cassava germplasm resistant to cassava mosaic disease is *M. glaziovii* Müll. Arg., also known as the Ceara rubber tree (Fei *et al*. 2023). According to Ceballos and de la Cruz (2024),

there are three sub-species of *M. esculenta* subsp. *esculenta* Crantz, subsp. *flabellifolia* (Pohl) Ciferri, and subsp. *Peruviana* Müll. Arg. Allem. The latter two sub-species are wild variations of *M. esculenta* subsp. *esculenta*, the cultivated type.

Despite the potential for interspecific hybridisation among the approximately 98 species that have been documented, natural reproductive isolation mechanisms appear to preserve the species' uniqueness in the wild (Rogers and Appan, 1973; Silva *et al*., 2017). Only one of these species, *Manihot esculenta* Crantz, or cassava, is widely grown for its starchy roots and has substantial economic importance. Early explorers and traders helped cassava spread throughout the world, giving rise to more than 100 regional names for the crop; in Latin America, it is commonly known as yuca in Spanish-speaking nations and mandioca in Portuguese-speaking areas (El-Sharkawy, 2003). In Brazil, bitter cassava (*mandioca*) and sweet cassava (*aipim*) are different (Ceballos and de la Cruz, 2024). *Manioc, yuca, tapioca, mandioca, shushu, mukshue, cassave, imanoka, maniba, kasaba, katelaboodin, manioc, manihot, yucca, mandioca,* sweet potato tree, and *tapioca* plant are some other names in various languages (Moore and Lawrence, 2024; Ceballos and de la Cruz, 2024). The native names for cassava in Kenya include *Muhogo* (Swahili), *Marieba* (Luo), *Manga/Mugazija* (Mijikenda), *Kimiogo* (Luhya), *Marebwa* (Kuria), *Amaregwa* (Kisii), *Manga* (Kamba) and *Mianga* (Kikuyu).

2.1.3 Botanical description of cassava

There are around 98 species of cassava (*Manihot esculenta* Crantz) in the genus *Manihot*, which range from sub-shrubs to shrubs and trees. Most of these species produce latex and contain cyanogenic glucosides (Nassar, 2000). In contrast to cultivated species, wild species have slender, fibrous roots; however, some species often have a small number of

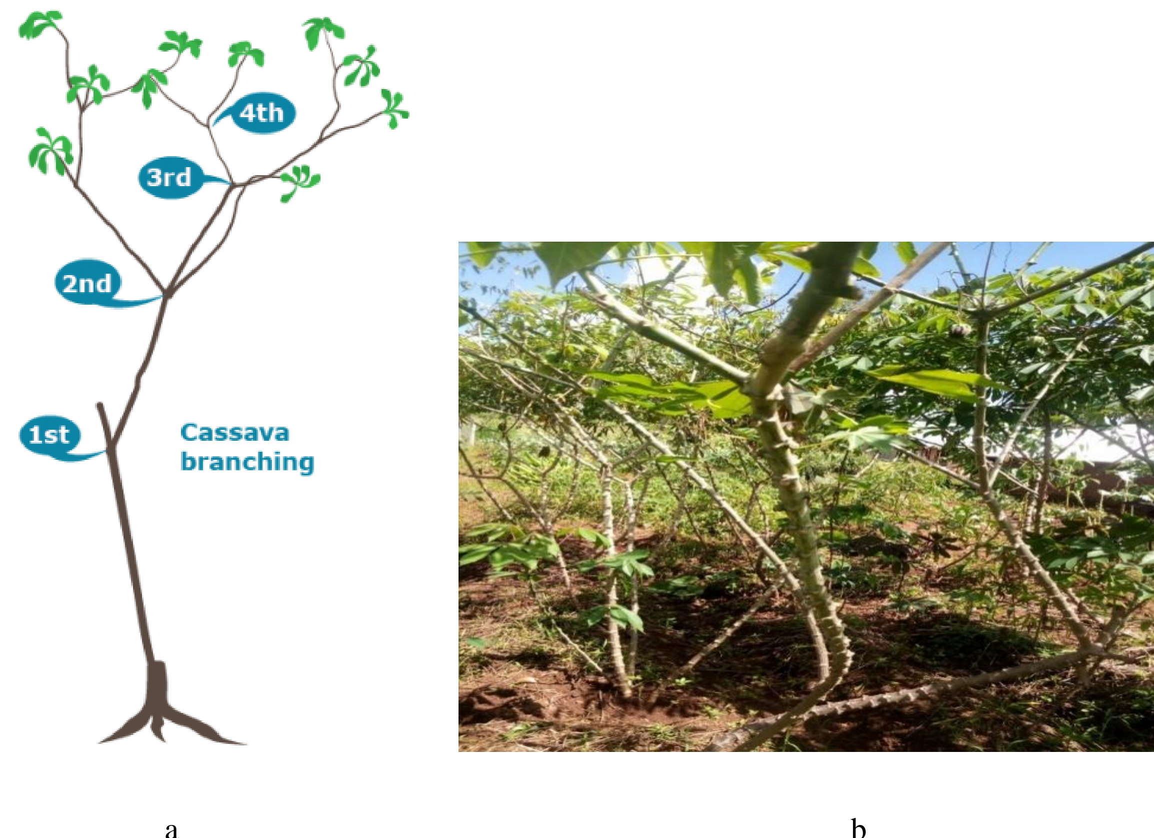
tuberous roots, which can have smooth or rough surfaces with a sub-epidermis that varies from red or yellow to white. The cortex of species with tuberous roots is white, cream, or yellow (Nassar, 2000). According to Nassar and Ortiz (2007), all species of the genus *Manihot* are indigenous to New World nations, particularly Brazil and Mexico, where they constitute unique centres of diversity. In their natural habitat, they often grow sparsely and hardly ever take over as the predominant plant. Although wild *Manihot* species are usually allogamous because of monoeceous or dioecious structure of the inflorescence, shifting towards autogamous plants in cultivated cassava. Based on the genus's polyploidy, observations of frequent hybridisation between the wild species and the cultigen as well as between the wild species themselves point to poor inter-species fertilisation barriers (Nassar, 2000).

2.1.3.1 The cassava stem

The growth habit of cassava (*Manihot esculenta* Crantz) significantly influences its agronomic performance and breeding potential. Cassava exhibits two main growth types namely; the erect type, which may branch at the apex and the spreading type, which is generally not cultivated due to its agronomic disadvantages (Hershey, 1987).

Branching in cassava (Figure 2.1) is strongly related to floral induction and is sometimes referred to as "reproductive branching." Following subsequent flowering occurrences, these branches may mature into higher order branches (Keating *et al*., 1982). Sub-shrubs have nearly acaulescent stems, while tree species have stems that are around 20 meters tall (Ceballos and Cruz, 2012). Native to the Brazilian savanna, shrub-type species often have stems that range in colour from grey to brown or reddish, and often die back to the crown during the dry season (Nassar and Ortiz, 2007). Typically, stems have a terminal

inflorescence at the branching point and branch dichotomously or trichotomously (USDA, 2003). Also see Figures 2.1 a and b. A characteristic that is uncommon in cultivated cassava is the juvenile stem's variable degree of pubescence, which is common in wild species (Nassar, 2000).



a b

Figure 2.1: (a) Cassava branching (b) Stems of cassava plants (Source: Iowa State University)

2.1.3.2 The cassava leaves

The lifespan of cassava leaves and the products of the entire plant are influenced by genotype and environmental conditions (Bolarinwa *et al.,* 2016). Cassava leaves are alternately arranged in a winding pattern along the stem. The leaves form a 2/5 spiral down the stem. The leaves' petiole and lamina are both simple and lobed. Each leaf is

covered by three to five stipules, each of which is around one millimetre long. The number of leaves is often odd and range from three to nine (i.e. three, five, seven or nine). Most African cassava varieties have elliptical or lanceolate lobes, according to studies (Bolarinwa *et al.,* 2016) (Figure 2.2).

Figure 2.2: Cassava leaves (this one has seven leaves) ***Source: (Iowa State University)***

All but three species possessed palmately lobed leaves, which alternate between sub-sessile and lengthy petiolated forms (Ceballos and Cruz, 2012).

2.1.3.3 The cassava roots

Cassava roots cannot be utilized for vegetative multiplication because they are true adventitious roots rather than modified stems (Fei *et al*., 2023). The storage root, which serves as the primary edible part of the cassava plant, accumulates starch within its parenchyma cells. Only three to ten of the many fibrous roots that cassava generates will usually go through secondary thickening and develop into store roots (Figures 2.3 a and b). According to Fei *et al*. (2023), the surviving fibrous roots continue to play a role in the intake of nutrients and water.

a b

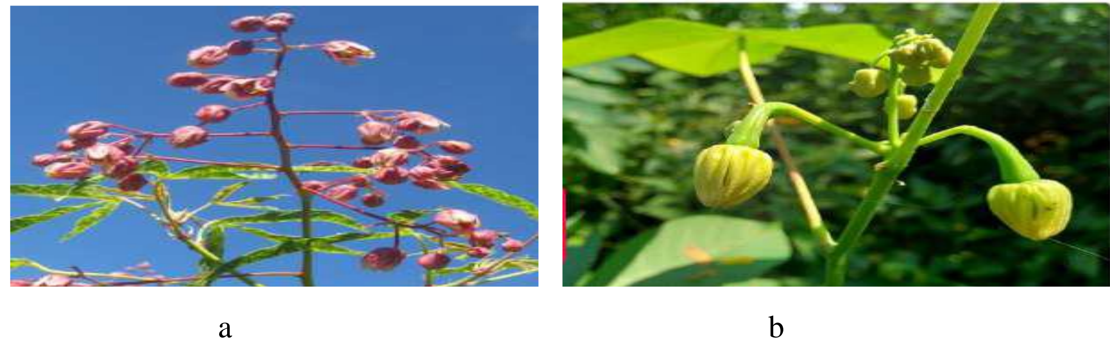
Figure 2.3: (a) Cassava tubers (SME 2; 9 months) (b) Cassava tubers (cross section)
Source: Swamy (2024)

Although roots up to three feet long have been discovered, they are typically 3-10 cm in diameter and 20- 40 cm long. At the base of the stem, the edible tuberous roots grow in

clusters of 48 (Figure 2.3 a). The pure white inside (Fig. 2.3 b) has a high starch content and is harder than potatoes. The thin reddish brown fibrous bark covering the roots can be scraped and peeled off (Figure 2.3 b). According to Moore and Lawrence (2024), the bark contains harmful hydrocyanic (prussic) acid that needs to be cleaned, scraped off, and heated to eliminate it.

2.1.3.4 Flowering in cassava

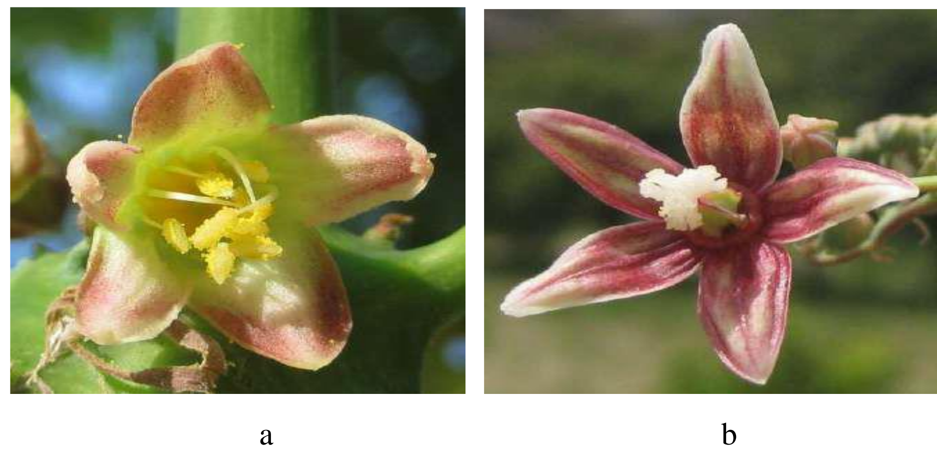
Cassava has a monoecious inflorescence, which means there are separate male and female flowers on the same plant (Figs. 2.5 a & b). These inflorescences are usually loose and organised in cymes or fascicles and they grow at the ends of fork-type branches that develop after floral initiation at the shoot apex. Staminate (male) flowers (Fig. 2.5 a) are more numerous and found higher up in the inflorescence, whereas pistillate (female) flowers (Fig. 2.5 b) are fewer and larger and found closer to the base. When male and female flowers on different inflorescences bloom simultaneously, the temporal separation of floral opening occurs; female flowers opening 1-2 weeks before male flowers on the same inflorescence thus increasing the likelihood of cross-pollination while preserving the ability for self-pollination (Perera *et al*. 2013; Hyde *et al.,* 2020). Cassava flowering (Figs. 2.4 a & b) is strongly influenced by environment and genotype. It can be difficult to synchronise blooming times in cassava breeding projects since certain early flowering genotypes can bloom a month after planting, while others may take 24 months (Pedri *et al*., 2018). Irrigation is necessary for crossing blocks during a lengthy drought since flowering rarely happens during long dry periods. The fork of the branches develops into the cassava inflorescence (Figs. 2.4 a & b) (Luna-Castellanos *et al*., 2018).



a b

Figure 2.4: (a) Cassava inflorescence (b)Cassava flower buds

Source; Swamy, (2024).



a b

Figure 2.5: (a) Cassava male flower (b) Cassava female flower

Source; Swamy, (2024)

a b

Figure 2.6: (a) Cassava fruits (b) Cassava fruit (dehisced) and seeds

Source; Swamy, (2024).

Cassava is regarded as a cross-pollinated species and insects carry out cross-pollination even when self-pollination occurs (CONAB, 2020). Compared to pollen from most other species, cassava pollen is significantly bigger and heavier, with an average size of 122-148 µm. According to Matos *et al*. (2016), cassava pollen has a brief lifespan; no more than two days. The presence of pollinating insects, environmental factors and genotype all affect the extent of self-pollination. Cassava suffers from a severe inbreeding depression, which results in saplings of selfed progeny which are weak and uncompetitive. A single seed is contained in each of the trilocular capsules that make up cassava fruit (Fig. 2.6 b). A cassava fruit requires 75-90 days to reach physiological maturity following pollination (Luna-Castellanos *et al*., 2018). According to Luna-Castellanos *et al*. (2018), the fruit (Fig. 2.6 a) is subglobose, green (to pale yellow, white, and dark brown), smooth and has six longitudinal wings. The seeds can grow up to 12 mm long. Bagging must be done before fruits mature to extract seed in controlled crosses since dehiscing ripe cassava fruit (Figure 2.6 b) is explosive (Njoku and Ano, 2018). It takes three to six months of dry storage at room temperature to break the dormancy of freshly collected seeds, which are typically dormant. In roughly 15 days, physiologically active cassava seeds (Figure 2.6 b) can easily sprout. The ideal temperature range for germination is between 30 and 35 degrees Celsius. The ideal storage conditions for cassava seeds are 4-5°C and 60% relative humidity. For seeds kept in such settings for a year, a germination rate of above 80% has been documented (Fei *et al*., 2023).

2.1.4. Cassava genome

According to Nassar (2009) and Soto *et al*. (2015), 2n = 36 is present in all *Manihot* species which have been investigated, including cassava (*M. esculenta* Crantz).

Backcrossed generations have good fertility, and interspecific hybrids between cassava and its wild relatives have fair regular meiosis (Sécond *et al.* 1997; OECD, 2014). According to Carvalho *et al*. (1999), electrophoresis demonstrates affinity between species of various sections as well as between some of them and cassava itself. While apomixis has provided a way to perpetuate novel hybrid types adapted to varied habitats, polyploidy appears to have had a role in the genus's rapid diversification (OECD, 2014). It is believed that two wild *Manihot* species; *Manihot glaziovii* (Ceará rubber) and *Manihot pruinosa* hybridised to produce cassava (*Manihot esculenta* Crantz), which was then vegetatively reproduced (Nassar, 2000). During meiosis, the 36 chromosomes in cassava create 18 bivalents. Nonetheless, the paleotetraploidy of cassava is supported by cytological and sequencing data (Fei *et al.,* 2023). The cytogenetics and genetics of cassava are both poorly understood. The Euphorbiaceae family typically has eight basic chromosomes, though they vary from 6 to 11. Polyploidy occurs in almost half of Euphorbia species (Soto *et al*., 2015). Analyses carried out between diakinesis and metaphase I reveal the presence of 18 tiny and comparable bivalents in cassava, though it is commonly thought of as a polyploid species. Similary, cassava includes late bivalent pairings, trivalents, and univalents. Accordingly, $2n = 2x = 36$, making this plant a functional diploid (Ceballos and de la Cruz, 2024). The Hi-C method has been used to reconstruct and make available the entire and haplotype-resolved African cassava (TME204) genome.

2.1.4.1 Genetic diversity in cassava

Genetic diversity is essential for agricultural improvement via breeding. Despite numerous research activities on cassava, there has been no thorough global assessment of

its genetic diversity. However, efforts have been made to assess diversity by utilising genebank collections and breeder germplasm from the International Institute of Tropical Agriculture (IITA) and the International Centre for Tropical Agriculture (CIAT), as well as elite germplasm and landraces from Eastern, Southern and Central Africa. For this evaluation, a GoldenGate SNP-based assay (Illumina Inc.) was utilised. Four separate populations were identified by the results of the ADMIXTURE and genetic distance analyses:

(i) West African germplasm, including IITA breeding lines and genebank accessions, which both exhibit somewhat lower diversity

(ii) A more mixed group from East, South and Central (ESC) Africa with moderate diversity

(iii) A smaller group of African introduction germplasm that is distantly related to others and

(iv) South American germplasm with high genetic diversity and presumed founder genotypes from Brazil (Ferguson *et al*., 2012).

Significant differences between South American and African cassava germplasm, as well as among African landraces, have been found by molecular marker-based research. Interestingly, landraces in West Africa are not the same as those in East, South and Central Africa (Fregene *et al.*, 2003). Due to concentrated breeding efforts for resistance to cassava mosaic disease (CMD), which frequently involve a small number of parental lines and cassava clonal multiplication, African breeding lines have a relatively narrow genetic base (Esuma *et al*., 2012).

Landraces often have more genetic variation than elite lines; this is also the case with cassava (Ferreira *et al*., 2015). It is believed that apomixis and both natural and artificial hybridisation events between wild *Manihot* species and cultivated cassava are the sources of the genetic variety in cassava (Amelework and Bairu, 2022). International research organisations maintain large collections of cassava germplasm: IITA in Nigeria maintains over 2,000 genotypes primarily of West African origin, whereas CIAT in Colombia maintains over 6,000 accessions, with roughly 30% of Brazilian provenance (Fukuda *et al*., 2002).

2.2 Characterization of cassava germplam

Each region where cassava is grown has a variety of the root vegetable. The phenotypic traits of the cultivars, for instance leaf characteristics, colour plus shape, branching-off behaviour, plant height, stem colour, root form, flowering and maturation time, have been used to identify them (Sanni *et al*., 2015; Saravanan *et al*., 2016). Based on genetic studies (Campos *et al.,* 2020), characterization may be advantageous for the acquisition of quality attributes. Cassava variants are a vital foundation of genomic materials for crop improvement because they offer a vast foundation of inherent discrepancy. The rapid creation of high-yielding cassava variants should be given top attention to ensure their long-term sustainability (Nduwumuremyi *et al*., 2017). Wide-ranging genetic variation among cassava cultivars offers advantageous traits for *in- situ* conservation besides research on inherent range and development. Notably, cassava cultivars are cultivated plants that embody a type of inherent material that needs to be conserved and kept, primarily for the transmission of qualitative features (Singh *et al*., 2017). To prevent

losses and ensure the safety of these inherent resources, actions that aim to maximize the maintenance and utilization of cassava germplasm are required (Soto *et al*., 2015).

Although physical and agronomic criteria have traditionally been used to assess genetic resources, these indicators do not always reflect the nutritional value or reveal the underlying genetic relationships among the germplasm. In fact, most of the morphological and biochemical descriptors exhibit continuous fluctuation and strong plasticity and the majority of them are only scoreable at maturity (Soto *et al.,* 2017). With a view to intensification of effectiveness of identifying and using plant germplasm to reduce malnutrition and starvation, utilisation of trustworthy and standardised inherent descriptors is crucial (Singh *et al*., 2017). Developing the best management techniques for the resource's long-term utilisation and conservation requires precise characterization, review, and assessment of the collections within Kenya's cassava germplasm inventory alongside an estimation of the degree of genetic heterogeneity in the resource.

2.2.1 Phenotypic characterization of cassava

Since Mendel, geneticists and breeders have utilized morphological qualities like leaves and flowers to track the separation of protein arrangement and hybrids, but most agronomic parameters are not connected to readily apparent phenotypic markers (Fasahat *et al.,* 2016; Oliveira *et al*., 2019). Plant phenotypic characterization frequently relies on the physical characteristics observed and noted in nature. Characterization is a useful tool for cultivar classification. The pure genetic makeup of a specific crop is typically the foundation for certification of novel cultivars (Avivi *et al.,* 2020). The detection of duplicates, investigations of inherent disparity arrangements, and association with traits of agronomic capability have all benefited from phenotypic characterization. These could

entail a protracted study of plant development, which could be expensive, labor-intensive, and susceptible to environmental factors. But in cassava breeding operations, the focus has been mostly on gathering and preserving gene pools and characterizing them to get rid of duplicates (Mithra *et al*., 2018). The variable traits are those brought about by environment-genotype interaction in big genotypes. The consistent traits, such as the branching type in cassava cultivars, characterize the species or variety. It is challenging to pinpoint the physical traits of cassava since it thrives in a variety of ecological settings. As a result, the environment's impact on genotype is always significant (Karim *et al*., 2020). Numerous spontaneous and artificial crossings have made it difficult to identify genotypes in most crops through the study of taxonomy and genetic linkages. Only genes impacting morphological features, like leaf shape and dwarfism, have been used in crop enhancement. Morphological descriptors have traditionally been used to characterize and categorize cassava germplasm. IBPGR (1998) and Fukuda *et al.,* (2010) developed a collection of morphological features of importance towards identifying the genotype of cassava. Assessments of both the quality and quantity of cassava's shoot and root components are included. Molecular methods have expanded this significant taxonomic tool. Phenotypic traits such as, branching-off pattern, plant height, colour of the stem and petiole, root shape and skin colour, maturity period, yield and the amounts of cyanogenic glycosides in the roots have been used to discriminate between different cultivars (Saravanan, 2016).

Cassava cultivars are typically identified based on morphological features (Fukuda *et al*., 2010). They have a huge range of botanical variations. Several varieties can be distinguished from one another by morphological traits like plant height; other factors to

consider include size, shape, and leaf colour. Large-scale linguistic variation is a barrier to the accurate identification of cultivars. The cultivated varieties of crops have been clearly labelled, as they are in most other parts of the world. Each site has its own distinctive group of names for various cultivars, each with a distinct significance (Fukuda *et al.,* 2010). Cassava classification by morphology has been crucial in clearing up cultivar uncertainties. Utilizing an adaptation of International Board for Plant Genetic Resource (IBPGR) scale, researchers have attempted to categorise them. In certain unique varieties, the architecture of the plant can differ greatly, depending on whether branches are present, their level of branching and the total number of lobes on the young leaves, which can range in colour from light green to purple. The accessions typically consist of huge numbers, occasionally lacking definite identification due to the influence of shifting environmental conditions (Fasahat *et al*., 2016; Zago *et al*., 2017).

In Brazil, 36 qualitative morphological descriptors were assessed with the intention of identifying different cassava cultivars (Tiago *et al*., 2020). In the dissimilarity study between the evaluated samples, the 45 ethno-varieties of cassava that were analyzed showed 97.35% polymorphism, revealing morphological divergence (Tiago *et al*., 2020). Similarly, Dissanayake *et al*., (2019) assessed the morphology of cassava variants in Sri Lanka and found that wild-accession and local cultivars had considerably different leaf morphologies. The mean internodal length of the stems amongst cultivars was considerably dissimilar among them, whilst stem thickness distinguished wild-accession varieties from the others. Morphological characterization was carried out on 29 cassava genotypes in Indonesia by Ridwan *et al.,* (2022). They used information on 14 morphological traits, such as the leaf colour, design, shape leaf petiole and lobe. Thus,

the authors employed leaf traits such lanceolate and elliptic-lanceolate shapes of the center leaflets, light green foliage colour, missing pubescence, the apical foliage, typical leaf retention, and green colour leaf vein. It was determined that 100 percent of them had seven lobes, a smooth lobe margin, a purple petiole colour, and their orientation was horizontal (Ridwan *et al*., 2022). In another study, Nadjiam *et al*., (2016) evaluated morphological features of cassava in Chad. More than 80% to 95% of the varieties had variation. These variations included an upright branching habit, greenish-yellow stems, light brown stem epidermis, complete stipules, no pubescence on apical foliage and roots that were simple to de-husk. All the cultivars under study displayed large foliar scars, gaps amongst foliage scars, light green stem cortex, no velum on matured foliage, and straight habit growth. It was also determined that the proportion of varieties having light green foliage to those with dark green foliage was roughly equal. Descriptive morphological traits of cassava cultivars were conducted in Ghana (Kumba *et al.,* 2020). These authors evaluated the characters such as external storage root colour, colour of root pulp, easiness to peel, colour of foliage vein, cassava mosaic disease, lobe margins, foliage colour, colour of apical leaves and shape of central leaflet. There were significant discrepancies among the genotypes under study. The majority of the genotypes had white root pulp and dark brown peripheral storage roots, the study found that colour was ostensibly the most representative and unique feature. Further, the study reported that the genotypes' above-ground leaf characteristics were light green apical foliage, light green leaves, green foliage veins, smooth lobe margins and pandurate centre leaflets.

The morphological characteristics of 159 entries of cassava in Centre-west, South-west, and West of Côte d'Ivoire were studied (Nzue *et al*., 2014). The study grouped the entries

into three clusters. It turned out that there were 16 duplicates and 143 morphotypes among the germplasm that had been collected (N'zue *et al*., 2014). Niyonzima *et al*., (2021) examined the morphological characteristics of cassava in Burundi and established that the distribution of leaf features varied between cassava cultivars. The stems were also found to be widely variable within the cassava accessions. Additionally, there was a wide variation in color of external storage root, color of the root cortex and the colour of the root pulp. Both Ward's approach and the hierarchical clustering based on morphological traits categorised accessions into three clusters; showing that there was phenotypic variation among them that might be used to improve the crop. Masinde *et al*., (2018) established that most of the cassava cultivated in Kenya are typically late branching, with varied plant heights. Most petioles and root skin were pink. The tint of the stems seemed to vary. The same cultivar may possess several distinct names due to arbitrary naming of the cassava cultivars, which frequently depended on the name of the person giving out cuttings. As a result, the morphological characterization may show morphological duplication and variability in the germplasm (Fukuda *et al*., 2010).

2.2.2 Genetic characterization of cassava germplasm

Phenotypic identification of plants is commonly based on the morphological traits assessed and recorded in the field (Fukuda *et al*., 2010). Molecular characterization is particularly important in cassava due to the crop's vegetative propagation and the presence of morphologically similar but genetically distinct varieties. This makes traditional phenotypic characterization insufficient for accurate identification and conservation efforts (Adjebeng-Danquah *et al*., 2020). Moreover, phenotypic characters are highly influenced by environmental factors. Genetic markers allow researchers to

overcome limitations associated with phenotypic characterization by providing precise, reliable data on genetic variation within and among cassava populations; thus, enhancing breeding programs (Agre *et al*., 2016; Amarullah, 2020). Simultaneous studies on phenotypic and genotypic characterization of cassava germplasm have been carried out in various cassava growing regions in the world. In America, studies by Olsen *et al.*, (2001) and Elias *et al.*, (2004) focused on the genetic diversity of cassava using molecular markers such as SSR. The studies demonstrated significant diversity in cassava germplasm and highlighted the complementarity between molecular and phenotypic characterization in improving cassava breeding programs. Similarly, in Asia, a study by Sivan *et al*., (2023) in India utilized both morphological descriptors and molecular tools like Random Amplified Polymorphic DNA (RAPD) markers to characterize local cassava varieties. The study found that molecular data helped in accurately identifying genetic relationships among cassava accessions, which was not fully achievable through phenotypic traits alone.

Molecular characterization is becoming crucial in overcoming challenges, in the identification of cassava genotypes, understanding genetic diversity and enhancing breeding programs (Charters & Wilkinson, 2000; Amarullah, 2020; Manze *et al*., 2021). Molecular markers, such as microsatellites (SSR), single nucleotide polymorphisms (SNP) and DNA sequencing technologies, have revolutionized the study of cassava germplasm. These tools allow for the accurate identification of varieties, differentiation of closely related genotypes and assessment of genetic diversity (Rabbi *et al*., 2022).

2.2.2.1 Molecular markers in characterization of cassava germplasm

Since they are quick and simple method for determining the degree of variation among germplasm, genetic markers like agro-morphological markers have been utilized frequently in early investigations (Brian *et al*., 2015; Andrade *et al*., 2017; Phumichai *et al*., 2022). In addition to phenotypic and protein-based indicators, molecular genetic markers centered on DNA sequence polymorphism are now frequently used (Singh *et al.,* 2017). The DNA-based markers are extensively utilized in cultivated crops over the past 20 years (Andrade *et al*., 2017). When feasible and practical, single-marker assays have been utilized to replace phenotypic assays in plant characterization to speed up the process (Platten *et al*., 2019; Parmer *et al*., 2017). In order to create an adapted elite variety, multiple genomic areas from numerous various individuals will progressively be purposefully combined (Gbaguidi *et al*., 2015). It is crucial to have a thorough understanding of the genetic variety found in both farmed besides wild germplasm, which is the foundation of unique genomic areas, alleles and phenotypes (Brian *et al*., 2015; Fasahat *et al*., 2016). Progressively from single marker assays to genome-wide marker profiles, genomic fingerprints containing genetic variability at hundreds of loci is of value in embracing molecular markers in this setting (Araus and Kefauver, 2018; Ozimati, 2019). Whole-genome sequencing will be necessary for genetic variation analysis to provide an accurate assessment of variations among individuals and relationships (Agre *et al*., 2016; Fasahat *et al.,* 2016).

An assortment of procedures can be utilized to reveal polymorphism of the nucleic acids at DNA level. The array of procedures are of 3 clusters; (i) Non- Polymerase Chain Reaction (PCR) based approach such as Restriction Fragment Length Polymorphism

(RFLP); (ii) PCR arbitrary priming such as Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Inter Simple Sequence Repeat (ISSR); (iii) Targeted PCR such as Sequence Tag Sites (STS), Cleaved Amplified Polymorphism (CAPs), Simple Sequence Repeats (SSR) and Single Strand Conformation Polymorphism (SSCP) (Wachira, 2002: Moyib *et al.,* 2007). Non-PCR based methods are not frequently utilized because they require a lot of labour and have a low throughput. Some researchers have chosen to use new polymorphic assay methods based on PCR, particularly the Start Codon Targeted (SCoT) markers (Collard and Mackill, 2009; Habiba *et al.,* 2021).

2.2.2.1.1 Polymerase Chain Reaction

A molecular biology technique called the polymerase chain reaction (PCR) allows DNA to be replicated enzymatically without the use of a living thing like yeast or *E. coli* (Collard *et al*., 2005). The method enables a little amount of DNA to be amplified steadily, similar to amplification utilizing living organisms (Souza, 2015). Although PCR is an *in vitro* procedure, it can be embraced to undertake a wide range of genetic modifications without being constrained by the shape of DNA (Temegne *et al.*, 2015). Several activities, including the identification of inherent fingerprints, duplicating of genetic factor and DNA computing, are frequently carried out using this method in medical and biological research facilities (Andrade *et al*., 2017). A DNA strand can be amplified in specified places using PCR. A sole gene, only a section of a protein sequence, or a non-coding region can be included in this. Only brief DNA segments, usually up to 10 kb in length, are typically amplified by PCR. The chromosomal DNA of

a eukaryotic cell is substantially larger than some techniques can copy segments, which can reach a maximum size of 47 kb (da Silva *et al.,* 2015).

Gel electrophoresis can be used to visualize the PCR products based on their size. In gel electrophoresis, DNA is injected into a gel matrix, and the gel is subsequently subjected to an electric current (Darkwa *et al*., 2020). Because of this, the smaller DNA strands move via gel medium more quickly towards the positive endpoint than the bigger strands do. A DNA ladder, which is also present in the gel and contains DNA fragments with known molecular weights, can be used to compare the size of the PCR product (Tiago *et al*., 2019). RAPD, AFLP, SSR, and SNP are just a few examples of the several PCR-based marker systems (Karim *et al*., 2020).

2.2.2.1.1.1 Random Amplified Polymorphic DNA

Any DNA sections that are amplified via utilization of brief oligodeoxynucleotide primers of any nucleotide arrangement are known as random amplified polymorphic DNA markers (RAPDs) (da Silva *et al*., 2015). Using random primers and genomic DNA, PCR is used to generate RAPDs (Asha *et al*., 2020b). In addition to the brief random oligomers (usually 10-mers), DNA Taq polymerase is employed to amplify DNA segments amongst closely spaced arrangements (2 kb). Changes in the DNA sequence's primer-binding location cause RAPD polymorphism. Gel electrophoresis can be used to separate the PRC products (Rabbi *et al*., 2017). Because the RAPD markers depend on dominant features, they are not sufficiently informative. Additionally, the reproducibility and transferability of this marker system between laboratories may be poor. RAPDs have been employed in research on Kenyan tea (Wachira *et al*., 1995) and cassava variability in Ghana (Asante and Offei, 2003).

2.2.2.1.1.2 Amplified Fragment Length Polymorphism

Any variation amongst homologous DNA segments from two different organisms that is picked up by the amplified restriction length polymorphism approach is known as amplified fragment length polymorphism (AFLP) (Fregene *et al*., 2000). According to Okeke *et al.,* (2017), the AFLP technique combines elements of RFLP analysis with PCR technology. With a pair of ceiling enzymes, typically a common and an infrequent cutter, the total genomic DNA is broken down. The DNA fragment is then joined at the 3' and 5' ends by adaptors with a known sequence. The adapter primers are used as complimentary primers to amplify the restriction fragments. After that, banding patterns can be seen at low resolution and the PCR-amplified sections be disjointed by gel electrophoresis. The complexity of AFLP fingerprints can be altered using a variety of enzymes and primers to fit the application (Elias *et al*., 2001). Selecting primers with selective bases requires caution. Additionally dominant, the AFLP markers are unable to discriminate between homozygotes and heterozygotes. They have been applied in research on cassava's genetic variability and heredity (Elias *et al*., 2001).

2.2.2.1.1.3 Simple Sequence Repeats

Simple Sequence Repeats (SSR), often referred to as Micro satellites, are a collection of DNA sequences that are extremely brief (2–10 bp), substantially recurrent, tandemly organized, and extremely distinct (hyper variable) and are found across the genomes of various fungi, plants, animals and humans (Asare *et al*., 2011). As in (AT)29, (CAC)16, or (GACA)32, the di-, tri-, or tetra-nucleotide repeats are organized in tandem arrays of 5–50 copies. According to Cardle *et al*., (2000), the SSRs are frequent in plants, occurring roughly every 6-7 kb. In order for PCR-amplify the DNA portion

encompassing the SSR, forward and reverse primers can be created using the conserved nucleotide sequences that flank these repeat motifs. SSR alleles, which are variable-length amplified products, can be automated (if the primers are fluorescently marked) or disjointed by gel electrophoresis and visualized using silver staining, auto-radiography, or other methods. SSR markers are perfect for genetic diversity research since they are co-dominant and can tell homozygotes from heterozygotes. SSR analysis can be automated and multiplexed, enabling genotyping on an extensive number of lines and concurrent study of many loci (Fregene *et al*., 2003; Zayed *et al.,* 2013).

DNA databases like EMBL and Gene Bank can be searched for SSRs, or small supplement (200–600 bp) genomic DNA libraries can be created and augmented for specific repeats (Rabbi *et al*., 2015). Primer pairs (of roughly 20 bp each) can be created from the sequence data using software applications. There is utilization of SSR markers to identify genetic variety in cattle (Guo *et al*., 2005) besides cassava globally (Tiago *et al*. 2016; Okeke *et al*. 2017; Prempeh, 2020; Asha *et al*. 2020b; Asha *et al*. 2020a; Kumba *et al*. 2020). The SSR has also distinguished itself as a significant tool for the investigation of inherent assortment in the description of entries besides cultivars of various species (Isshiki *et al*., 2008; Marcos *et al*., 2009). Simple sequence repeat markers were created and utilized to assess the inherent assortment of cassava (Reynolds, 2017) and create the inherent association map of cassava (Ogbonna *et al*., 2020). They are units of tandem nucleotides, and according to Udoh *et al*., (2017), they are good targets and a way to measure genetic diversity within species. Heterozygotes and homozygotes can be delineated from each other through usage of codominant markers like SSR and RFLP (Rabbi *et al*., 2017).

2.2.2.1.1.4 Single Nucleotide Polymorphism

Even in low diversity species, single nucleotide polymorphisms (SNPs) are helpful in identifying and separating specific genetic differences (Agre *et al.,* 2015). Among the competing markers for diversification research are SSR and SNP markers. Microsatellites (SSR) may be constrained by stutter bands, which cause quasi-scoring in ladders missing conspicuous bands and make scoring challenging (Park *et al*., 2009), as well as by low interspecies transferability (Grattapaglia and Kirst, 2008). Microsatellites are more difficult to measure per locus than single nucleotide polymorphisms. In the genomes of plants, animals, and microorganisms, SNPs are the most prevalent marker system. They are regarded as the most recent wave molecular marker for a variety of applications (Niyonzima *et al*., 2021). Even in low diversity species, the SNPs are helpful in identifying and separating specific genetic differences (Ferri *et al.,* 2010). Instead of using the traditional technique alone, the use of SNPs has sped up inherent assortment research and advancements in selection (Kawuki *et al*., 2019; Niyonzima *et al*., 2021). Instead of solely using traditional techniques and other molecular tools, the use of SNPs accelerated the pace of evaluating genetic assortment and selection gains.

2.2.2.1.1.5 Diversity Arrays Technology (DArT)

Later, cassava SNP markers and Diversity Arrays Technology (DArT) markers based on SilicoDArT were created and presented as a technique for genotyping large germplasm entries (Alam *et al.*, 2018), however, in Kenyan cassava cultivar genotypes, this has not been embraced yet. According to Xia *et al*., (2005), DArT accomplishes much in polyploid species, requires no prior knowledge of DNA sequences, and uses fewer resources than SNP platforms. The foundation of DArT is in the discoveries of DNA

polymorphism that the technique makes by examining the presence or lack of particular pieces in representations of genomic DNA samples (Wenzl *et al.,* 2008; Kilian *et al*., 2012). It is the technique of genotyping without using sequence data that may simultaneously identify genetic variation at numerous genomic loci (Xiao *et al*., 1998; Xia *et al.,* 2005; Wenzl *et al*., 2008).

2.2.2.1.1.6 Start Codon Targeted (SCoT) Markers

Since DNA is not immediately impacted by the environment, molecular characterization by markers centered on DNA amplification is greatly advised (Mbah *et al*., 2019). This increases the trustworthiness of the results. Start Codon Targeted (SCoT) markers stand out amongst the molecular markers centered on polymerase chain reaction (PCR) as a crucial tool for investigation of the genomic variation in description of accessions besides cultivars of most species (Habiba *et al.,* 2021).

Start-codon-targeted (SCoT) markers are based on polymorphisms in the short, highly conserved region of plant genes surrounding the ATG of the start codon (Collard and Mackill, 2009). These markers can distinguish genetic variation in specific genes linked to a specific trait (Deng *et al,* 2015). They have been preferred since they are a simple, novel, cost-effective, highly polymorphic, and reproducible molecular marker that are independent of sequence information. In addition, detection is carried out using agarose-gel-based techniques, making them simple and relatively cheap to use (Collard and Mackill, 2009). Studies have revealed that the short flanking regions of the ATG start codon are highly conserved across plant species and have led to the widespread use of SCoT markers in quantitative trait locus (QTL) mapping, marker-assisted breeding, bulked segregate analysis, and genetic variation studies (Collard and Mackill, 2009). The

SCoT markers have been used in genetic diversity analysis, phylogenetic relationships and DNA fingerprinting of economically important food crops and medicinal plants including yam (*Dioscorea spp*.) (Owiti *et al.,* 2023), mango (Luo *et al*., 2011), grape (Guo *et al*., 2012), orchid (Bhattacharyya *et al.*, 2013), durum wheat (Etminan *et al*., 2016), rose (Agarwal *et al*., 2019), Diospyros (Deng *et al*., 2015), *Elymus sibiricus* (Zhang *et al*., 2015), *Vigna unguiculata* (Igwe *et al*., 2017), *Taxus media* (Hao *et al*., 2018), Dendrobium (Feng *et al*., 2019), *Chrysanthemum morifolium* (Feng *et al*., 2016), coconut (Rajesh *et al*., 2015), *Orchidaceae* (Sun and Zhang, 2020) and *Physalis species* (Feng *et al*., 2018). Studies have indicated that SCoT markers have good capabilities in genetic research due to their ability to reveal polymorphisms in conserved regions and their high reliability as compared with other molecular systems (Etminan *et al*, 2016; Aboulila and Mansour, 2017). Therefore, the current study determined the genetic diversity among sampled cassava germplasm in Kenya using SCoT molecular markers.

2.2.3 Cyanide concentration in cassava germplasm

Despite being a significant starchy tuberous root crop growing in the tropics, cassava has inherent cyanide toxicity that raises serious public health concerns. According to Ndubuisi and Chidiebere (2018), cyanogenic glucosides, particularly linamarin, are the cause of the toxicity. Because of this, eating cassava has occasionally caused fatalities as well as chronic illnesses like Konzo (Cliff *et al*., 2011; Siritunga and Sayre, 2017). According to Ndubuisi and Chidiebere (2018), cyanogenic glycosides are a class of chemical molecules that, after being broken down by enzymes, release hydrogen cyanide. In cassava, cyanogens can be found in three different forms: (i) free cyanide, (ii) cyanohydrins, and (iii) cyanogenic glucosides (linamarin and lotaustralin). More than

80% of the cassava cyanogenic glucosides are linamarin. Linamarin is a ß-glucoside of acetone cyanohydrin and ethylmethyl-ketone cyanohydrins (Andersson *et al*., 2017).

Linamarin's ß-linkage is easily destroyed by enzymes, but it can only be structurally disrupted by high pressure, high temperature, or the use of mineral acids (Ogbonna *et al*., 2020). This ß- bond can be broken by the natural cassava enzyme linamarase. Optimal conditions for the enzymatic process are 25^{0}C and a pH range of 5.5 to 6.0. The cassava plant contains linamarin in all its components, albeit it is being more prevalent in the roots and leaves. Detoxification is possible if the enzyme and the substrate are combined (Saravanan, 2016). Crops besides cassava such as sorghum, almonds, lima beans, flax, and white clover also synthesise and store cyanogenic glycosides (Njoku and Ano, 2018). The typical magnitude of cyanogenic glycoside level in cassava ranges from 1 to 1300 mg per kg of dry weight (Ndubuisi and Chidiebere, 2018), depending on genetic, physiological, climatic and edaphic factors. According to Njoku and Ano (2018), the concentrations of cyanogenic glycosides in cassava roots are characteristically lesser compared to those in the foliage and stems. Cassava roots exhibit 10-500 mg of cyanide per kilogramme of dry matter (Bolarinwa *et al.,* 2016), whilst the foliage has been found to possess 53-1300 mg (Zidenga *et al*., 2017).

The occurrence or lack of harmful amounts of cyanogenic glucosides, characterizes cassava varieties biochemically as bitter or sweet (Moyib *et al*., 2015). Those variants with extremely minimal cyanogenic content (15-50 milligrams per kilogramme of fresh weight of roots) confined primarily to the peel are referred to as sweet varieties, whereas the bitter variants are characterised by their high magnitude of cyanogenic content (100-400 mg per kilogramme of fresh weight of roots) spread all through the storage roots

(Bolarinwa *et al*., 2016). Consuming raw or improperly processed cassava may culminate in cyanide poisoning, which can cause both acute and chronic health issues (Saravanan, 2016). World Health Organisation (WHO) recommends that the acceptable threshold for hydrogen cyanide in cassava flour is 10 ppm, or 10 mg/kg (WHO, 2016). Chronic exposure to cyanide has been associated with health risks including tropical ataxic neuropathy, goiter and Konzo- a paralytic disease, when adequate detoxification is not conducted (Cliff *et al*., 2011; Alitubeera *et al.*, 2019; FAO, 2020; Nyamekye, 2021). Usually, cassava cultivars occur in 3 clusters in regard to feature of cyanogenic glucoside composition which include: cassava with a strong potential to produce HCN (at least 10 mg per 100g of fresh weight), HCN values in intermediate types range from 5 to 10 mg per fresh weight and also less than 5 mg of HCN can be produced per 100g of fresh weight from cassava.

The cyanide concentration in cassava varies significantly among cultivars, as it is influenced by both genetic (Ndubuisi and Chidiebere, 2018) and environmental factors (Ospina *et al*., 2024). Nyirenda (2021) clarified that every cassava variety contains cyanogenic glycosides in varying quantities. In general, cyanogenic compounds are higher within the cortex (~804 ppm) than in the root parenchyma (~305 ppm) (Ospina *et al*., 2024). Hawashi *et al*., (2019) explained that cassava leaves contain 5 to 20 times more cyanogenic glycosides and are considered potentially more toxic if consumed than the root tubers. The toxic hydrogen cyanide (HCN) is released as a result of enzymatic hydrolysis by β-glucosidase following the maceration of plant tissues as they are consumed by gut microflora, leading to the breakdown of cyanogenic glycosides (Kwok, 2008). This process can be represented by the following biochemical equation:

$$\text{Cyanogenic Glycoside} + H_2O \xrightarrow{\beta\text{−Glucosidase}} \text{Aglycone} + \text{Glucose} \qquad (1)$$

$$\text{Aglycone} \xrightarrow{\alpha\text{−Hydroxynitrile Lyase}} \text{Hydrogen Cyanide (HCN)} + \text{Ketone/Aldehyde} \qquad (2)$$

A complex molecule (cyanogenic glycoside) is broken down into two parts (Aglycone and Glucose) (Equation 1). One of those parts (Aglycone) is still unstable and breaks down further into two more pieces (Hydrogen Cyanide and a Ketone/Aldehyde) (Equation 2). This leads to cyanide toxicity, which could manifest as nausea, headache, confusion, feebleness, unconsciousness and eventually death in both animals and humans (Mosayyebi *et al*., 2020). The acute threshold fatal doses of cyanide ranges from 0.5–3.5 mg/kg body weight, whereas the chronic cyanide dose for daily consumption is 0.02 mg cyanide/kg body weight (Harenčár *et al*., 2021).

Given that cassava roots present highly attractive nutritional characteristics as a readily available source of essential vitamin C and A, and dietary fibers (Ndubuisi and Chidiebere, 2018), high carbohydrates (66.1–72.1%), and total energy (385.9–397.9 kcal/100 g) (Modesto Junior *et al*., 2019), this variability in and potential of dietary HCN exposure to consumers, underscores the need for determining the cyanide levels in different cassava cultivars to ascertain consumer safety (FAO, 1991). Furthermore, even though, it has been established that improved cassava cultivars generally have lower cyanide concentrations compared to traditional varieties, whose cyanide levels often exceed the safe limit of 10 mg/kg set by the World Health Organization (FAO, 1991), and while certain cultivars are generally classified as sweet (or low cyanide) or bitter (having high cyanide), such classifications can be ambiguous, as the threshold for

bitterness is subjective and depends on consumer tolerance (Ndubuisi and Chidiebere, 2018).

2.2.3.1 Methods used in cyanide analysis in cassava

In the literature, there are many techniques for cyanide evaluation, but they all have three things in common: (a) the extraction technique (b) the hydrolysis of cyanogenic glycosides and (c) the analytical measurement of the free cyanide (Brito *et al*., 2009; Ndubuisi and Chidiebere, 2018). Because cyanide in cassava species demonstrates such a wide range of toxicities, cyanide toxins have been formally divided into three classes: (i) free cyanide (HCN, alkaline and alkaline earth cyanides) (ii) weak acid dissociable cyanide (WAD) - (a collective term for free cyanide and metal-cyanide complexes such as (Ag(CN)2-, Cu(CN)43-, Cd(CN)42-, Zn(CN)42-, Hg(CN)42-, Ni(CN)42-) which easily release HCN under slightly acidic environmental conditions) and (iii) total cyanide each being potential source of HCN regardless of its origin (U.S EPA,1992).

If the necessary measures are not taken, interference issues could affect all three of the regularly employed techniques (Bradbury *et al*., 1999). The extraction of cyanogenic chemicals from plants, their hydrolysis, and analytical assessment of the released cyanide are the three steps required by the approach for linamarin and their metabolic analysis (Cagnon *et al*., 2002; Phoncharoen *et al*., 2019). Since linamarase is inert at low pH, cyanogenic chemicals are extracted from the plant using an acid dilution solution (Bradbury *et al*., 1994; Hershey, 2017). After removing linamarin, cyanide is subjected to a series of hydrolyses to turn it into free cyanide (IITA, 2020). One can mention the titration of cyanide with $AgNO_3$ (AOAC, 1990; Saravanan, 2016) and the reaction with alkaline picrate (Egan *et al*., 1998) as approaches for the third step. The König reaction,

in which free cyanide (CN-) is oxidized into cyanogen halide by chloramine T or N-chloro succinimide, is the basis for the most popular colouring technique (FAO/WHO, 2020).

2.2.3.2 Alkaline titration method

Onyesom *et al*., (2008) used the technique to find out how much cyanide was present in cassava fermented with *Cymbopogon citratus*. Alkaline titration was embraced to compute the cyanide concentration per sample sample (AOAC, 1990). The end point of the titration was attained when the solution transformed from being clear to a faint turbid in nature. Then, five percent of potassium iodide was employed as the indicator. The equation $1cm^3$ 0.02M $AgNO_3$ = 1.08 mg HCN was utilized in computing the amount of cyanide per sample. It is commonly utilized cyanide measurement techniques in the gold extraction sector. It is more appropriately referred to as "titratable cyanide" than "free cyanide" when the prevailing ion is CN (Heath *et al*., 1999). The procedure entails adding silver ions to the solution in order to complex with the "free cyanide".

2.2.3.3 Picric acid in solution method

The endogenous linamarase enzyme has been employed to hydrolyze linamarin and release CN- during the autolysis of cassava roots by Baltha and Cereda (2006) to determine the amount of free and potential cyanide. By modifying the Smith method, which relies on the colorimetric reaction of picrate solution, as described by Winton and Winton (1958), the amount of free cyanide was ascertained. The authors discovered that the maximum levels of cyanide were produced after a 15-minute reaction, showing an asymptotic curve for colour development.

It is feasible to conclude, based on the findings of a study by Brito *et al*. (2009), that both qualitative and quantitative approaches ought to be utilized in identifying free and latent cyanide in various regions of the cassava entries. The technique offers reasonable accuracy, good cyanide preciseness, and good sensitivity. After linamarin is hydrolyzed, the resulting HCN interacts with picric acid to create a charge transmission multifaceted that has a brown colour.

2.2.3.4 Picrate paper method

A picrate paper kit was created by Bradbury *et al*., (1999) to estimate the total cyanogens in cassava root. In regard to the extent of 0 to 800mg HCN equivalent kg^{-1} cassava, a linear Beer's law relationship between absorbance and cyanogens will be found. A case study on a pair of instances involving fatal cassava poisoning in Kenya's Kitui district, where two children perished, employed the picrate paper method. Cyanide levels in raw and cooked cassava were measured. The outcomes revealed significant amounts of HCN for the raw and cooked samples of 46.0 mg/Kg and 52.3 mg/Kg respectively (Njue *et al*., 2011). The method was also employed by Oliver and Hernaez (2006) to assess the cyanide content of cassava in the Philippines, and according to them, it was successful. This straightforward procedure uses equipment and substances that are easily accessible. The technique quantifies cyanide that is dissociable in weak acids. It focuses on the colorimetric measurement of a coloured product created by the reduction of sodium picrate by cyanide. Only free cyanide can affect this decrease of picric acid. The picrate reactions, which were created by a number of writers, including Bradbury *et al*., (1999) and Brimer (1994), are the foundation of the picrate methodology.

Picric acid solution and sodium carbonate solution - or picric acid in a solution of sodium carbonate, are impregnated into sheets of absorbent paper (or TLC sheets) using this technique (Brimer *et al.,* 1983). In the presence of sodium carbonate, picrate combines with cyanide to form isopurpurin (and related chemicals), which are coloured and may be detected using picrate paper and a UV-Vis spectrophotometer. Thus, in this investigation, the quantity of cyanide in cassava was determined using the picrate paper method, one of the most efficient, trustworthy, and simple methods. The current study determined cyanide concentration levels among cassava (*Manihot esculenta* Crantz) germplasm in Kenya. Quantitative analysis of cyanide content across cassava germplasm, such as presented in the present work, can provide critical data as a basis for the development of more accurate classification systems, while supporting breeding programs that are focused on producing safer cassava varieties with cyanide levels, and guide formulation of recommendations for safer cassava consumption (Cardosso *et al.,* 2005).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Phenotypic (Morphological) characterization of cassava germplasm

3.1.1 Germplasm collection and multiplication

A survey was undertaken in the seven purposefully selected major cassava growing regions/counties in Kenya to collect cassava samples. The regions/ counties were Coast (Kilifi County), Eastern and Central (Makueni and Nakuru Counties), Western (Busia County), Nyanza (Migori, Homabay and Kisumu Counties). Within each selected county, purposeful stratified random sampling approach was adopted, where sub-counties served as strata to ensure representative coverage of the major cassava growing zones. Sampling was conducted along transects established parallel to major roads, collecting accessions from farms located both adjacent to and within accessible distances from these transects. Sampling points were systematically spaced at an equidistant intervals of 1 km along each transect, starting at the edge of the sub-county on the main road to maintain uniform distribution and avoid clustering. During the survey, samples were collected from cassava farmers who were visited at random in each stratum. A single stem from one plant was collected to represent the accession. A single stem from one plant was collected to represent the accession. The local name of the accession was obtained from the farmer and/ or from the supplier of the planting materials. The stem was cut into pieces, 15 cm long each and placed in a collection bag.

A total of 131 accessions were collected. Care was taken to avoid damaging the stems. All the samples collected were taken to Rongo University farm in Migori County for multiplication. The accessions were planted in single rows, each of at least five stem cuttings. The spacing was 1m between rows and 1 m within rows.

3.1.2 Establishment of experimental plots

Plants from the multiplication plots were harvested 12 months after planting. The stems of each accession were cut into pieces of length 15 cm and placed in a collection bag. The accessions were planted at Rongo University farm (-0.826279^o, 34.614186^o) in Migori County. Each entry was planted three rows with five cuttings in each row. The spacing was 1 m between plants and 1 m between rows (Plate 3.1). Hand weeding was done at 2, 4 and 6 months after planting. No fertilizer or pesticides were applied on the crop.

a b

Plate 3.1: Planting (a) and establishment of the cassava accessions (b; 2MAP)

3.1.3 Data collection

Data on phenotypic traits were collected from the middle row of each accession plot. Phenotypic characterization was done using the selected morphological and agronomic descriptors (21 qualitative traits and 4 quantitative traits) for the characterization of cassava as described by (Fukuda *et al*., 2010) (Table 3.1). The observations were made at 3, 6, 9 and 12 months after planting.

Table 3.1: Selected qualitative and quantitative phenotypic descriptors for the characterization of 131 cassava genotypes

SN	Trait descriptor	Score code	Sampling time	Trait type
1	Colour of apical leaves	3 = light green; 5 = dark green; 7 = purplish green; 9 = purple	3 MAP	a
2	Shape of central leaflet	1 = ovoid; 2 = elliptical-lanceolate; 3 = obovate-lanceolate; 4 = oblong-lanceolate; 5 = lanceolate; 6 = straight or linear; 7 = pandurate; 8 = linear-piramidal; 9 = linear-pandurate; 10 = linear-hostalobalate	6 MAP	a
3	Petiole colour	1 = yellowish-green; 2 = green; 3 = reddish-green; 5 = greenish-red; 7 = red; 9 = purple		a
4	Leaf colour	3 = light green; 5 = dark green; 7 = purple green; 9 = purple		a
5	Number of leaf lobes	3 = three lobes; 5 = five lobes; 7 = seven lobes; 9 = nine lobes; 11 = eleven lobes		b
6	Length of leaf lobe	Measurement of two middle leaf lobes from three middle plants		b
7	Width of leaf lobe	Measure the width of the widest part of the same lobes in SN 6 above		b
8	Ratio of lobe length to lobe width of central lobe	Calculation		b
9	Lobe margins	3 = smooth; 7 = winding		a
10	Petiole length	Measure two leaves per plant		b
11	Colour of leaf vein	3 = green; 5 = reddish-green in less than half of the lobe; 7 = reddish-green in more		a

		than half of the lobe; 9 = all red		
12	Orientation of petiole	1 = inclined upwards; 3 = horizontal; 5 = inclined downwards; 7 = irregular		a
13	Flowering	0 = absent; 1 = present		a
14	Pollen	0 = absent; 1 = present		a
15	Prominence of foliar scars	3 = semi-prominent; 5 = prominent	9 MAP	a
16	Colour of stem cortex	1 = orange; 2 = light green; 3 = dark green		a
17	Colour of stem epidermis	1 = cream; 2 = light brown; 3 = dark brown; 4 = orange		a
18	Colour of stem exterior	3 = orange; 4 = greeny-yellowish; 5 = golden; 6 = light brown; 7 = silver; 8 = grey; 9 = dark brown		a
19	Distance between leaf scars	3 = short < (8 cm); 5 = medium (8-15 cm); 7 = long > (15 cm)		b
20	Growth habit of stem	1 = straight; 2 = zig-zag		a
21	Colour of end branches of adult plant	3 = green; 5 = green-purple; 7 = purple		a
22	Extent of root peduncle	0 = sessile; 3 = pedunculate; 5 = mixed	12 MAP (at harvest)	a
23	Root shape	1 = conical; 2 = conical-cylindrical; 3 = cylindrical; 4 = irregular		a
24	External colour of storage root	1 = white or cream; 2 = yellow; 3 = light brown; 4 = dark brown		a
25	Colour of root pulp (parenchyma)	1 = white; 2 = cream; 3 = yellow; 4 = orange; 5 = pink		a
26	Colour of root cortex	1 = white or cream; 2 = yellow; 3 = pink; 4 = purple		a
27	Cortex: ease of peeling	1 = easy; 2 = difficult		a
28	Texture of root epidermis	3 = smooth; 5 = intermediate; 7 = rough		a
29	Root taste	1 = sweet; 2 = intermediate; 3 = bitter		a
30	Cortex thickness	1 = thin; 2 = intermediate; 3 = thick		a

Key: Qualitative trait = a, Quantitative trait = b

3.1.4 Data analysis

The phenotypic variation among the studied genotypes for agro-morphological traits was explored using the Multivariate analysis technique (Karim *et al*., 2020). Multivariate analysis of each of the four cluster's data matrix, comprising of principal component analysis (PCA) was processed using IBM SPSS statistics software version 25. In the PCA, Eigenvalues and load coefficient values were generated from the data set. The relevance of trait contribution to the variation accounted by each principal component, was based on the absolute Eigenvector Arbitrary Cut-off value of 0.30 (Richman, 1988). The PCA and correlation matrices were utilized to assess the relationships among the traits.

3.1.4.1 Delimitation of cassava accession clusters based on PCA

The delimitation of cassava accession clusters based on phenotypic traits was carried out by analyzing the clustering patterns, which were consequent from the agro-morphological data. This involved analyzing the Principal Components within a cluster that attributed to the similarities within the hierarchical clustering methods.

3.2 Molecular characterization of the cassava germplasm

To assess the genetic structure of cassava germplasm, molecular marker technique was applied. Start-codon-targeted (SCoT) markers based on polymorphisms in the short, highly conserved region of plant genes surrounding the ATG of the start codon were used to study the inherent diversity of the cassava germplasm by describing their polymorphisms.

A total of 40 samples of the cassava accessions were proportionately taken from four distinct clusters derived from the phenotypic characterization study (Nyamwamu *et al*., 2023). Twenty samples representing 21% were taken from cluster 1, eight samples representing 38% of the accessions from cluster 2, four samples representing 100% of accessions from cluster 3 while eight samples representing 71% of the accessions were also taken from cluster 4 (Table 3.2).

3.2.1 Planting and sample collection

The selected varieties (Table 3.2) were planted at the Rongo University farm. Each variety was planted in two rows at spacing of 1 m between plants and 1 m between rows. For each accession, at least 10 plants were planted. Two months after planting, from each accession, two newly emerged apical leaf tissues from a single stem, measuring about 6 cm were cut, packed in labelled polythene bags size 3 and then placed in a cool box for transportation to the Centre for Biotechnology and Bioinformatics (Biochemistry) Laboratory, University of Nairobi for molecular analysis.

Table 3.2: Names of cassava varieties, locations and the counties collected from

Cluster 1			
Accession No.	**Variety**	**Location collected**	**County**
114	Nyakanyamkago	Sigiria	Migori
104	Nyar-ICIPE	Sigiria	Migori
073	Busia-004	Busia	Busia
112	Agriculture-020	Ranen	Migori
113	Agriculture-021	Maram	Homa bay
105	Obaro dak-002	Nyamarere	Migori

018	Katune	Kiboko	Makueni
020	Kazanzwara	Kiboko	Makueni
025	Nyaeta	Kehancha	Migori
040	Nyagire	Maram	Homa bay
044	Unknown variety-003	Maram	Homa bay
061	Machoberi	Kegonga; Kehancha	Migori
074	Nyarkogutu-002	Ngothe	Migori
075	Nyarkadera	Kadera	Migori
087	Nyatanga-004	Rongo	Migori
088	F-19	Mtwapa	Kilifi
099	MM96/0067	Mtwapa	Kilifi
010	Agriculture-001	Ranen	Migori
006	Mbale-002	Mida Creek	Kilifi
009	Mary Kaluorore	Ranen	Migori
Cluster 2			
096	Agriculture-017	Mtwapa	Kilifi
007	Agriculture-001	Ranen	Migori
014	Kamgundho	Rapogi	Migori
016	KBK-4	Kiboko	Makueni
062	Adhiambo Lera	Awendo	Migori
089	Mtwapa-009	Mtwapa	Kilifi
098	MM96/0067	Mtwapa	Kilifi
004	Mbale-001	Miida Creek	Kilifi
Cluster 3			
015	Kasukali	Kiboko	Makueni
117	Toji	Kendu bay	Homa bay
058	Agriculture-010	Kegonga, Kehancha	Migori
068	Madam	Opapo	Migori
Cluster 4			
063	Unknown variety-003	Rakwaro	Migori
109	Selele-007	Sigiria	Migori
125	Selele-009	Ranen	Migori

024	Selele-002	Rongo	Migori
035	Selele-004	Rakwaro	Migori
036	Nyatanga-002	Uriri	Migori
039	Selele-005	Maram	Migori
064	Selele-006	Busia	Busia

3.2.2 The DNA extraction

The cassava leaf samples from the 40 samples were preserved in a freezer at -86°C. From each sample, 0.50 mg of the frozen leaves were ground using a mortar and pestle. Genomic DNA extraction protocol; cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990) was adopted. The pre-heated CTAB extraction buffer, maintained at 65°C, was added to the ground leaf material. The mixture was poured into 1.5 ml Eppendorf tubes, flicked to mix, and placed in a water bath set at 65°C for an hour. Then, the Eppendorf tubes were removed from the water bath and centrifuged at 14,000 rpm for 10 minutes at room temperature (approximately 25°C). The supernatant, or the top layer, was carefully poured into clean Eppendorf tubes (Collard and Mackill, 2009).

In a fume hood, chloroform: isoamyl alcohol, in a 24:1 ratio, was added to the tubes, then allowing the mixture to gently agitate for 5 minutes. The tubes were then centrifuged again at 14,000 rpm for 15 minutes at room temperature. After centrifugation, the upper phase was carefully transferred into new, clean Eppendorf tubes. To precipitate the DNA, 750 μL of ice-cold isopropanol was added. The tubes were then centrifuged at 13,000 rpm for 10 minutes at room temperature. Once the centrifugation was complete, the supernatant was discarded.

The pellets remaining in the tubes were washed using 500 μL of 70% ethanol, cooled to -20°C, by gently flicking the tubes. After the washing, the ethanol was decanted and any remaining liquid was carefully removed using a pipette, ensuring the pellet was not disturbed. The pellets were then dried under a vacuum for 15 minutes. Once dry, 100μL of nuclease free water (NF-H_2O) was added to dissolve the pellets followed by treatment with RNase through the addition of 0.5 mg/mL Ribonuclease A and incubated in a water bath at 37ºC for 30 min. The pellets were then removed from incubation for DNA quality and quantity check (Figure 3.3).

Plate 3.2: DNA extraction

3.2.3 DNA quality check

Using a digital scale, 0.32 mg of agarose was weighed. Thereafter, 80 ml of 1-Tris–Boric-EDTA (TBE) buffer was measured with a 100 ml graduated cylinder and added to the agarose in a 500 ml conical flask. To this mixture, 0.5 µL of ethidium bromide stain

was added. A conical flask containing the mixture was placed in a fume hood to cool for 20 minutes. Once cooled, the molten agarose solution was poured into a gel tray, into which a well comb had already been inserted. The gel was allowed to solidify at room temperature for 30 minutes before the comb was carefully removed. The gel box was then filled with 1x TBEX buffer until the gel was fully submerged.

The gel box was placed into an electrophoresis tank, which was set to run. Following this, a DNA ladder was loaded into the first well. For each DNA sample, 0.7 µL was pipetted and mixed with 0.3 µL of loading dye before being loaded into the subsequent wells. The electrophoresis tank was allowed to run for one hour. The gel was removed from the tank and placed in UV spectrophotometer (Model: NanoDrop™ 2000, Thermo Fisher Scientific, USA) for the estimation of the quality and quantity of the extracted DNA samples by resolving these DNA samples. A final DNA concentration of 50 ng/μL was prepared which was stored at -20 ^{0}C until use. DNA extraction was done from each of the 40 samples of the cassava accessions for subsequent SCoT analysis.

Plate 3.3: DNA quality check

3.2.4 Optimization of primer conditions and SCoT-PCR amplification

The SCoT-PCR analysis was carried out at the Centre for Biotechnology and Bioinformatics (Biochemistry) Laboratory, University of Nairobi according to protocols established by Collard and Mackill, (2009) with some minor adjustments besides optimization of the annealing temperature of the SCoT primers and the duration of PCR thermal cycling conditions. Twenty SCoT primers were tested for their ability to prime to DNA of cassava samples. The primers that either failed to amplify or produced faint bands were excluded from the study. Fifteen SCoT primers (Table 3.3) that produced consistent amplification and clear banding patterns were used for analysis of the genetic diversity of the forty sample cassava accessions (Appendix I).

PCR reactions were performed in 25 μL volume using 12.5 μL one Taq Quick-Load 2_ Master Mix with a standard PCR buffer (New England Biolabs, Hertfordshire, UK), 10 mM of the primer, 50 ng of template DNA, and the reaction mixtures were topped up to 25 μL with nuclease-free water. PCR amplifications were performed in a Veriti thermocycler (Bio-Rad, Singapore) with the following thermocycling conditions: initial denaturation at 94 ^{0}C for 3 min followed by 35 cycles of denaturation at 94 ^{0}C for 1 min, primer annealing at 50 ^{0}C for 1 min, and extension at 72 ^{0}C for 1.5 min (Collard and Mackill, 2009).

The amplification process was completed with a 5 min final extension at 72 ^{0}C and the PCR products were maintained at 4 ^{0}C. The PCR reaction for each SCoT primer was performed at least twice using DNA from two individual samples of the same accession. Where the PCR amplifications and banding patterns were not consistent, a third PCR

amplification was carried out (Collard and Mackill, 2009). Only clear and reproducible bands were used in the data analysis.

Table 3.3: Scot Primers used in this study

Serial number	Name	Sequences	%G/C	Annealing Temp (^0C)	Amplicon size (b/p)
1	SCoT 2	CAACAATGGCTACCACCC	56	50	200-2500
2	SCoT 6	CAACAATGGCTACCACGC	56	50	200-3000
3	SCoT 9	CAACAATGGCTACCAGCA	50	56	200-3000
4	SCoT 11	AAGCAATGGCTACCACCA	50	51	300-3000
5	SCoT 12	ACGACATGGCGACCAACG	61	52	350-3000
6	SCoT 15	ACGACATGGCGACCGCGA	67	60	100-3000
7	SCoT 16	ACCATGGCTACCACCGAC	56	52	100-3000
8	SCoT 21	ACGACATGGCGACCCACA	61	60	200-3000
9	SCoT 23	CACCATGGCTACCACCAG	61	52	1300-3000
10	SCoT 26	ACCATGGCTACCACCGTC	61	52	200-4000
11	SCoT 29	CCATGGCTACCACCGGCC	72	58	200-3000
12	SCoT 32	CCATGGCTACCACCGCAC	67	60	100-3000
13	SCoT 33	CCATGGCTACCACCGCAG	67	58	200-5000
14	SCoT 34	ACCATGGCTACCACCGCA	61	60	200-3000
15	SCoT 35	CATGGCTACCACCGGCCC	72	58	200-3000

Source: MACROGEN, Netherlands.

3.2.5 Visualization of amplified PCR products

Resolving of the PCR products was through electrophoresis on a 1.5% ethidium-bromide-stained agarose gel in 1X TBE buffer. Electrophoresis was carried out at 70 V for 60 min and PCR products were visualized using a Gel-Doc TM XR+ Imaging System (Bio-Rad, Gmbh, FeldKirchen, Germany). Resolved PCR amplified SCoT fragments were

visualized on gels and scored as binary data, for presence (1) or absence (0). Only reproducible and well-defined bands were scored. Summary of genetic parameters including total and number polymorphic and monomorphic bands were determined for each SCoT primer.

3.2.5.1 Data analysis

The molecular weights of the PCR products were estimated using a Gene Ruler 1 kb Plus DNA marker (Fischer Thermo Scientific, Waltham, MA, USA). All PCR-amplified SCoT fragments were detected on gels and scored as binary data for presence (1) or absence (0). Only reproducible and well-defined bands were scored. Polymorphic and monomorphic bands were determined for each SCoT primer. The genetic similarity, resolving power for each primer, and genetic distances based on Nei's coefficients between pairs were analyzed using Popgene software, version 3.5 (www.ualberta.ca/fyeh/popgene.pdf, accessed on 25 August 2024).

Polymorphism information content (PIC) per locus was computed using Power Marker (version 3.25). Principal coordinate analysis (PCoA) and analysis of molecular variance (AMOVA) were performed using GENALEX 6.5 software (Peakall and Smouse, 2006) (accessed on 26 August 2024). The distance matrices were generated based on Jaccard's similarity coefficient. Similarity matrices were subjected to cluster analysis through the unweighted pair group method with arithmetic mean (UPGMA) and a dendrogram was constructed using FigTree software (Version 1.4.2; accessed on 27 August 2024). The data from the 15 polymorphic SCoT markers were subjected to population structure analysis based on the admixture model clustering method in the software package STRUCTURE 2.3.4 (Pritchard *et al*., 2000). This model was run by varying the number

of assumed population (K) from 1 to 10 (K = group numbers formed according to the STRUCTURE software (Version 2.3.4; accessed on 29 August 2024). A burn-in period of 10,000 and Markov Chain Monte Carlo (MCMC) replications of 100,000 after each burn-in was used. The optimum population (K) which best estimated the structure of the 40 cassava accessions was predicted using the Evanno's method (Evanno *et al*., 2005) through the online-based software STRUCTURE HARVESTER (Version A.2) (Earl and VonHoldt, 2012) (accessed on 29 August 2024). The model was repeated for the K at maximum DK with a burn-in period of 100,000 and an MCMC of 100,000 after each burn-in with ten alterations.

3.2.5.2 Comparison of phenotypic and genotypic characterization

A comparison was made between the analysed data obtained from the phenotypic and genotypic studies. The clustering of the 40 accessions obtained from the phenotypic study was compared with their clustering in the genotypic study. Correlation matrices were derived.

3.3.1 Determination of cyanide concentration among the cassava germplasm

3.3.1.1 The plant materials

A total of 32 samples of popular cassava germplasm were taken from 4 clusters derived from earlier morphological characterization studies (Nyamwamu *et al*., 2023). Young leaves and tubers were collected from pre-planted 12-months plants. These were separately packed in labelled polythene bags size 3 and transported to scienc laboratory at Rongo University for cyanide content analysis.

3.3.1.2 Sample preparation

3.3.1.2.1 Processing of cassava leaves

The petioles were removed from the leaf samples. Exactly 30 g of each sample was weighed using analytical balance (Pioneer Model PX 5231/E (0.001G Precision, Max. 520G) manufactured by Ohaus Corporation, New Jersey, USA). Samples were ground into a paste using a mortar and pestle as described by Onukwa, (2005). The resulting paste was placed in tubes for analysis.

3.3.1.2.2 Processing of cassava peels (cortex)

The tubers were cleaned thoroughly using tap water to remove any soil or debris. Each sample was sectioned using a sharp blade into distinct parts: (a) peels and (b) pulp. Thirty grams of the peels were weighed and ground into a paste using a mortar and pestle as described by Onwuka, (2005). The resulting paste was transferred into labeled reaction tubes for analysis.

3.3.1.2.3 Processing of cassava pulps (parenchyma)

Similarly, exactly 30 g of each of the pulp samples were weighed and ground into a paste and placed in labeled reaction tubes for later analysis.

3.3.1.3 Qualitative determination of cyanide in leaves, peels and pulp using picrate paper method

Cyanide test kit Quantofix (A007858/91318/0242.5) was supplied by MACHEREY-NAGEL GmbH $ Co. KG, Germany.

Using a micropipette, 5 mL of distilled water was added to each reaction vial containing the samples in order to assist with the extraction of cyanide from the samples. One level

spoon of CN-1 buffer was added to each vial to stabilize the pH, support the release of cyanide gas and to ensure consistent reaction conditions across the samples. Five drops of CN-2 were then added to each of the reaction vials to acidify the mixtures and promote the release of HCN gas from the cyanogenic compounds from the cassava samples. Picrate paper strips were carefully placed into each reaction vial using forceps, with the strips suspended just above the sample mixture while care was taken to avoid any direct contact between the strips and the liquid. Each vial was then sealed tightly using a stopper to contain any cyanide gas released. The reaction vials were incubated in a water bath at 30–35°C for 30–60 min. The vials were then opened, and the color of the picrate papers matched against a picrate color chart (Plate 3.4). The total amount of cyanide in each sample was read in mg/L CN^- from the color chart (Plate 3.5) and recorded.

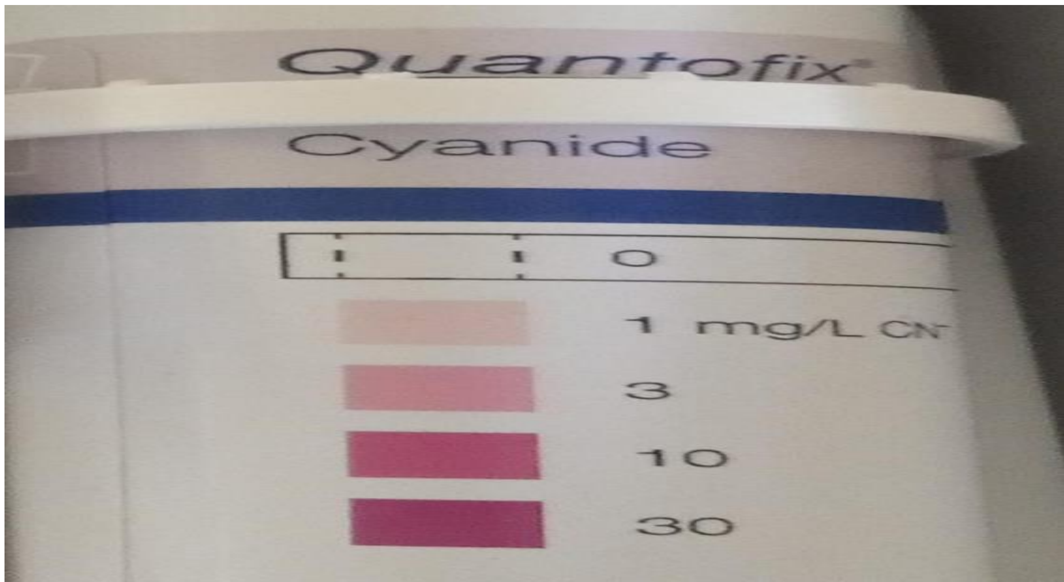


Plate 3.4: Cassava Cyanogen Kit color chart for cyanide determination

Source: *Adopted from Cyanide test kit Quantofix (A007858/91318/0242.5); MACHEREY-NAGEL GmbH $ Co. KG, Germany.*

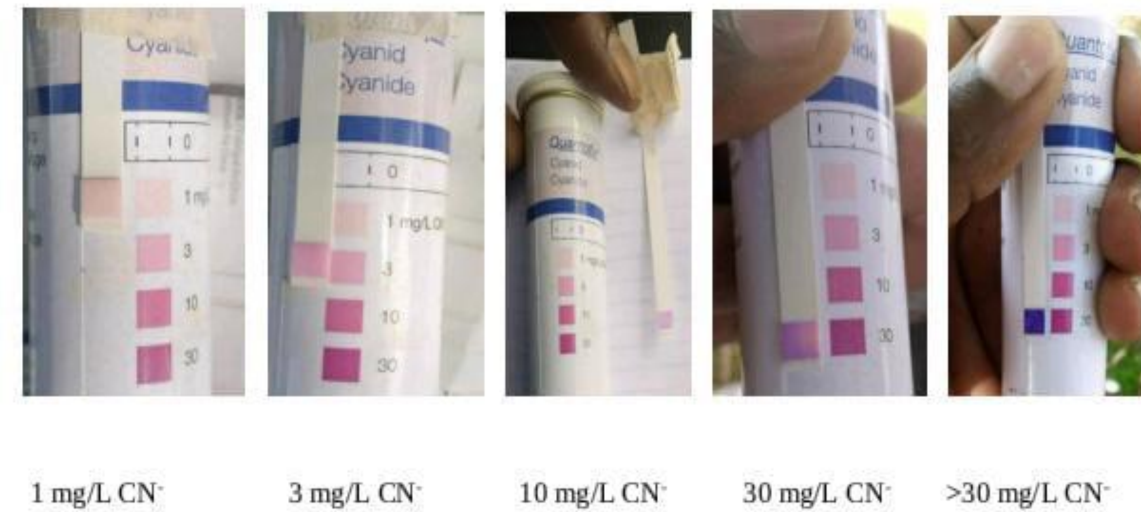


Plate 3.5: Sample color chart readings in mg/L CN-

3.3.4.4 Determination of cyanide using UV-visible Spectrophotometer

Following the qualitative assay, each picrate paper strip was carefully removed from the reaction vials using forceps and soaked in a cuvette containing 5 mL of distilled water for 3 min while gentle swirling to allow the color to transfer from the paper into the solution. The cuvettes containing the color extract solutions were then placed into the spectrophotometer and absorbance of the picrate solution read at 510 nm using UV-visible spectrophotometer (Model BK-D590; Libra S12, Biobase, China) (Bradbury *et al*., 1999).

3.4.3 Analysis of cyanide content using the spectrophotometer method

Calculation of the total cyanide content (ppm) in each of the cassava samples examined (leaves, peels and pulps), was determined by the equation (Bradbury *et al*., 1999; Rezaul *et al*., 2002):

$$Total\ cyanide\ (ppm) = absorbance\ \times 396 \quad (3)$$

where the constant 396 is a conversion factor that relates the absorbance obtained from a spectrophotometric analysis to the cyanide concentration in parts per million (ppm).

Correlation analysis was carried out to determine the strength and direction of the linear relationship between picrate paper (color chart) and spectrophotometer. The level of significant difference was determined at $p < 0.01$ using SPSS program (version 27) IBM.

3.3.1.2 Surveys to analyse cyanide concentration in cassava germplasm *in situ* in Migori County

Field surveys were carried out among cassava-growing farmers in Suna West and Kuria Sub-Counties, Migori County. These are the main cassava growing areas in the County. In each sub county, 16 cassava growing farmers were sampled. Farmers were sampled at random. The sampling process involved selecting mature cassava tubers that were ready for harvest. Each sample collected was labeled with an identifier corresponding to the name given to it by the farmer. Within the farm, one plant was uprooted. The tubers were processed as described in Section 3.3.1.2.3. The picrate paper method was used to determine the cyanide concentation as described in Section 3.3.1.3.

CHAPTER FOUR

RESULTS

4.1 Phenotypic (morphological) characterization of cassava germplasm

The study utilized 25 principal components (PC) to phenotypically characterize the 131 collected cassava accessions. These PCs included 21 qualitative traits (the Shape of central leaflet, Lobe margins, Colour of stem epidermis, Colour of stem exterior, Leaf colour, Orientation of petiole, Extent of root puduncle, Colour of root cortex, Root shape, Root taste, Cortex thickness, Colour of root pulp (parenchyma), Texture of root epidermis, Color of apical leaves, Cortex ease of peeling, Colour of end branches of adult plant, Flowering, Colour of stem cortex, Colour of leaf vein, Petiole colour, External colour of storage root and also 4 quantitative traits (number of leaf lobes, average ratio of lobe length to lobe width, average petiole length and also the distance between leaf scars (Table 4.1).

4.1.1 Principal component analysis of phenotypic characters

The eigenvalues and percentage variations of the principal component analysis are as provided in Table 4.1. Eigenvalues are the special set of scalar values that is linked with the set of linear equations most probably in the matrix equations. Eleven PCs that accounted for 71.58% of the total variation among the genotypes were identified. The first PC axis with eigenvalue of 3.27 accounted for 13.07% of the total variation whereas the second, third, fourth and the fifth PC axes with eigenvalues of 2.39, 2.04, 1.74 and 1.54 accounted for 9.55%, 8.15%, 6.97% and 6.15% of the total variation, respectively. The 6th, 7th, 8th, 9th, 10th and 11th PC axes with eigenvalues of 1.41, 1.27, 1.16, 1.11, 0.99

and 0.98 accounted for 5.64%, 5.08%, 4.65%, 4.45%, 3.95% and 3.93% of the total variation, respectively (Table 4.1).

Table 4.1: Principal component analysis, eigenvalues and percentage variations of twenty-five phenotypic traits of 131 cassava genotypes

Principal component (PC)	Characteristics	Initial Eingen values		
		Total	% of variance	Cumulative %
1	Shape of central leaflet	3.27	13.07	13.07
2	Lobe margins	2.39	9.55	22.62
3	Colour of stem epidermis	2.04	8.15	30.76
4	Colour of stem exterior	1.74	6.97	37.73
5	Leaf colour	1.54	6.15	43.88
6	Orientation of petiole	1.41	5.64	49.53
7	Extent of root puduncle	1.27	5.08	54.61
8	Colour of root cortex	1.16	4.65	59.26
9	Root shape	1.11	4.45	63.70
10	Root taste	0.99	3.95	67.65
11	Cortex thickness	0.98	3.93	71.58
12	Distance between leaf scars	0.88	3.50	75.09
13	Colour of root pulp (parenchyma)	0.82	3.27	78.36
14	Texture of root epidermis	0.77	3.08	81.43
15	Average petiole length	0.66	2.64	84.07
16	Color of apical leaves	0.62	2.49	86.57
17	Cortex ease of peeling	0.57	2.26	88.82
18	Colour of end branches of adult plant	0.51	2.02	90.84
19	Flowering	0.46	1.83	92.67
20	Colour of stem cortex	0.42	1.66	94.35

21	Number of leaf lobes	0.35	1.40	95.74
22	Colour of leaf vein	0.34	1.35	97.09
23	Petiole colour	0.30	1.20	98.29
24	External colour of storage root	0.24	0.98	99.27
25	Average ratio of lobe length to lobe width	0.18	0.73	100.00

4.1.2 Development of clusters

The dendrogram obtained using phenotypic characters separated the 131cassava genotypes into four major clusters (1, 2, 3 and 4) at similarity index of 0.5 (Figure 4.1).

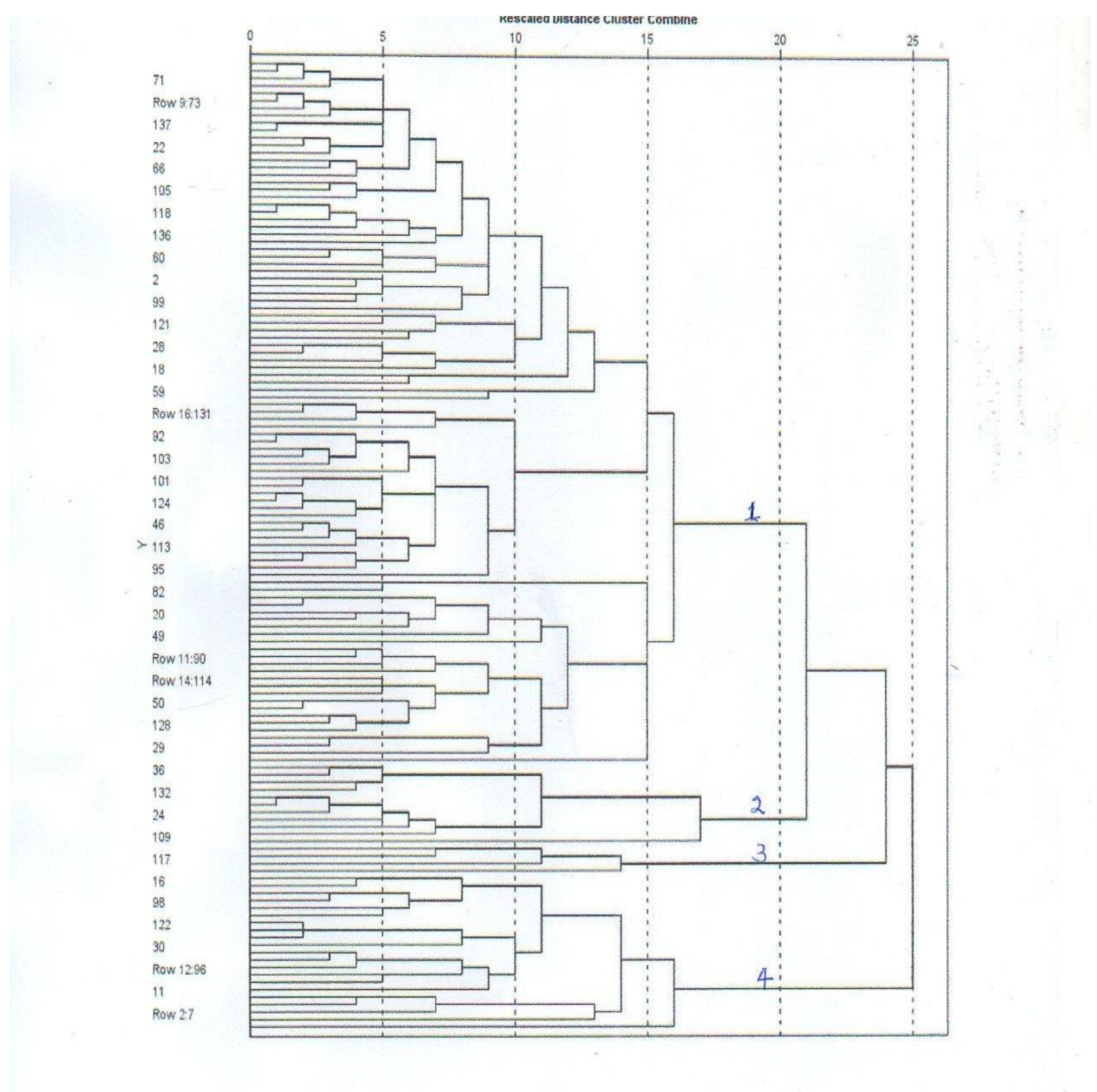


Figure 4.1: Dendrogram illustrating 131 Cassava genotypes based on average linkage (between groups) cluster analysis

The cassava cultivars analyzed revealed larger degree of morphological variations based on 25 phenotypic qualitative and quantitative descriptors used (Table 4.2). The results showed that 95 cassava genotypes accounting for 72.5% of the accessions were grouped in cluster 1. Cluster 2 had 21 accessions representing 16% of the total number of entries.

Cluster 3 and 4 had 4 genotypes (3.1%) and 11 genotypes (8.4%) respectively (Table 4.2).

Table 4.2: Names of cassava accessions, locations and the counties they were collected from

Cluster 1			
Accession No.	**Variety**	**Location collected**	**County**
114	Nyakanyamkago	Sigiria	Migori
131	MM96/0039	Chakol	Busia
104	Nyar-ICIPE	Sigiria	Migori
083	Mygera	Rongo	Migori
090	Adhiambo lera-002	Mtwapa	Kilifi
023	Nyatanga	Rabuor	Kisumu
033	Nyarkokaro	Rakwaro	Migori
053	Amakuria	Masaba- Kehancha	Migori
073	Busia-004	Busia	Busia
106	Agriculture-019	Maram	Homa bay
107	Nyatonge-002	Sigiria	Migori
108	Mygera-002	Sigiria	Migori
110	Obayo dak-003	Ranen	Migori
111	Otia	Sigiria	Migori
112	Agriculture-020	Ranen	Migori
113	Agriculture-021	Maram	Homa bay
115	Konono	Ranen	Migori
116	Agriculture-022	Pembe	Migori
118	Agriculture-23	Kitere	Migori
119	Agriculture-024	Sigiria	Migori
120	Unknown	Maram	Homa bay
121	Obaro dak-004	Nyamarere	Migori

124	Mufutu	Migori	Migori
126	Nyaodendo	Sigiria	Migori
127	NyarMaseno	Busia	Busia
128	MM96/4878	Busia	Busia
129	Yellow - 002	Chakol	Busia
130	MH95/0183	Chakol	Busia
134	Magana	Chakol	Busia
135	MH96/0031	Chakol	Busia
136	Migyera - 003	Chakol	Busia
137	Bwana Terana	Chakol	Busia
138	MH95/2480	Kolwa	Kisumu
100	Agriculture-018	Ranen	Migori
101	Bwong	Ranen	Migori
102	Obaro dak-001	Maram	Homa bay
103	Nyarkanyamkago	Ranen	Migori
105	Obar dak-002	Nyamarere	Migori
012	Agriculture-003	Rapogi	Migori
018	Katune	Kiboko	Makueni
019	KME-4	Kiboko	Makueni
020	Kazanzwara	Kiboko	Makueni
021	KBK-20	Kiboko	Makueni
022	Agriculture-004	Busia	Busia
025	Nyaeta	Kehancha	Migori
026	Wild cassava-001	Ranen	Migori
028	Kienyeji	Maram	Homa bay
029	Wild cassava -002	Rusinga island	Homa bay
034	Unknown variety	Kegonga-Kehancha	Migori
037	Buria	Maeta, Kehancha	Migori
038	NyaogutuNgalo	Maram	Homa bay
040	Nyagire	Maram	Homa bay
041	Agriculture 006	Opago	Migori

044	Unknown variety-003	Maram	Homa bay
045	Nyasega	Rakwaro	Migori
046	Nyasuna	Kokuro	Migori
047	Nyatanga-003	Opapo	Migori
048	Nyakakelo	Kanga	Migori
049	Obiero Abele	Ranen	Migori
050	Opoto	Uriri	Migori
051	Nyakasamwel	Awendo	Migori
052	Nyarkagutu	Ngothe	Migori
054	Agriculture-007	Suba; Kuria	Migori
055	Agriculture-008	Busia	Busia
056	Agriculture-009	Masaba; Kehancha	Migori
057	MM96/4466	Subukia	Nakuru
059	Busia-001	Busia	Busia
060	Busia-002	Busia	Busia
061	Machoberi	Kegonga; Kehancha	Migori
066	Agriculture-011	Kegonga; Kehancha	Migori
067	Agriculture -012	Awendo	Migori
071	Agriculture -013	Dede	Migori
074	Nyarkogutu-002	Ngothe	Migori
075	Nyarkadera	Kadera	Migori
076	Agriculrure-014	Uriri	Migori
078	Unknown variety-004	Kehancha	Migori
080	Agriculture-015	Busia	Busia
081	Agriculture-016	Rongo	Migori
082	Waite-002	Kegonga; Kehancha	Migori
085	Achuth	Uriri	Migori
086	Nyanchama	Rongo	Migori
087	Nyatanga-004	Rongo	Migori
088	F-19	Mtwapa	Kilifi
091	Katsuhanzala	Mtwapa	Kilifi

092	Kasukari	Mtwapa	Kilifi
093	Karembo	Mtwapa	Kilifi
094	Mtwapa-002	Mtwapa	Kilifi
095	Tajirika	Mtwapa	Kilifi
097	Kibanda meno-003	Mtwapa	Kilifi
099	MM96/0067	Mtwapa	Kilifi
010	Agriculture-001	Ranen	Migori
002	Mzungu	Mida Creek	Kilifi
003	Miida	Mida Creek	Kilifi
006	Mbale-002	Mida Creek	Kilifi
009	Mary Kaluorore	Ranen	Migori
Cluster 2			
123	Rateng	Pembe	Migori
096	Agriculture-017	Mtwapa	Kilifi
043	Odiero	Rongo	Migori
007	Agriculture-001	Ranen	Migori
122	Ratena	Nyamarere	Migori
013	Yellow-001	Rapogi	Migori
014	Kamgundho	Rapogi	Migori
016	KBK-4	Kiboko	Makueni
017	KBK-21	Kiboko	Makueni
030	Nyarkawuor	Uriri	Migori
031	Wild cassava-003	Kendu bay	Homa bay
032	Agriculture-005	Maeta-Kehancha	Migori
042	Unknown variety-002	Rakwaro	Migori
062	AdhiamboLera	Awendo	Migori
072	Busia-003	Busia	Busia
077	Nyakasani	Awendo	Migori
089	Mtwapa-009	Mtwapa	Kilifi
098	MM96/0067	Mtwapa	Kilifi
011	Selele rachar	Rapogi	Migori

004	Mbale-001	Miida Creek	Kilifi
008	Nyaranen	Ranen	Migori
Cluster 3			
015	Kasukali	Kiboko	Makueni
117	Toji	Kendu bay	Homa bay
058	Agriculture-010	Kegonga, Kehancha	Migori
068	Madam	Opapo	Migori
Cluster 4			
063	Unknown variety-003	Rakwaro	Migori
109	Selele-007	Sigiria	Migori
125	Selele-009	Ranen	Migori
132	Fumbachai	Chakol	Busia
024	Selele-002	Rongo	Migori
027	Selele-003	Maram	Homa bay
035	Selele-004	Rakwaro	Migori
036	Nyatanga-002	Uriri	Migori
039	Selele-005	Maram	Migori
064	Selele-006	Busia	Busia
084	Nyasuna	Masaba, Kehancha	Migori

4.1.3 Delimitation of cassava germplasm clusters based on phenotypic traits

4.1.3.1 Cassava phenotypic cluster one

Cassava accessions analysed revealed larger degrees of morphological variations based on the 25 phenotypic qualitative and quantitative descriptors used (Table 4.1). The results depicted that 95 cassava genotypes which accounted for 72.5% of the accessions, and were grouped in Cluster 1 (Table 4.2).

The eigenvalues and percentage variations of the PCA are presented in Table 4.3. Five PCA that accounted for 61.7% of the total variation, were identified among these accessions. Based on these results, the first principal component (PC1) was the colour of the root cortex, with a correlation of rotated component matrix of 0.985; accounting for 15.4% of the total variation. The second PC was cortex thickness, with a correlation of rotated component matrix of 0.994, accounting for 13.7% of the total variation, among the accessions. The third PC was orientation of petiole with a correlation of rotated component matrix of 0.987; accounting for 12.9% of the total variations. The fourth PC was root taste with correlation of rotated component matrix of 0.986; accounting for 10.2% among the genotypes. The fifth PC was leaf colour, with a correlation of rotated component matrix of 0.989 and a percentage of variance of 9.5 (Table 4.3). The data highlighted in yellow are the key PCs (PC1–PC5), which are the primary PCs explaining the variance among the cassava accessions, drawing attention to the most critical traits being analysed. The data in red prints corresponded to the correlation values of the rotated component matrix, emphasising the strength of the association between each phenotypic trait and its respective PC (Table 4.3).

Table 4.3: Principal component analysis, eigenvalues and percentage variations of eleven phenotypic traits of 95 cassava genotypes

Principal Component (PC)	Initial Eingen values			Correlation of Rotated Component Matrix				
	Total	**% of Variance**	**Cumulative %**	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
1. Colour of root cortex	1.697	15.428	15.428	0.985	-0.013	0.005	0.017	0.008
2. Cortex thickness	1.504	13.674	29.101	-0.013	0.994	0.033	-0.011	-0.002
3. Orientation of petiole	1.429	12.94	42.006	0.005	0.034	0.987	-0.027	0.018
4. Root taste	1.210	10.190	52.796	0.017	-0.011	-0.027	0.986	0.074
5. Leaf colour	1.047	9.515	61.711	0.008	-0.002	0.017	0.073	0.989
6. Shape of central leaflet	0.897	8.159	69.870	0.046	0.006	0.109	0.018	0.025
7. Lobe margins	0.845	7.684	77.554	0.123	-0.021	0.043	-0.085	0.006
8. Colour of stem epidermis	0.746	6.778	84.334	-0.070	0.093	-0.047	-0.017	0.032
9. Colour of stem exterior	0.654	5.949	90.281	0.089	0.031	-0.005	0.064	0.080
10. Extent of root puduncle	0.547	4.973	95.253	0.035	0.027	0.007	0.094	-0.082
11. Root shape	0.522	4.747	100.000	0.030	-0.045	0.092	0.047	-0.043

4.1.3.2 Cassava phenotypic cluster two

Cluster No. 2 had 21phenotypes (Table 4.2) representing 16% of the total number of entries. Four PCs that accounted for 69.2% of the total variation among the accessions, were identified (Table 4.6). Colour of stem exterior was the PCA 1, with a correlation of rotated component matrix of 0.936; accounting for 23.9% of the total variation. The shape of central leaflet, root taste and extent of root puduncle were the second, third and fourth PCs that showed correlation of rotated component matrices of 0.932, 0.975 and 0.963 respectively (Table 4.4). The data sheded in yellow indicates the key PCs (PC1–PC4), which contribute to the major variations among the cassava accessions. The correlation values of the rotated component matrix were represented by the data in red colour, which highlighted how strongly each phenotypic characteristic was associated with its corresponding PC (Table 4.4).

Table 4.4: Principal component analysis, eigenvalues and percentage variations of eleven phenotypic traits of 21 cassava genotypes

Principal Component (PC)	Initial Eingen values			Correlation of Rotated Component Matrix			
	Total	% of Variance	Cumulative %	PC1	PC2	PC3	PC4
1. Colour of stem exterior	2.394	23.940	23.940	0.936	-0.124	0.045	-0.019
2. Shape of central leaflet	1.845	18.453	42.393	-0.124	0.932	-0.129	-0.076
3. Root taste	1.499	14.989	57.383	0.043	-0.116	0.975	-0.054
4. Extent of root puduncle	1.179	11.788	69.171	-0.021	-0.064	-0.056	0.963
5. Leaf colour	0.856	8.561	77.732	-0.010	0.018	-0.090	-0.147
6. Lobe margins	0.732	7.324	85.055	-0.155	-0.098	-0.071	-0.051
7. Orientation of petiole	0.529	5.290	90.345	-0.097	0.127	0.015	-0.077
8. Colour of stem epidermis	0.384	3.835	94.180	0.311	-0.316	0.203	-0.167
9. Root shape	0.361	3.609	97.789	-0.152	-0.121	-0.063	0.169
10. Cortex thickness	0.221	2.211	100.000	-0.005	-0.021	-0.012	-0.017

4.1.3.3 Cassava phenotypic cluster three

Cluster 3 had 4 phenotypic clusters (3.1%) as shown Table 4.2. The second PC which was orientation of leaf petiole, showed a correlation of rotated component matrix of 0.968, and accounted for 38.5% of the total variation. The fourth and fifth PCs were root taste and leaf colour with each showing a correlation of rotated component matrix of -0.968; accounting for 0.0% of the total variation among the genotypes. The nineth and tenth PCs were colour of stem epidermis and cortex thickness; which showed correlation of rotated component matrices of -0.713 and -0.964, respectively; accounting for 0.0% of the total variation of the genotypes (Table 4.5). The primary PCs (PC1–PC2), which contributed to major variation among the cassava accessions, are indicated by the data in yellow. The data in red prints indicated the correlation values of the rotated component matrix, highlighting the degree to which each phenotypic trait is linked to its respective PC.

Table 4.5: Principal component analysis, eigenvalues and percentage variations of eleven phenotypic traits of 4 cassava genotypes

Principal Component (PC)	Initial Eingen values			Correlation of Rotated Component Matrix	
	Total	% of Variance	Cumulative %	PC1	PC2
1. Colour of stem exterior	6.150	61.495	61.495	0.997	0.080
2. Orientation of petiole	3.850	38.505	100.000	0.968	-0.250
3. Shape of central leaflet	0.000	0.000	100.000	0.968	-0.250
4. Root taste	0.000	0.000	100.000	-0.968	0.250
5. Leaf colour	0.000	0.000	100.000	-0.968	0.250
6. Extent of root puduncle	0.000	0.000	100.000	-0.267	0.964
7. Root shape	0.000	0.000	100.000	-0.267	0.964
8. Lobe margins	0.000	0.000	100.000	0.701	0.713
9. Colour of stem epidermis	0.000	0.000	100.000	-0.701	-0.713
10. Cortex thickness	0.000	0.000	100.000	0.267	-0.964

4.1.3.4 Cassava phenotypic cluster four

Cluster 4 had 11 phenotypic clusters (8.4%) as presented in Table 4.2. The four PCs accounted for a total variation of 79.1% among the genotypes identified (Table 4.6). The data in yellow shade represent the main PCs (PC1–PC4) that contributed to major variance among the cassava accessions. The degree to which each phenotypic characteristic was connected to its corresponding PC, is indicated by the data in red prints, which represents the correlation values of the rotated component matrix.

Table 4.6: Principal component analysis, eigenvalues and percentage variations of eleven phenotypic traits of 11 cassava genotypes

Principal Component (PC)	Initial Eingen values			Correlation of Rotated Component Matrix			
	Total	% of Variance	Cumulative %	PC1	PC2	PC3	PC4
1. Lobe margins	2.521	28.008	28.008	0.919	0.247	-0.041	-0.086
2. Cortex thickness	1.853	20.593	48.601	-0.929	-0.012	0.022	-0.194
3. Colour of stem exterior	1.575	17.499	66.099	0.180	0.920	-0.047	-0.231
4. Leaf colour	1.170	13.003	79.103	-0.133	-0.622	0.516	-0.373
5. Shape of central leaflet	0.949	10.549	89.651	-0.023	-0.080	0.978	-0.037
6. Extent of root puduncle	0.456	5.070	94.722	0.073	-0.122	-0.064	0.972
7. Orientation of petiole	0.413	4.590	99.312	0.120	-0.148	0.035	-0.102
8. Root shape	0.051	0.564	99.876	-0.001	-0.011	-0.040	0.114
9. Colour of stem epidermis	0.011	0.124	100.000	0.088	-0.096	-0.232	0.095

4.2 Molecular characterization of cassava germplasm in Kenya

4.2.1 SCoT marker analysis

Out of the 20 SCoT markers tested for their ability to amplify cassava DNA samples, 15 markers which showed polymorphic amplification fingerprint patterns were used to analyze the genetic diversity of the 40 samples of the cassava accessions. The 15 SCoT markers generated a total of 119 amplified DNA fragments with an average of 8 bands per marker (Table 4.7).

Out of the 119 amplified fragments, 107 (90%) were polymorphic. Similarly, out of the 15 SCoT markers, two markers namely SCoT12 and SCoT15 were the most polymorphic with 10 and 13 polymorphic bands respectively. The lowest numbers of polymorphic bands (3) were amplified using SCoT11 (Table 4.7). The polymorphic information content (PIC) value of all the SCoT markers ranged from 0.27 (SCoT11) to 0.37 (SCoT16, SCoT21, SCoT23 and SCoT29) with an average of 0.35 (Table 4.7). Nei's gene diversity (heterozygosity) varied from 0.28 (SCoT11) to 0.50 (SCoT16 and SCoT 21) with an average of 0.45 while Shannon information index ranged from 0.10 to 0.35 with an average value of 0.25. The highest and lowest Rp were 5.75 (for SCoT15) and 1.20 (for ScoT11), respectively (Table 4.7).

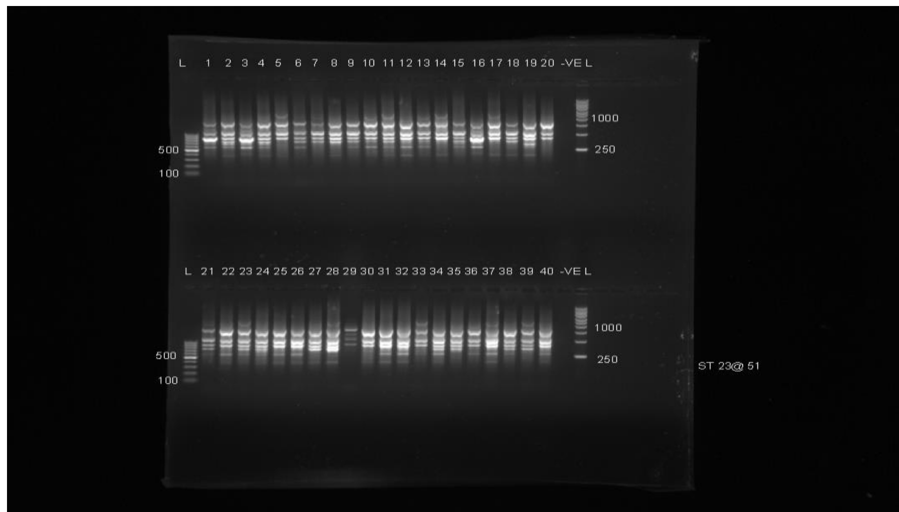


Figure 4.2: SCoT Marker 23 and 1kb ladder in Agarose Gel Electrophoresis with amplification profile

Figure 4.2 shows the results of an agarose gel electrophoresis for SCoT Marker 23 and a 1 kb ladder**,** which served as a molecular weight reference. The amplification profile was generated from cassava accessions 1-40. The 1 kb ladder was used to estimate the size of the DNA fragments by comparison to known fragment sizes. Each lane in the gel represents DNA samples from different cassava accessions, with the bands indicating successful amplification of specific DNA fragments by the SCoT markers used (Appendix I). The clarity, size, and intensity of the bands gave understandings into genetic variability among the cassava accessions founded on their DNA profiles.

Table 4.7: Total and number of polymorphic bands, gene diversity and resolving power per SCoT marker used for the analysis of the 40 cassava accessions

No.	SCoT Marker	Code of SCoT Marker	NAB	NPB	%P	PIC	H	I	Rp	D	E	MI
1	SCoT2	ST2	10	9	90	0.36	0.48	0.217	4.2	0.84	4.0	0.004
2	SCoT 6	ST6	5	5	100	0.34	0.44	0.217	2.35	0.55	3.4	0.007
3	SCoT 9	ST9	5	5	100	0.36	0.47	0.306	3.05	0.86	1.9	0.004
4	SCoT11	ST11	6	3	50	0.24	0.28	0.104	1.2	0.31	5.0	0.006
5	SCoT12	ST12	10	10	100	0.35	0.44	0.219	3.85	0.89	3.3	0.004
6	SCoT15	ST15	13	13	100	0.33	0.42	0.256	5.75	0.91	3.9	0.003
7	SCoT16	ST16	7	6	85.7	0.37	0.50	0.330	3.2	0.74	3.6	0.006
8	SCoT 21	ST21	9	9	100	0.37	0.50	0.259	5.0	0.74	4.6	0.006
9	SCoT 23	ST23	10	8	80	0.37	0.50	0.274	5.0	0.72	5.3	0.006
10	SCoT26	ST26	7	6	85.7	0.33	0.42	0.196	2.6	0.51	4.9	0.007
11	SCoT29	ST29	7	6	85.7	0.37	0.50	0.206	1.9	0.74	3.6	0.006
12	SCoT32	ST32	9	8	88.8	0.35	0.46	0.239	3.0	0.87	3.3	0.004
13	SCoT33	ST33	6	6	100	0.36	0.46	0.332	4.0	0.87	2.2	0.004
14	SCoT34	ST34	6	6	100	0.36	0.46	0.352	3.9	0.60	3.8	0.007
15	SCoT35	ST35	9	7	77.7	0.36	0.47	0.200	2.65	0.86	3.4	0.005
		Total	**119**	**107**	**89.57**			**3.705**	**51.65**			
		Mean	**7.93**	**7.13**		**0.35**	**0.45**	**0.247**	**3.44**	**0.734**	**3.75**	**0.005**

NAB=Number of amplified bands; **NPB**=Number of polymorphic bands; **%P**=Percentage polymorphism; PIC=Polymorphic information content; **H=**Nei's gene diversity (Heterozygosity)= **I** = Shannon information index**; Rp**=Resolving power; **D=**Discriminating power; **E**=effective multiple ratio; **MI**=Marker index

4.2.2 Genetic diversity and cluster analysis of the cassava accessions

The genetic diversity and relationships among the studied cassava accessions were determined by Jaccard's similarity coefficient. Based on Jaccard's (J) similarity coefficient and using the UPGMA method, the dendrogram (Figure 4.3) divided the 40 cassava samples into 2 main clusters (I and II) at a Jaccard's similarity coefficient of 0.35. The two major clusters namely I and II each was composed of 20 cassava accessions respectively. Cluster I comprised of 20 accessions that were divided into sub-clusters, sub-cluster I(a) with 19 cassava accessions and sub-cluster I(b) with only 1 accession. All the 19 cassava accessions (75, 109, 112,113, 87,98, 88,114, 113, 68, 73,63, 89, 99, 105, 96, 125, 64 and 104 in sub-cluster I(a) were obtained from Migori, Homa Bay, Busia and Kilifi counties (Nyanza/western and Coastal zones) while the 1 cassava accession (74) in sub-cluster I(b) was obtained from Migori county. Cluster 2 comprises of 20 accessions (4, 6, 40, 7, 9, 14, 15, 16, 25, 18, 20, 24, 35, 36, 44, 58, 39, 61 62 and 10) and were also obtained from Makueni, Kilifi, Homa bay and Migori counties (Central/eastern, Coastal and Nyanza/western zones) as shown in Figure 4.3.

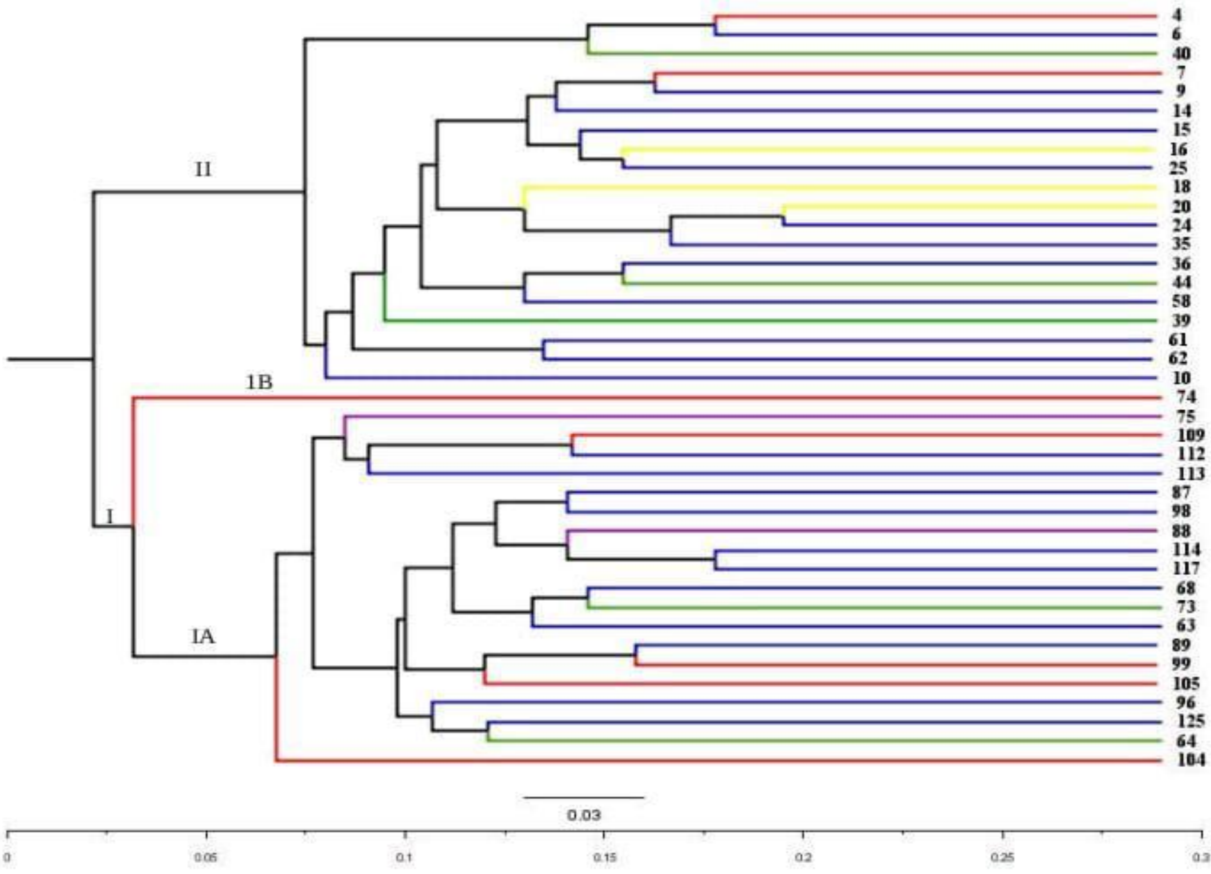
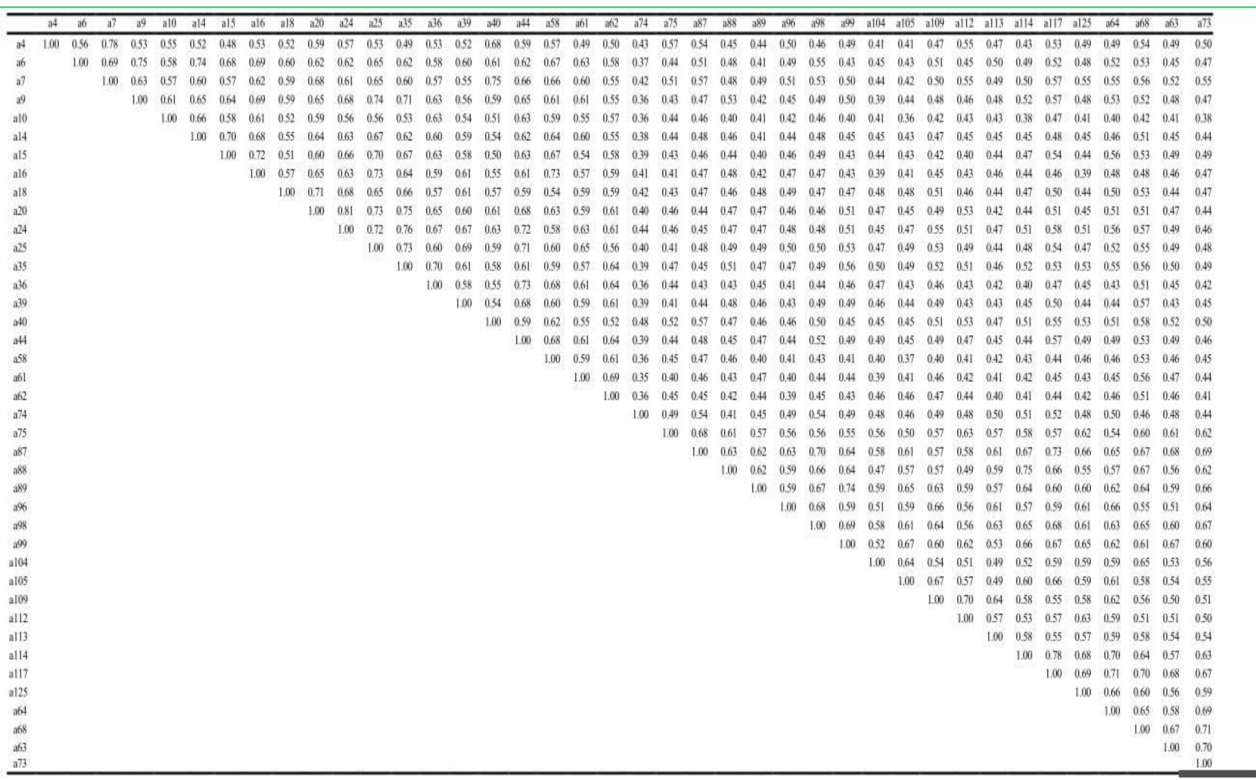


Figure 4.3: Dendrogram based on UPGMA showing the relationships among the 40 cassava accessions using SCoT marker data based on Jaccard's similarity index. Cassava accessions represented by similar colors in the dendrogram were obtained from the same county.

The similarity coefficient among the 40 accessions ranged from 0.35 to 0.78 with an average of 0.57. The highest similarity coefficient of 0.78 was recorded between accessions 4 & 7 and as well as between 114 &117 while the lowest value of 0.35 was obtained between 61 and 74 (Table 4.8).

Table 4.8: Pairwise genetic similarity among the 40 cassava accessions revealed by 15 SCoT Primers



	a4	a6	a7	a9	a10	a14	a15	a16	a18	a20	a24	a25	a35	a36	a39	a40	a44	a58
a4	1.00	0.56	0.78	0.53	0.55	0.52	0.48	0.53	0.52	0.59	0.57	0.53	0.49	0.53	0.52	0.68	0.59	0.57
a6		1.00	0.69	0.75	0.58	0.74	0.68	0.69	0.60	0.62	0.62	0.65	0.62	0.58	0.60	0.61	0.62	0.67
a7			1.00	0.63	0.57	0.60	0.57	0.62	0.59	0.68	0.61	0.65	0.60	0.57	0.55	0.75	0.66	0.66
a9				1.00	0.61	0.65	0.64	0.69	0.59	0.65	0.68	0.74	0.71	0.63	0.56	0.59	0.65	0.61
a10					1.00	0.66	0.58	0.61	0.52	0.59	0.56	0.56	0.53	0.63	0.54	0.51	0.63	0.59
a14						1.00	0.70	0.68	0.55	0.64	0.63	0.67	0.62	0.60	0.59	0.54	0.62	0.64
a15							1.00	0.72	0.51	0.60	0.66	0.70	0.67	0.63	0.58	0.50	0.63	0.67
a16								1.00	0.57	0.65	0.63	0.73	0.64	0.59	0.61	0.55	0.61	0.73
a18									1.00	0.71	0.68	0.65	0.66	0.57	0.61	0.57	0.59	0.54
a20										1.00	0.81	0.73	0.75	0.65	0.60	0.61	0.68	0.63
a24											1.00	0.72	0.76	0.67	0.67	0.63	0.72	0.58
a25												1.00	0.73	0.60	0.69	0.59	0.71	0.60
a35													1.00	0.70	0.61	0.58	0.61	0.59
a36														1.00	0.58	0.55	0.73	0.68
a39															1.00	0.54	0.68	0.60
a40																1.00	0.59	0.62
a44																	1.00	0.68
a58																		1.00

	a61	a62	a74	a75	a87	a88	a89	a96	a98	a99	a104	a105	a109	a112	a113	a114	a117	a125	a64	a68	a63	a73
a4	0.49	0.50	0.43	0.57	0.54	0.45	0.44	0.50	0.46	0.49	0.41	0.41	0.47	0.55	0.47	0.43	0.53	0.49	0.49	0.54	0.49	0.50
a6	0.63	0.58	0.37	0.44	0.51	0.48	0.41	0.49	0.55	0.43	0.45	0.43	0.51	0.45	0.50	0.49	0.52	0.48	0.52	0.53	0.45	0.47
a7	0.60	0.55	0.42	0.51	0.57	0.48	0.49	0.51	0.53	0.50	0.44	0.42	0.50	0.55	0.49	0.50	0.57	0.55	0.55	0.56	0.52	0.55
a9	0.61	0.55	0.36	0.43	0.47	0.53	0.42	0.45	0.49	0.50	0.39	0.44	0.48	0.46	0.48	0.52	0.57	0.48	0.53	0.52	0.48	0.47
a10	0.55	0.57	0.36	0.44	0.46	0.40	0.41	0.42	0.46	0.40	0.41	0.36	0.42	0.43	0.43	0.38	0.47	0.41	0.40	0.42	0.41	0.38
a14	0.60	0.55	0.38	0.44	0.48	0.46	0.41	0.44	0.48	0.45	0.45	0.43	0.47	0.45	0.45	0.45	0.48	0.45	0.46	0.51	0.45	0.44
a15	0.54	0.58	0.39	0.43	0.46	0.44	0.40	0.46	0.49	0.43	0.44	0.43	0.42	0.40	0.44	0.47	0.54	0.44	0.56	0.53	0.49	0.49
a16	0.57	0.59	0.41	0.41	0.47	0.48	0.42	0.47	0.47	0.43	0.39	0.41	0.45	0.43	0.46	0.44	0.46	0.39	0.48	0.48	0.46	0.47
a18	0.59	0.59	0.42	0.43	0.47	0.46	0.48	0.49	0.47	0.47	0.48	0.48	0.51	0.46	0.44	0.47	0.50	0.44	0.50	0.53	0.44	0.47
a20	0.59	0.61	0.40	0.46	0.44	0.47	0.47	0.46	0.46	0.51	0.47	0.45	0.49	0.53	0.42	0.44	0.51	0.45	0.51	0.51	0.47	0.44
a24	0.63	0.61	0.44	0.46	0.45	0.47	0.47	0.48	0.48	0.51	0.45	0.47	0.55	0.51	0.47	0.51	0.58	0.51	0.56	0.57	0.49	0.46
a25	0.65	0.56	0.40	0.41	0.48	0.49	0.49	0.50	0.50	0.53	0.47	0.49	0.53	0.49	0.44	0.48	0.54	0.47	0.52	0.55	0.49	0.48
a35	0.57	0.64	0.39	0.47	0.45	0.51	0.47	0.47	0.49	0.56	0.50	0.49	0.52	0.51	0.46	0.52	0.53	0.53	0.55	0.56	0.50	0.49
a36	0.61	0.64	0.36	0.44	0.43	0.43	0.45	0.41	0.44	0.46	0.47	0.43	0.46	0.43	0.42	0.40	0.47	0.45	0.43	0.51	0.45	0.42
a39	0.59	0.61	0.39	0.41	0.44	0.48	0.46	0.43	0.49	0.49	0.46	0.44	0.49	0.43	0.43	0.45	0.50	0.44	0.44	0.57	0.43	0.45
a40	0.55	0.52	0.48	0.52	0.57	0.47	0.46	0.46	0.50	0.45	0.45	0.45	0.51	0.53	0.47	0.51	0.55	0.53	0.51	0.58	0.52	0.50
a44	0.61	0.64	0.39	0.44	0.48	0.45	0.47	0.44	0.52	0.49	0.49	0.45	0.49	0.47	0.45	0.44	0.57	0.49	0.49	0.53	0.49	0.46
a58	0.59	0.61	0.36	0.45	0.47	0.46	0.40	0.41	0.43	0.41	0.40	0.37	0.40	0.41	0.42	0.43	0.44	0.46	0.46	0.53	0.46	0.45
a61	1.00	0.69	0.35	0.40	0.46	0.43	0.47	0.40	0.44	0.44	0.39	0.41	0.46	0.42	0.41	0.42	0.45	0.43	0.45	0.56	0.47	0.44
a62		1.00	0.36	0.45	0.45	0.42	0.44	0.39	0.45	0.43	0.46	0.46	0.47	0.44	0.40	0.41	0.44	0.42	0.46	0.51	0.46	0.41
a74			1.00	0.49	0.54	0.41	0.45	0.49	0.54	0.49	0.48	0.46	0.49	0.48	0.50	0.51	0.52	0.48	0.50	0.46	0.48	0.44
a75				1.00	0.68	0.61	0.57	0.56	0.56	0.55	0.56	0.50	0.57	0.63	0.57	0.58	0.57	0.62	0.54	0.60	0.61	0.62
a87					1.00	0.63	0.62	0.63	0.70	0.64	0.58	0.61	0.57	0.58	0.61	0.67	0.73	0.66	0.65	0.67	0.68	0.69
a88						1.00	0.62	0.59	0.66	0.64	0.47	0.57	0.57	0.49	0.59	0.75	0.66	0.55	0.57	0.67	0.56	0.62
a89							1.00	0.59	0.67	0.74	0.59	0.65	0.63	0.59	0.57	0.64	0.60	0.60	0.62	0.64	0.59	0.66
a96								1.00	0.68	0.59	0.51	0.59	0.66	0.56	0.61	0.57	0.59	0.61	0.66	0.55	0.51	0.64
a98									1.00	0.69	0.58	0.61	0.64	0.56	0.63	0.65	0.68	0.61	0.63	0.65	0.60	0.67
a99										1.00	0.52	0.67	0.60	0.62	0.53	0.66	0.67	0.65	0.62	0.61	0.67	0.60
a104											1.00	0.64	0.54	0.51	0.49	0.52	0.59	0.59	0.59	0.65	0.53	0.56
a105												1.00	0.67	0.57	0.49	0.60	0.66	0.59	0.61	0.58	0.54	0.55
a109													1.00	0.70	0.64	0.58	0.55	0.58	0.62	0.56	0.50	0.51
a112														1.00	0.57	0.53	0.57	0.63	0.59	0.51	0.51	0.50
a113															1.00	0.58	0.55	0.57	0.59	0.58	0.54	0.54
a114																1.00	0.78	0.68	0.70	0.64	0.57	0.63
a117																	1.00	0.69	0.71	0.70	0.68	0.67
a125																		1.00	0.66	0.60	0.56	0.59
a64																			1.00	0.65	0.58	0.69
a68																				1.00	0.67	0.71
a63																					1.00	0.70
a73																						1.00

4.2.3 Nei's Gene Diversity Index (NGDI) and Shannon Diversity Index (SDI)

Nei's gene diversity index and Shannon diversity index were calculated and used to evaluate the genetic diversity of the cassava accessions under study. The estimates of the genetic diversity in each population were summarized as presented in Table 4.9. Nei's gene diversity index of the cassava accessions from the five counties ranged from 1.143 to 1.478 and Shannon's diversity index ranged from 0.099 to 0.411. Within the five counties from where the cassava accessions were sampled, cassava from Kilifi County exhibited the highest level of variability (NDGI= 1.478) whereas accessions from Homa

bay exhibited the lowest level of variability (NGDI= 1.143 and SDI=0.213) as shown in Table 4.9.

Table 4.9: Nei's gene Diversity Index (NGDI) and Shannon Diversity Index (SDI) of the 40 sampled cassava accessions

Place (County) of Cassava Collection	NGDI	SDI
Busia	1.340	0.099
Kilifi	1.478	0.288
Migori	1.269	0.411
Makueni	1.246	0.214
Homa bay	1.143	0.213

4.2.4 Population structure analysis

The maximum peak value of DK (500) was observed at K = 2 (Figure 4.4). Using STRUCTURE software, the Bayesian-model-based clustering analysis grouped the 40 cassava accessions into two distinct genetic groups at K = 2, designated as A and B (Figure 4.5). However, at K = 3, some cassava accessions occurred as an admixture group (Figure 4.4).

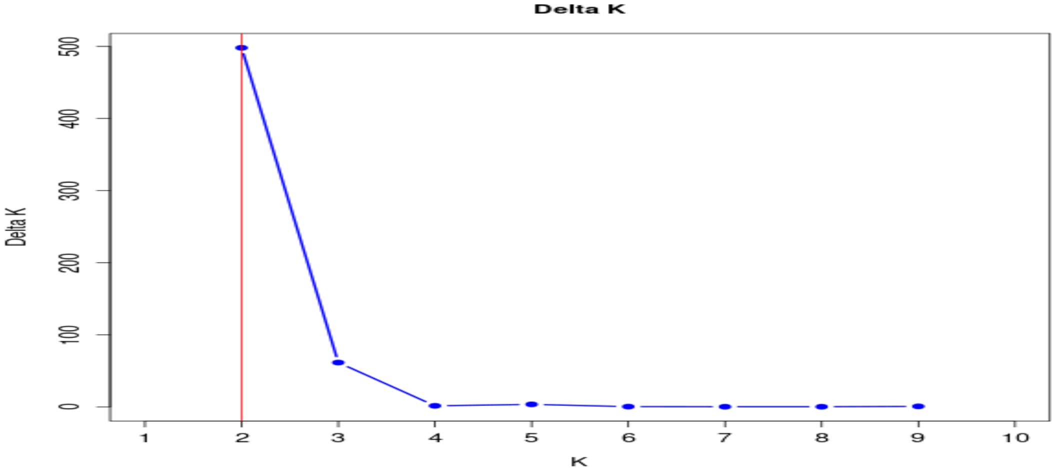


Figure 4.4: The estimated membership fraction using LnP(D)-derived delta K (DK) with cluster (K) ranged from 1 to 10 for K = 2.

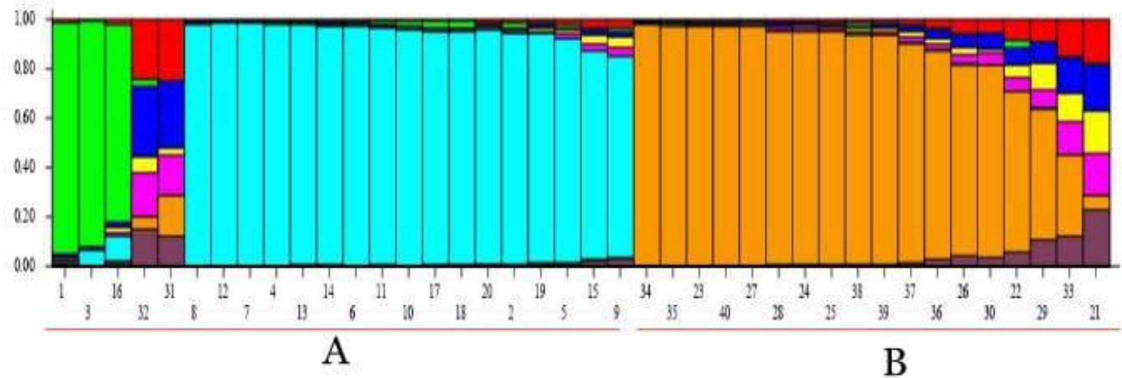


Figure 4.5: Population structure of 40 cassava accessions inferred STRUCTURE analysis based on SCoT marker data. Each color represents a single genetic group, namely A and B. Each solid bar represents a single accession, while each color represents a genetic group.

4.2.5 Analysis of Molecular Variance (AMOVA)

Analysis of molecular variance (AMOVA) was used to evaluate the population differentiation among and within the cassava accessions. The SCoT markers' data

revealed 16% of the genetic variation among the population and 84% of the variation within the population (Table 4.10).

Table 4.10: Analysis of Molecular Variance (AMOVA) based on SCoT markers for the 40 cassava accessions

Source of Variance	Df	SSD	MSD	Variance component	Percentage of Variation	P-Value
Among Population	4	132.1	33.0	3.0	16	0.002
Within Population	35	540.9	15.5	15.5	84	
Total	39	673.0		18.4	100	

*Significant at p≤0.002.

4.2.5 Principal Coordinates Analysis (PCoA)

In order to ascertain the genetic relationships between and within the populations and also assess the consistency of differentiation between the populations using cluster analysis, the principal coordinate analysis was carried out. The 40 cassava accessions were classified similarly by the PCoA analysis and the cluster analysis (Figure 4.6). The percentage of the total variation explained by the first three dimensions of the PCoA axis was 35.69% (first axis = 22.99%, second axis = 6.54%, and third axis = 6.16%). Accessions from Busia, Homa bay, Makueni, and Migori counties (Western, Nyanza and eastern zones) were clustered together (Figure 4.6).

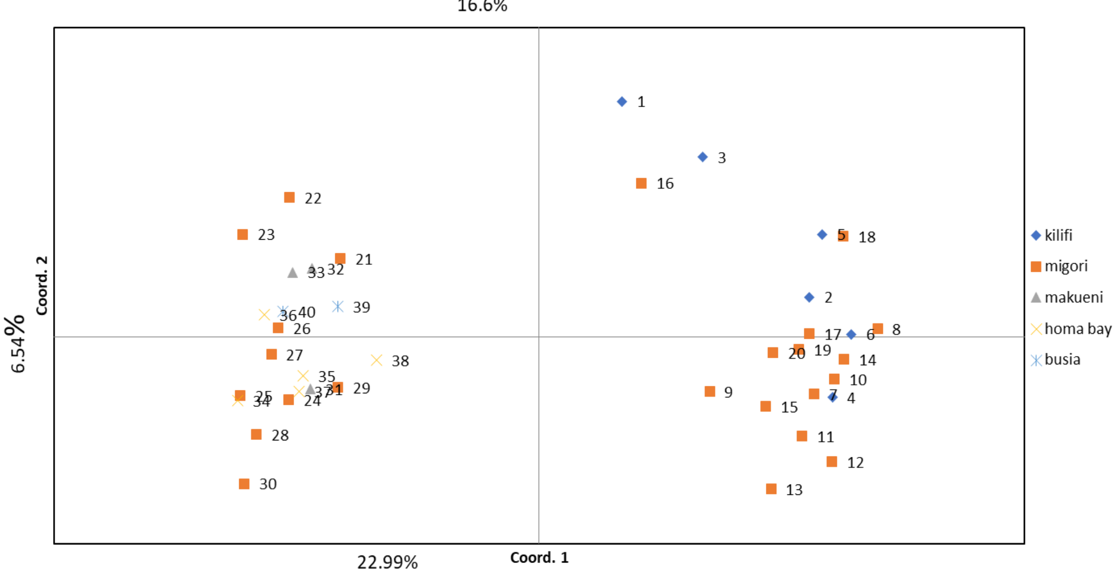


Figure 4.6: Bi-plots derived from principal coordinate analysis of 40 cassava accessions using SCoT data. The numbers plotted represents individual cassava samples. The colors represent county/site of collection of the accessions: red= Migori, Blue= Kilifi, Green=Makueni, Light blue= Busia, Purple= Homa bay.

4.3 Comparative analysis of phenotypic and molecular characterization of cassava (*Manihot esculenta* Crantz) germplasm in Kenya

Molecular characterization separated the 40 cassava accessions into two distinct clusters (Table 4.11). Cluster 1 had 20 acceassions while cluster 2 had 20 accessions. In each of these two clusters, the varieties were taken from various counties and locations. Phenotypic characterization grouped the 40 accessions into four clusters (Table 4.11). Cluster 1 had 20 accessions, cluster 2 had 8 accessions while cluster 3 had 4 accessions. The last cluster 4 had 8 accessions (Table 4.11). Correlation between genotypic and phenotypic characterization was 0.04225771.

Table 4.11: Derived clusters of the 40 cassava accessions based on phenotypic (agro-morphological) and genotypic (molecular) characterization

Accession no.	Variety	Location collected	County	Genotype cluster no.	Phenotype cluster no.
104	Nyar-ICIPE	Sigiria	Migori	1	1
064	Selele-006	Busia	Busia	1	4
125	Selele-009	Ranen	Migori	1	4
096	Agriculture-017	Mtwapa	Kilifi	1	2
105	Obar dak-002	Nyamarere	Migori	1	1
099	MM96/0067	Mtwapa	Kilifi	1	1
089	Mtwapa-009	Mtwapa	Kilifi	1	2
063	Unknown variety-003	Rakwaro	Migori	1	4
073	Busia-004	Busia	Busia	1	1
068	Madam	Opapo	Migori	1	3
117	Toji	Kendu bay	Homa bay	1	3
114	Nyakanyamkago	Sigiria	Migori	1	1
088	F-19	Mtwapa	Kilifi	1	1
098	MM96/0067	Mtwapa	Kilifi	1	2
087	Nyatanga-004	Rongo	Migori	1	1
113	Agriculture-021	Maram	Homa bay	1	1
112	Agriculture-020	Ranen	Migori	1	1
109	Selele-007	Sigiria	Migori	1	4
075	Nyarkadera	Kadera	Migori	1	1
074	Nyarkogutu-002	Ngothe	Migori	1	1
010	Agriculture-001	Ranen	Migori	2	1
062	AdhiamboLera	Awendo	Migori	2	2
061	Machoberi	Kegonga; Kehancha	Migori	2	1

058	Agriculture-010	Kegonga, Kehancha	Migori	2	3
044	Unknown variety-003	Maram	Homa bay	2	1
036	Nyatanga-002	Uriri	Migori	2	4
035	Selele-004	Rakwaro	Migori	2	4
024	Selele-002	Rongo	Migori	2	4
020	Kazanzwara	Kiboko	Makueni	2	1
018	Katune	Kiboko	Makueni	2	1
025	Nyaeta	Kehancha	Migori	2	1
016	KBK-4	Kiboko	Makueni	2	2
015	Kasukali	Kiboko	Makueni	2	3
014	Kamgundho	Rapogi	Migori	2	2
009	Mary Kaluorore	Ranen	Migori	2	1
007	Agriculture-001	Ranen	Migori	2	2
040	Nyagire	Maram	Homa bay	2	1
006	Mbale-002	Mida Creek	Kilifi	2	1
004	Mbale-001	Miida Creek	Kilifi	2	2
039	Selele-005	Maram	Migori	2	4

4.4 Cyanide concentration levels among cassava (*Manihot esculenta* Crantz) germplasm in Kenya

4.4.1 Spectrophotometer analysis

The results of analyses of cyanide levels among leaves, peels and pulps of various cassava accessions as determined by the two methods is illustrated in Table 4.12. The average cyanide levels observed in leaves, peels and pulp was 5.89 mg/LCN$^-$, 7.42

mg/LCN$^-$ and 8.20 mg/LCN$^-$respectively. The highest cyanide concentration in the leaves was observed in the varieties Nyarkadera (Accession 075) collected from Migori County (26.93 mg/LCN$^-$) and Karembo (Accession 093) collected from Kilifi County (24.55 mg/LCN$^-$) while the lowest cyanide content in the leaves was observed in the varieties Kasukali (Accession 015), Nyakanyamkago (Accession 114) and Nyatanga-02 (Accession 036) all of which had 0.40 mg/LCN$^-$ (Table 4.12). The highest cyanide concentration in the peels was observed in the varieties; Kazanzwara collected from Makueni County (17.82 mg/LCN$^-$) and Wild cassava - 02 collected from Homa bay County (16.63 mg/LCN$^-$) while the lowest cyanide content in the peels was observed in the varieties Kasukali and Karembo which had 1.19 mg/LCN$^-$ and 1.58 mg/LCN$^-$ respectively (Table 4.12). The spectrophotometer analysis for the pulp indicated that the highest cyanide content was in the varieties Nyatanga (26.93 mg/LCN$^-$) and NyaRanen (26.53 mg/LCN$^-$). Similarly, the lowest cyanide content in the pulp was observed in the varieties Mzungu (0.40 mg/LCN$^-$) and Kasukali (0.79 mg/LCN$^-$) (Table 4.12).

4.4.2 Picrate paper test method

Results presented in Table 4.16 showed that the average cyanide content in the leaves, peels and pulp were 3.13 mg/LCN$^-$, 5.44 mg/LCN$^-$ and 7.97 mg/LCN$^-$ respectively.

Cyanide in leaves: Several varieties including Nyarkadera (Accession 075), Karembo (Acc. 093), Nyakanyamkago (Acc. 114) Fumbachai (Acc. 132) all showed the highest level of cyanide concentration of 10 mg/LCN$^-$. Results presented in Table 4.12 indicated that 12 varieties had low score of 1 mg/LCN$^-$ in the leaves.

Cyanide in peels: The highest score for cyanide content in the peels was 10 mg/LCN^-. A total of 14 varieties were recorded with this score. However, 10 varieties had a score of 1 mg/LCN^- while 8 varieties had a score of 3 mg/LCN^- (Table 4.12).

Cyanide in pulp: The pulp showed high cyanide levels of up to 30 mg/L CN^-. In this category there were 5 varieties which included Adhiambo lera (Acc. 062), Nyatanga (Acc. 099), Obaro dak - 001 (Acc. 102), Nyaeta (Acc. 025) and Kasukali (Acc. 015). In the same analysis, 9 varieties had a score of 1 mg/L CN^- (Table 4.12).

Table 4.12: Cyanide determination in cassava leaves, peels and pulps

Accession No.	Accession	County collected	Spectrophotometric analysis (Aggregate cyanide (mg/L CN^-) = Absorbance x 396)			Picrate method (Color chart reading) (mg/L CN^-)			
			Leaves	Peels	Pulp	Leaves	Peels	Pulp	Pulp
075	Nyarkadera	Migori	26.93	3.56	2.38	10	10	3	3
093	Karembo	Kilifi	24.55	1.58	9.11	10	1	10	10
061	Machoberi	Migori	13.07	6.34	3.56	3	1	3	3
074	Nyarkogotu-002	Homa Bay	9.90	11.48	5.15	1	3	10	10
068	Madam	Migori	8.32	2.34	10.30	1	10	3	3
090	Adhiambo lera	Kilifi	7.92	2.77	3.17	3	3	1	1
020	Kazanzwara	Makueni	7.92	17.82	5.54	3	10	3	3
018	Katune	Makueni	7.92	13.86	14.65	3	3	3	3
062	Adhiambo lera	Migori	7.92	14.26	7.13	3	10	30	30
099	Nyatanga	Migori	6.34	10.30	26.93	3	10	30	30
029	Wild cassava-002	Homa bay	5.94	16.63	5.94	3	10	1	1
002	Mzungu	Kilifi	5.94	1.98	0.40	3	1	3	3
127	NyarMaseno	Busia	5.94	9.90	6.34	3	10	10	10
004	Mbale-001	Kilifi	5.54	9.50	3.96	3	3	1	1
089	Mtwapa-009	Kilifi	5.15	3.56	4.76	3	1	3	3

035	Selele-004	Migori	3.96	13.86	6.34	3	1	1	1
088	F 119	Kilifi	3.96	4.75	2.36	1	10	3	3
026	Wild cassava -001	Migori	3.96	11.88	5.54	3	10	1	1
067	Agriculture-012	Migori	3.56	3.56	1.98	3	3	1	1
102	Obaro dak-001	Homa bay	3.56	8.71	2.77	3	1	30	30
008	NyaRanen	Migori	3.56	4.36	26.53	3	10	10	10
014	Kamgundho	Migori	3.17	4.75	2.38	1	3	10	10
084	Nyasuna	Migori	3.17	5.54	9.50	1	1	1	1
132	Fumbachai	Busia	2.77	3.17	11.09	10	1	1	1
122	Ratena	Migori	1.98	2.77	20.20	1	1	3	3
025	Nyaeta	Migori	1.58	3.56	4.75	1	10	30	30
060	Busia-002	Busia	1.19	10.30	17.42	1	10	3	3
091	Kastuhanzala	Kilifi	0.79	9.11	18.22	1	10	1	1
024	Selele-002	Migori	0.79	15.04	5.94	1	10	3	3
015	Kasukali	Makueni	0.40	1.19	0.79	1	1	30	30
114	Nyakanyamkago	Migori	0.40	5.94	13.07	10	3	10	10
036	Nyatanga-02	Migori	0.40	3.17	4.36	1	3	3	3
Average			**5.89**	**7.42**	**8.20**	**3.13**	**5.44**	**8.00**	**7.97**

1 mg/kg OR 1 mg/L = 1 ppm. NOTE: WHO, (2016) guidelines on cyanide cponent; 0-10 mg/L= low content; >10 mg/L=High content

4.4.3 Correlation analysis between findings from picrate paper method (color chart readings) and cyanide content from spectrophotometer analysis

4.4.3.1 Correlation analysis in leaves

Correlation analysis was carried out on the two methods used for cyanide analysis. The results presented in Table 4.13 indicated that the correlation coefficient on the two methods for cyanide analysis on the leaves was 0.548** (p=0.001) while the correlation between the two methods on the cyanide content in peels was 0.570** (p=0.001) (Table 4.14). The highest correlation among these two methods was observed in the cyanide determination in the pulp which showed a correlation of 0.936. Correlation analysis generated a correlation coefficient of 0.548 (Table 4.13).

Table 4.13: Correlation analysis on leaves

		Picrate paper method	Spectrophotometer method
Picrate paper method	Pearson Correlation	1	.548**
	Sig. (2-tailed)		.001
	N	32	32
Spectrophotometer method	Pearson Correlation	.548**	1
	Sig. (2-tailed)	.001	
	N	32	32

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

4.4.3.2 Correlation analysis in peels

Correlation analysis generated a correlation coefficient of 0.570 (Table 4.14).

Table 4.14: Correlation analysis on peels

		Picrate paper method	Spectrophotometer method
Picrate paper method	Pearson Correlation	1	.570**
	Sig. (2-tailed)		.001
	N	32	32
Spectrophotometer method	Pearson Correlation	.570**	1
	Sig. (2-tailed)	.001	
	N	32	32

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

4.4.3.3 Correlation analysis in pulps

Correlation analysis was computed and generated a correlation coefficient of 0.936 (Table 4.15).

Table 4.15: Correlation analysis on pulps

		Picrate paper method	Spectrophotometer method
Picrate paper method	Pearson Correlation	1	.936**
	Sig. (2-tailed)		.000
	N	32	32
Spectrophotometer method	Pearson Correlation	.936**	1
	Sig. (2-tailed)	.000	
	N	32	32

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

4.4.4 Cyanide concentration levels in cassava sample accessions collected from farmers in Migori County, Kenya

Results presented in Table 4.16 indicated that 13 Accessions (206-230) exhibited the highest detectable level of 30 mg/L CN^-. Two Accessions (225 and 227) showed a moderate concentration of 10 mg/L CN^-. Eleven accessions had the lowest recorded concentration of 1 mg/L CN^- (Table 4.16).

Table 4.16: Cyanide concentration levels in cassava samples collected from farmers in Migori county, Kenya

Accession No.	Accession name	Color chart readings (concentration in mg/LCN^-)
		Leaves/peels
206	Waite Nyaabasi-002	30
207	Waite Nyabasi-003 (peels)	30
208	Waite Nyabasi-004	30
209	Waite Nyabasi-005 (peels)	30
210	Sudhe-001	30
211	Sudhe-002 (peels)	30
212	Machicha-001	30
213	Machicha-002 (peels)	30
214	Dar- saalam-001	30
215	Dar- saalam-002 (peels)	30
228	Agriculture-001	30
229	Nyakade-001	30
230	Nyakade-002 (peels)	30
225	Bondeni-002	10
227	Bondeni-004	10

200	Nyasakwa	3
201	IFAD	3
205	Waite Maeta-001	3
217	Rateng yellow-002 (peels)	3
226	Bondeni-003	3
231	Grade-001	3
203	Nguruna-001	1
204	Nguruna-002	1
216	Rateng yellow-001	1
218	Rateng black	1
219	Roteng	1
220	Rabuor	1
221	Pith Nyadundo-001	1
222	Pith Nyadundo-002	1
223	Sagero	1
224	Bondeni-001	1
232	Kanyamwa	1

1 mg/kg OR 1 mg/L = 1 ppm.

NOTE: WHO, (2016) guidelines on cyanide cponent; 0-10 mg/L= low content; >10 mg/L=High content

CHAPTER FIVE

DISCUSSION

5.1 Phenotypic characterization of cassava (*Manihot esculenta* Crantz) germplasm in Kenya

Numerical taxonomic studies are important for discovering and documenting new character and character states (Rahman, 2013). Cluster analysis (CA) and principal component analysis (PCA) are two techniques commonly used in numerical classification (Sonibare *et al*. 2004). The PCA is usually used as an exploratory tool in systematic. There are as many components as original variables, and these components are linear combinations of the original variables. Most of the variance is usually summarized by the first few components and PCA thus reduces a larger number of variables to fewer variables, which are often easier to interpret and is thus described as a dimension reducing method (Rahman, 2013). Cluster analysis (CA) is an exploratory tool for classifying objects with no statistical assumptions about the data. Cluster analysis produces a hierarchical classification of entities (taxa) based on the similarity matrix. Results are usually presented in the form of trees or dendrograms (Henderson, 2006).

In this study, eleven principal components accounting for 71.58% of the total variation among the genotypes were identified. Similar studies have been carried out in other regions. Studies carried out in Sierra Leone (Karim *et al.* 2020) identified a total of seven principal components (PCs) in the qualitative and four PCs in the quantitative trait sets accounted for 79.03% and 72.30% of the total genetic variation in 102 cassava genotypes, respectively. In the same study five clusters were obtained based on qualitative agronomic traits. The estimation of descriptive statistics of 25 different morphological

traits studied in the present study revealed the existence of morphological diversity among cassava landraces. From the small sample of 131 genotypes, it was possible to characterize them into four clusters with a majority (72.5%) falling into one cluster. This signified that a large population of cassava grown in Kenya have similar characteristics as only 27.5% are characterized in the other three clusters. This makes it difficult for farmers to select the distinctive planting materials. These findings concur with those found in Brazil whereby among the 45 cassava varieties studied, they presented 97.35% polymorphism, which established morphological divergence amongst the evaluated samples (Tiago *et al*., 2020).

The three wild cassava accessions included in this study were characterized in clusters where cultivated cassava was identified. These results differed with the findings of Dissanayake *et al*. (2019) in Sri Lanka who carried out morphological assessment of cassava cultivars and established that the leaf morphology of wild-accessions and landrace cultivars were significantly different from the rest of the cultivars. Stem morphology among the cultivars was significantly different mainly by the mean inter-nodal length of the stems whereas wild-accession cultivars were significantly different from the rest by the diameter of the stems.

In this study, it was expected that the wild cassava germplasm would be clustered in a distinct group. In Indonesia, details of 14 morphological characteristics for 29 cassava genotypes were utilized in cassava landrace characterization. It was revealed that majority of the genotypes had purple petiole color and horizontal orientation, smooth lobe margin, and seven lobes (Ridwan *et al*., 2022). In Burundi, Niyonzima *et al*., (2021)

assessed landrace cassava morphological traits and noted that stem, root and leaf traits distribution differed among cassava cultivars.

The TMS cassava varieties released by KALRO and named *Agriculture* by farmers were coded Agriculture – 001, Agriculture 021. The genotypes appeared in clusters 1, 2 and 3; signifying that the varieties that were in different clusters were different varieties. This is a first step in the identification of these genotypes. There were also varieties whose names the farmers did not know (*Unknown variety*). This is a common occurrence especially for farmers who are planting cassava for the first time or who are recently introduced to cassava farming. For them, the name of the variety is not important. This observation also applied to the genotypes collected from Mtwapa (Kilifi County). It was expected that this study would shed light on their identities. Further genetic studies need to be carried out to correlate the phenotypic and genotypic traits for proper characterization.

5.1.1 Delimitation of cassava germplasm clusters in Kenya based on phenotypic traits

5.1.1.1 Cassava phenotypic cluster one

The analysis of cassava accessions revealed substantial morphological variation. The use of 25 phenotypic qualitative and quantitative descriptors allowed for the identification of key traits that contributed to this variation, which were additionally assessed using Principal Component Analysis (PCA). The PC1, which focused on the colour of the root cortex, was the most influential, accounting for 15.4% of the variation. This aligns with

related studies highlighting the value of root characteristics, such as colour as significant phenotypic traits in cassava characterisation (Nweke *et al*., 2016; Enesi *et al*., 2022).

In addition to root colour, PC2, which described cortex thickness, accounted for 13.7% of the variation and was found to be strongly correlated (0.994) with the phenotypic trait. This finding concurs with research by Adejumo *et al*., (2019), who also identified cortex thickness as a crucial trait for cassava characterisation, as it can influence both the storage and culinary quality of the roots. Furthermore, the orientation of the petiole (PC3) and root taste (PC4) were significant contributors to the variation observed in Cluster 1, corroborating earlier findings that emphasised the role of petiole orientation in leaf morphology and root taste in determining consumer preferences and culinary uses of cassava (Agre *et al*. 2016; Chaengsee *et al*, 2020).

The fifth principal component (PC5), leaf colour, accounted for 9.5% of the variance, which is supported by results from related studies indicating that leaf colour is a reliable phenotypic marker for distinguishing cassava genotypes (Elias *et al*, 2001; Rimoldi *et al*, 2010). The correlations between these morphological traits and their respective PCs affirmed the strength of certain phenotypic features in determining genetic diversity and their potential applications in breeding programmes aimed at improving cassava productivity and quality.

The variation in this Cluster, as reflected in the PCA, highlighted the critical phenotypic traits of cassava, particularly those related to root characteristics and leaf morphology, which have been widely discussed in previous studies (Nweke *et al*., 2016; Adejumo *et al*., 2019; Enesi *et al*., 2022). Thus, these findings provide valuable insights for the

selection and development of improved cassava varieties with enhanced quality and adaptability.

5.1.1.2 Cassava phenotypic cluster two

Cluster 2's phenotypic diversity can be attributed to four key PCs, which accounted for 69.2% of the total variation among the cassava accessions (Table 4.4). Principal component one (PC1), which primarily captured the colour of the stem exterior had the highest correlation with the data, reflecting 23.9% of the total variation. The strong correlation of 0.936 between stem colour and PC1 aligns with related studies emphasising the value of stem colour as a morphological trait in cassava. This is often linked to specific biochemical properties such as anthocyanin content, which can influence drought tolerance and disease resistance (Tao *et al*. 2020). This characteristic, therefore, plays a central role in understanding genetic diversity in cassava. The shape of the central leaflet is a well-documented morphological trait used to classify and distinguish cassava varieties. As noted by Ridwan *et al*., (2022), leaf morphology has significant implications for photosynthetic efficiency and overall plant vigor. Leaf shape variability in this study highlights the potential for leaf morphology to serve as a reliable phenotypic marker for cassava breeding programs aimed at improving yield and resilience.

The study established that the root taste, represented as PC3 with a correlation of 0.975, is a prominent phenotypic trait which contributed to the variability within this cluster. The correlation affirmed the significant role of root characteristics, particularly taste, in cassava consumer preference and acceptability, which are crucial for cassava's

marketability (Okonkwo *et al*, 2021). Findings from related studies have revealed that root taste directly influences the selection of cassava varieties for both local consumption and international trade, reinforcing the relevance of this trait in cassava breeding (Kengkanna *et al*, 2019).

Moreover, the root peduncle, while often overlooked in many cassava studies, plays a role in the overall architecture of the plant and can affect harvesting efficiency and yield potential. Another study by Rattanasopa *et al*, (2022) noted that the extent of the root peduncle may influence the transport of nutrients from the soil, further linking this morphological feature to plant performance.

Data from the rotated component matrix, further highlighted the strong association between these phenotypic characteristics and their respective PCs, reinforcing the importance of each trait in explaining the genetic diversity observed in cassava accessions. These findings are in tandem with studies that utilise principal component analysis to capture the main sources of phenotypic variation and provide a clearer understanding of the interrelationships among various traits (Afonso *et al*, 2014).

5.1.1.3 Cassava phenotypic cluster three

Cluster 3 suggested that the two primary phenotypic characteristics, the colour of the stem exterior and the orientation of the leaf petiole, were the main contributors to the total variation among the genotypes in this Cluster. PC1, associated with the colour of the stem exterior, accounted for 61.5% of the variation, showing a high correlation of 0.997 in the rotated component matrix (Table 4.5). This suggested that stem colour is a key distinguishing trait within this cluster, which is in tandem with previous studies that have

highlighted the value of stem colouration in cassava genotypic variation (Ogbonna *et al*., 2021).

Principal component 2, related to the orientation of the leaf petiole and accounted for 38.5% of the variation with a correlation of 0.968 (Table 4.5). This high correlation further emphasised the relevance of leaf morphology, particularly the petiole's orientation, in characterising variation within cassava genotypes (Karim *et al*., 2020). Interestingly, the PC4 and PC5 (root taste and leaf colour), both showed a correlation of -0.968, but accounted for 0.0% of the total variation. This suggested that these traits were not significant in differentiating the genotypes within this cluster. This finding contrasted with previous studies in which root taste was identified as an important trait in cassava breeding and characterisation (Agre *et al*., 2016). However, the absence of variation attributed to these traits in this specific cluster could indicate that root taste and leaf colour may not be as influential in determining the genetic diversity of this group, or that these traits were less variable within the analysed genotypes.

The ninth and tenth principal components, which were associated with the colour of the stem epidermis and cortex thickness, also showed low correlations of -0.713 and -0.964, respectively, and accounted for non-significant variation. The non-significant variation from these components suggested that these phenotypic traits were not useful in distinguishing between the genotypes in this Cluster; thus, reinforcing the idea that not all morphological traits contribute equally to the total phenotypic variation in cassava (Asare *et al*., 2011). Related studies on cassava morphological traits similarly highlighted that

some traits, such as epidermal colour or cortex thickness, may exhibit limited variability across certain genotypes (Ceballos *et al*., 2016).

These results offer valuable intuitions into the phenotypic traits driving variation within this cluster, with colour of the stem exterior and leaf petiole orientation emerging as the most significant characteristics. This conforms to earlier studies that emphasised the importance of stem and leaf morphology in cassava genotypic classification (Oliveira *et al.,* 2015; Karim *et al.,* 2020).

5.1.1.4 Cassava phenotypic cluster four

Cluster 4, consisted of 11 genotypes (8.4%); exhibited significant variation among cassava accessions based on principal component analysis. The lobe margin variation is known to be an important trait in cassava, as it can be linked to both genetic and environmental factors (Chavarriaga-Aguirre *et al*., 1998). The prominence of this trait is consistent with studies that have found morphological traits related to leaf shape and margins as key indicators of genetic diversity in cassava (Alves *et al*., 2019). The principal component 2, representing cortex thickness, accounted for 20.6% of the variation (Table 4.6). This trait is particularly important for cassava's processing qualities, as thicker cortex can impact the tuber's overall yield and suitability for various uses (Noerwijati *et al*., 2014). The variation in cortex thickness in this Cluster, could have practical implications for cassava breeding programmes focused on improving the crop's performance, particularly in terms of root quality for both consumption and industrial uses. Principal component 3, representing stem exterior colour, explained 17.5% of the total variation. Stem colour, although less frequently studied, has been noted as an

important phenotypic trait in cassava, potentially affecting resistance to pests and diseases (Hillocks *et al*., 2012). The variation in this trait within this Cluster could reflect underlying genetic diversity that may influence cassava's adaptability to different agro-ecological conditions.

Leaf colour in cassava has been linked to physiological traits such as photosynthetic efficiency and resistance to biotic stressors (De Souza and Long, 2018). The contribution of leaf colour to the total variation suggests that this trait plays an important role in differentiating the genotypes within this cluster, which may have implications for cassava cultivation under varying environmental conditions. The correlation values in the rotated component matrix, provide deeper insights into how these traits interrelate, and they are essential for understanding the genetic structure of cassava accessions within this cluster. Thus, the PCA results have revealed that lobe margins, cortex thickness, stem exterior colour, and leaf colour are key traits contributing to genetic differentiation among cassava genotypes. The findings conform to those of previous studies that have emphasised the importance of these phenotypic characteristics in cassava's genetic diversity and breeding potential (Chavarriaga-Aguirre *et al*., 1998; Oliveira *et al.,* 2015). This study has established a reduction of phenotypic characters from 25 to 11 in some clusters (Cluster 1, Table 4.3) and even fewer in others (Cluster 4, Table 4.6). This provides breeders with a more streamlined approach to identifying important phenotypic traits, which can be tailored according to environmental factors. Thus, breeders do not need to study all the 25 phenotypic characters but can prioritise the ones that contribute significantly to phenotypic variation, as evidenced by this present study. This approach offers a more targeted strategy for breeding programmes, as fewer characters simplify the

selection process; while still capturing significant genetic diversity (Oliveira *et al*., 2015). The environment and specific phenotypic traits may influence the selection of characters, so flexibility is required in defining the key traits for different cassava accessions (Shirima *et al*., 2022).

This study provides a significant distinction from the analysis of the Correlation of the Rotated Component Matrix which is different from most studies on cassava accession characterisation which hitherto focus on unrotated components. The rotated matrix helps achieve clearer and more interpretable factor loadings by minimising the complexity of factor structures (Jolliffe and Cadima, 2016). This rotation ensures that the resulting components are better aligned with distinct phenotypic characters, enhancing the identification of meaningful clusters. For example, in the current study, the rotated matrix revealed that PC1 was primarily associated with root cortex colour (correlation of 0.985); while other PCs highlighted cortex thickness, petiole orientation, root taste, and leaf colour. These results are of great significance since they allow for clearer distinctions between clusters, contributing to cumulative variance by aligning specific phenotypic traits to the most significant principal components.

The decrease in number of phenotypic characters from 11 in Cluster 1 (Table 4.3) to 9 in Cluster 4, (Table 4.6) highlighting how phenotypic traits are influenced and grouped by both environmental and genetic factors. As the cumulative percentage variance among clusters reaches 70% or more, the importance of certain characters decreases (Jolliffe, 2002). For instance, in Cluster 4, phenotypic traits such as stem exterior colour and root taste, with correlations of 0.936 and 0.975, respectively; played significant roles in explaining the variation among accessions, but the reduction to nine characters suggests

that some phenotypic traits became redundant or contributed minimally to variation (Joaqui *et al*., 2016). This phenomenon is common in PCA, where only a few PCs explain the bulk of variance, allowing for the exclusion of less influential characters while still capturing essential genetic diversity (Sánchez *et al*., 2018). Therefore, this study provides an insight into optimising phenotypic trait selection while maintaining high levels of explained variance, which could serve as a model for future cassava breeding research. Related studies have observed that the site effect, likely influences the phenotypic variation observed in cassava variety clusters, as environmental factors can impact traits such as root colour, leaf morphology, and stem characteristics (Adejumo *et al*, 2019; Malik *et al*, 2020). Therefore, differences in agro-ecological conditions across selected sites may have had a hand in genetic diversity and variability in cassava accessions within the Kenyan germplasm. All clusters demonstrated the importance of root characteristics, leaf morphology, and stem color in defining phenotypic diversity in cassava. This conforms with previous studies highlighting these traits as key determinants in cassava characterization (Fukuda *et al*., 2010). However, the relative importance of these traits varies significantly across clusters. For example, root cortex colour is crucial in Cluster 1, while stem exterior colour dominated in Clusters 2 and 3. Cluster 4 uniquely emphasises lobe margins. These findings offer valuable insights towards cassava breeding programmes. Focusing on the key traits identified within each cluster, breeders can develop more targeted and efficient breeding strategies for specific objectives, such as improving root quality, enhancing disease resistance or increasing yield potential.

5.2 Molecular characterization of cassava (*Manihot esculenta* Crantz) germplasm in Kenya

Genetic variability in cassava (*Manihot esculenta* Crantz) should be considered while developing conservation and utilisation plans, as well as accelerating breeding initiatives. Molecular markers are effective and precise techniques for revealing and estimating genetic diversity, as well as determining population structure in most plant species (Okogbenin *et al*., 2007; Rabbi *et al*., 2022). A few of gene-based markers have been produced to aid in the exploration of genetic diversity and population structure analyses in agricultural plants, including SCoT molecular markers (Sánchez *et al.,* 2018; Sheat *et al*., 2021).

SCoT markers have been employed in genetic variation research, phylogenetic analyses and DNA fingerprinting of commercially vital agricultural and medicinal plants such as yam (*Dioscorea spp*.) (Owiti *et al*., 2023), mango (Luo *et al*., 2011), grape (Guo *et al*., 2012), orchid (Bhattacharyya *et al.*, 2013), durum wheat (Etminan *et al*., 2016), rose (Agarwal *et al*., 2019), *Diospyros* (Deng *et al*., 2015), *Elymus sibiricus* (Zhang *et al*., 2015), *Vigna unguiculata* (Igwe *et al*., 2017), *Taxus media* (Hao *et al*., 2018), *Dendrobium* (Feng *et al*., 2019), *Chrysanthemum morifolium* (Feng *et al*., 2016), coconut (Rajesh *et al*., 2015) and *Physalis* species (Feng *et al*., 2018).

Additionally, studies have indicated that SCoT markers have good capabilities in genetic research due to their ability to reveal polymorphisms in conserved regions and their high reliability as compared with other molecular systems (Etminan *et al*, 2016; Aboulila and Mansour, 2017). Therefore, the current study determined the genetic diversity among sampled cassava germplasm in Kenya using SCoT molecular markers. As a result, the

current study used SCoT molecular markers to estimate the genetic diversity of cassava germplasm samples collected in Kenya. Notably, this is the first investigation that uses SCoT markers to assess the genetic diversity and population structure of diverse cassava accessions while determining phylogenetic links between them. The 15 SCoT markers amplified 119 bands, of which 107 (89.9%) were polymorphic, demonstrating the informative nature of the SCoT markers. The high amount of polymorphism (89.9%) indicated a considerable genetic diversity amongst cassava genotypes.

In other related studies, Zhao *et al*. (2017) highlighted the high reliability and ability of SCoT markers to detect polymorphisms in conserved regions, making them suitable for evaluating genetic diversity in various plant species. Their study on *Gossypium hirsutum* L. demonstrated that SCoT markers discovered a high magnitude of polymorphism, with over 80% polymorphic bands, like the 90% polymorphism observed in cassava accessions (Xiong *et al.* 2011). In line with this study's findings, Owiti *et al*. (2023) observed a relatively high magnitude of genetic diversity (95%) using SCoT markers to detect polymorphisms in yams (*Dioscorea spp*.). Additionally, Guo *et al*., (2012) reported a comparable polymorphism rate of 88% in *Carthamus tinctorius* L., emphasizing the utility of SCoT markers in revealing genetic variability and population structure in plant species. The consistent high polymorphism rates across different studies affirm the robustness of SCoT markers in genetic diversity studies.

In contrast, other molecular markers like Random Amplified Polymorphic DNA (RAPD) and Inter-Simple Sequence Repeats (ISSR) have shown varied levels of polymorphism, generally lower than SCoT markers. For example, Rodrigues *et al*., (2019) reported a polymorphism level of 76% in cassava using RAPD markers, which is significantly lower

than the 89.9% observed with SCoT markers in the current study. Similarly, Akinwale *et al.*, (2010) found that ISSR markers exhibited 72% polymorphism in their study on cassava, further supporting the assertion that SCoT markers provide more reliable and higher resolution in detecting genetic diversity among cassava genotypes. However, Que *et al*. (2014) obtained 92.85% polymorphism using SCoT markers in assessing the genetic diversity of sugarcane accessions within a local sugarcane germplasm collection from China. The high polymorphism revealed by SCoT markers in the present study not only conforms while exceeding the polymorphism rates reported in most of these previous studies, demonstrating their superior efficacy in genetic research and diversity studies. According to Hodge *et al*. (2017), Nei's gene diversity and Shannon's information index are significant tools for studying genetic variety in plant species. In this study, the median Nei's gene diversity (expected heterozygosity) and Shannon information index were 0.45 and 0.25, respectively, demonstrating considerable genetic variety across cassava accessions. The findings demonstrated that the germplasm evaluated in this study has modest genetic variation, which can be used to improve cassava.

Similarly, in a related investigation, Owiti *et al.* (2023) found that Nei's gene diversity (expected heterozygosity) and Shannon information index was 0.33 and 0.49, respectively, reflecting modest genetic variety for Kenyan yam accessions. The findings revealed that the germplasm tested had adequate genetic heterogeneity. Additionally, Sichalwe *et al*. (2024) reported moderate genetic diversity values in Ugandan cassava germplasm which ranged grom 0.28 to 0.31. Their study emphasized that such genetic diversity is essential for maintaining the adaptability and resilience of cassava populations, especially in the face of pests and diseases like cassava mosaic disease. A

related study by Prempeh *et al*. (2020) on cassava accessions in Ghana obtained gene diversity varying from 0.50 to 0.500 with an average of 0.36. These findings are consistent with the moderate genetic diversity observed in this current study, further affirming the importance of such diversity for cassava breeding programs aimed at enhancing yield, disease resistance, and climate adaptability. In line with these findings, Goncalves *et al*., (2017) assessed 51 cassava accessions of Minas Gerais state of Brazil and reported observed heterozygosity of 0.65 indicating moderate genetic variance with gene diversity of 0.48 and PIC of 0.4.

The PIC value showed a marker's informativeness in detecting polymorphism and is commonly used to identify genetic differences between crop accessions. PIC values less than 0.25 represent minimal polymorphism, 0.25 to 0.50 implied intermediate polymorphism, and values greater than 0.50 indicate high polymorphism (Botstein *et al*., 1980; Ge, 2013). In this work, the PIC values varied from 0.27 (SCoT11) to 0.37 (SCoT16, SCoT21, SCoT23 and SCoT29), with a mean of 0.35, confirming that the SCoT markers utilised in the current investigation were extremely valuable and thus exhibit strong discriminatory potential, as described by Botstein *et al*. (1980). In support of these findings, in a related study conducted by Adjebeng-Danquah *et al*. (2020), PIC value varied between 0.030 to 0.780 when 89 cassava accessions were assayed with 35 SSRs. Furthermore, these findings showed that the SCoT markers utilised in this study are highly informative and efficient, making them suitable for species authentication through the development of species-specific sequence-characterized amplified region (SCAR) markers. The genetic links between the tested cassava accessions were evaluated by computing the similarity coefficients, and the accessions were sorted according to

their commonalities using the UPGMA method. The similarity coefficient varied between 0.35 to 0.78, with an average of 0.57, showing modest genetic variation across the 40 cassava accessions. The maximum similarity coefficient of 0.78 was found between accessions 4 and 7, as well as between 114 and 117, signifying that each of these accessions are quite genetically close. The lowest similarity value of 0.35 was found between 61 and 74, indicating that these accessions were more genetically varied and suitable for cassava development. The dendrogram created using the UPGMA approach divided the 40 cassava accessions into two primary clusters/groups with a similarity coefficient of 0.35. The population structure was analyzed using STRUCTURE software to perform clustering, and the results showed that K = 2 had the best delta K (DK) interference.

According to Pritchard *et al*. (2000), the Bayesian clustering technique helped to identify population structure and distribute people or portions of genetic material among multiple clusters. STRUCTURE program assigned distinct accessions to various populations based on the discovered allele frequencies. The 40 cassava accessions used in this study were divided into two populations and an admixture group using population structure analysis. The principal coordinate analysis (PCoA) yielded equivalent results to the cluster analysis and STRUCTURE approaches. Analysis of molecular variance (AMOVA) revealed a considerable amount of genetic variance (84%), indicating the possibility of effective selection from a mini-core collection of the tested population due to the diversity of genetic lineages. High genetic variability within the population indicates that different cassava accessions have diverged within a same population, suggesting that genetic resources are present at each collecting county. In line with these

findings, a study by Pedri *et al*. (2019) genetic diversity of cassava landraces cultivated in northern Mato Grosso State, Brazil, using microsatellite markers found that 92% of the total genetic variance was attributed to within-population variability, which also indicated a high potential for effective selection from the genetic resources available. Similarly, in the genetic diversity study of cassava landraces in Ghana using SNP markers, Prempeh *et al.* (2020) reported 99% within and only 1% between group variation. Additionally, Sichalwe *et al.* (2024) and Kawuki *et al*. (2018) observed that 66.02% and 85% respectively, of the genetic variance in Ugandan cassava germplasm was within populations, suggesting that significant genetic diversity exists even within small, localized populations. This trend is not unique to cassava; for instance, Ramu *et al*. (2013) and Ramu *et al.* (2017) reported that in sorghum, most of the genetic diversity was found within populations rather than between them, emphasizing the importance of preserving within-population genetic resources for breeding programs.

These studies consistently demonstrated that high genetic variability within populations is a common characteristic in crops with wide geographical distributions, such as cassava, and affirms the importance of utilizing this diversity in breeding and conservation efforts. Notably, within-population selection allows for the identification of genotypes that are better adapted to specific climatic conditions or management approaches, resulting in bigger genetic gains during crop development. Thus, the current study found significant moderate genetic diversity among cassava germplasm in Kenya. The moderate level of genetic variation in cassava accessions identified by SCoT molecular markers may be beneficial for broadening the genetic foundation of cassava breeding projects. Parental selection may capitalise on the outcomes of genetic diversity and genetic distance to

increase the breeding value of cassava accessions and permit crosses among accessions that are further apart to produce hybrids with higher inheritable traits than their parents. The accessions in this study were grouped into two major genetic clusters that included many intermediates. Since these two genetic clusters would be treated as separate evolutionary units, choosing parents should be based on the larger inter-cluster distance. The UPGMA dendrogram and PCoA revealed no relationship between the locations of the cassava accessions' growing zones and the clusters. When compared to samples from other counties, the UPGMA dendrogram revealed that most cassava accessions from the same cassava growing zone had a higher level of genetic similarity. As a result, crosses between the various geographical zones' cassava accessions should be made. The knowledge these results offer on Kenyan cassava accessions is critical for the preservation of genetic resources and the start of germplasm enhancement initiatives.

5.3 Comparative analysis of phenotypic and molecular characterization of cassava (*Manihot esculenta* Crantz) germplasm in Kenya

The findings from this study affirmed the importance of integrating phenotypic and molecular characterization in understanding cassava germplasm diversity in Kenya. While phenotypic characterization provides valuable insights into regional adaptation and observable traits, it is often limited by inconsistencies, such as farmer-assigned naming conventions and environmental influences. The same cassava variety may have different names across regions, leading to misclassification and variability (Darkwa *et al*. 2020). Additionally, morphological traits, such as plant height and root shape, are subject to environmental and developmental stage variability, which reduces their reliability for

accurately representing genetic relationships (Niyonzima *et al*. 2021). Consequently, morphological characterization is best suited as a preliminary, exploratory tool to guide deeper molecular investigations (Feldberg *et al*. 2011). The Kenyan study's low correlation (0.0423) between molecular and agro-morphological characterizations echoes findings by Adu *et al*. (2018), who highlighted that phenotypic traits often fail to reflect underlying genetic diversity.

Molecular characterization, by contrast, offers a robust and stable assessment of genetic diversity, unaffected by environmental or developmental variables (Darkwa *et al*. 2020). In this study, molecular markers revealed hidden genetic relationships, such as grouping accessions like Kazanzwara-Kiboko and Katune-Kiboko in genotypic Cluster #2, despite their classification in phenotypic Cluster #1. These discrepancies highlight the limitations of phenotypic characterization and the superior precision of molecular techniques, as corroborated by Esuma *et al*. (2016) in Uganda and Pierre *et al*. (2022) in Burundi. Furthermore, the Kenyan findings resonate with the work of Asare *et al.* (2011), which demonstrated the value of molecular markers in uncovering genetic diversity critical for traits like pest resistance and yield improvement. The Selele varieties in phenotypic Cluster #4 exemplified this disconnect, as they were genetically diverse when analyzed molecularly.

Integrating both phenotypic and genotypic approaches is essential for comprehensive cassava breeding strategies and germplasm management. While phenotypic traits provide insights into regional adaptation and observable performance, molecular markers uncover genetic variations that inform breeding decisions and prevent genetic bottlenecks (Asare

et al. 2011). This dual approach aligns with Feldberg *et al*. (2011), who emphasized that combining these methods captures a holistic understanding of genetic resources, aiding in accurate identification, conservation and crop improvement efforts. The observed discrepancies in clustering between phenotypic and molecular assessments underline the need for both techniques to be used synergistically, as phenotypic analysis alone cannot reliably identify genetic diversity. The integration of these approaches not only enhances breeding decisions but also ensures the effective conservation of cassava genetic resources, a crucial step for improving crop resilience and productivity.

5.4 Cyanide Concentration among cassava (*Manihot esculenta* Crantz) germplasm in Kenya

5.4.1 Cyanide concentration levels in leaves, peels and pulp among the cassava accessions

All cassava varieties contain cyanide at different concentrations with the “sweet” varieties containing low cyanide while the “bitter” varieties containing high cyanide concentrations. The WHO-recommended threshold of safe cyanide concentration for food is 10 mg/L CN^- (WHO, 2016). Analysis of cyanide in cassava is essential due to its potential toxicity to humans and livestock. Different methods are used for cyanide quantification in cassava, including titration, the picrate paper method, and spectrophotometric analysis (Bradbury *et al*. 1999). The current study compared the picrate paper method with spectrophotometric analysis, showing low correlation, particularly in peels and leaves (Table 4.12). The spectrophotometric method showed that the leaves had the least average cyanide concentration (5.89 mg/L CN^-) while the peels

had average cyanide concentration of 7.42 mg/L CN^-. The pulp had the highest average cyanide concentration of 8.20 mg/L CN^-. The same trend was observed using the picrate paper method. This general trend of higher cyanide in roots (pulp and peels combined) compared to leaves aligns with studies by Selmar, (1993) and Nambisan & Sundaresan, (1994). These authors noted that leaves are the primary site of cyanogenic glucoside synthesis, but these compounds are often translocated and accumulate in the roots. However, the current study's contrasts with other studies. Moriasi *et al*. (2017), studying cassava in Kenya, found the highest cyanide levels in the cortex of both edible and poisonous cassava varieties. Also, Udeme *et al*. (2017) reported the highest levels in the peels of yellow cassava varieties intended for livestock feed, while Fekadu *et al*. (2017), while examining sweet cassava in Ethiopia, found the cortex to have the highest concentration, followed by parenchyma (pulp), and then the pith. In a related study, Ospina *et al.* (2024) also found the highest concentration in the cortex, followed by leaves and then parenchyma. The study noted that this pattern was likely due to the cortex's primary function in storage and transport, allowing for higher accumulation of substances. The leaves, being sites of active metabolism, exhibited moderate concentrations as they continuously process and redistribute compounds. In contrast, the parenchyma, mainly responsible for structural support and basic physiological functions, retained the lowest concentration due to its reduced involvement in storage and transport (Cardoso *et al*. 2005).

This current study's observation of significant variation in cyanide levels among different cassava varieties strongly agrees with previous findings (Aalbersberg and Limalevu, 1991; Cardoso *et al.*, 2005; Ndubuisi and Chidiebere *et al*., 2018) which consistently

demonstrated that cassava varieties exhibited a wide range of inherent capacities for cyanide production and thus, some varieties are naturally low-cyanide, while others are inherently high-cyanide. The wide range of cyanide concentrations observed in the current study, from very low to high levels, affirms the importance of screening cassava germplasm for cyanide content, as different varieties can pose varying levels of risk for consumption (Hahn and Keyser, 1985; Akiyama *et al*. 2006). This variability is further exemplified by studies such as Diallo *et al*. (2014), who reported varying levels of cyanide in different parts of Senegalese cassava cultivars, with some varieties exhibiting high toxicity levels in the roots.

Researchers have embraced varied methods for cyanide analysis such as titration, spectrophotometer and picrate paper and this could have led to the different results obtained. The correlation between cyanide concentration measurements obtained through the picrate paper test (color chart) and spectrophotometry varied across different cassava plant parts. In both leaves and peels, a moderate positive correlation was observed; r = 0.547 and r = 0.570, respectively. This suggests a discernible, though not strong, linear relationship between the two methods. While increases in color chart readings tended to correspond with increases in spectrophotometer readings, the moderate correlation indicates some variability and suggests that the two methods are related but not perfectly interchangeable for these tissues. This implies that while the picrate test can provide a general indication of cyanide levels in leaves and peels, it might not be as precise as spectrophotometry.

However, in the pulp, a very strong positive correlation (r = 0.936) was found between the two methods. This near-perfect correlation suggests a high degree of consistency

between the color chart readings and spectrophotometer readings for pulp samples. The strong relationship implies that the picrate paper method can serve as a reliable proxy for spectrophotometry when measuring cyanide concentrations in cassava pulp, especially in resource-limited settings where spectrophotometers may not be readily available. The differing correlations across tissues might be due to variations in the distribution of cyanogenic glycosides and the impact of other compounds available in the different plant parts that could interfere with the colorimetric reaction. However, it's crucial to acknowledge that the picrate paper method, while useful for screening, might not be as precise as spectrophotometry. The discrepancies between methods are reflected in the current study's data, where absolute cyanide values obtained from the two methods varied. This difference in precision was also noted by Azucena-Topor *et al.* (2008), who used spectrophotometry to validate color chart readings.

5.4.2 Cyanide concentration levels in cassava sample accessions collected from farmers in Migori County, Kenya

The observed variation in cyanide concentration among cassava accessions in Migori County reflects a similar pattern noted in earlier studies on cassava characterization using cyanide levels. For instance, a study by McMahon *et al*. (1995) noted that cyanide content varies significantly across cassava genotypes, environmental conditions, and processing methods. High cyanide concentrations, such as the 30 mg/L CN^- recorded in accessions like Waite Nyabasi-002 (Accs. 206) and Dar-salaam-001 (Accs. 214) (Table 4.16) align with findings by Cardoso *et al*. (2005), who reported elevated levels in bitter cassava accessions that are customarily embraced for industrial purposes or in regions

with low rainfall. These high levels suggest that certain cassava accessions in Migori may require additional detoxification steps before consumption to ensure food safety. Conversely, the low concentrations (1-3 mg/L CN^-) recorded in varieties such as Rateng black (Accs. 218), Roteng (Accs. 219) and Kanyamwa (Accs. 232) reflect traits typically associated with sweet cassava accessions, which are safer for direct human consumption. These findings conform with those of Burns *et al.,* (2012), which showed that sweet cassava varieties often have cyanide levels below 10 mg/L CN^-, posing minimal risk to consumers. The study also highlights the importance of considering cyanide concentrations in peels, which were consistently higher in certain accessions. This observation corroborates findings by Amelework *et al*. (2021), who emphasized that cassava peels contain higher cyanogenic glycosides than tuberous flesh, suggesting the need for proper disposal or processing of peels to prevent accidental poisoning by either livestock or humans. Therefore, the use of the picrate paper method in this field study demonstrated its practicality for on-farm cyanide analysis. This method is extensively acknowledged for its simplicity, cost-effectiveness, and suitability for resource-limited settings (Egan *et al*. 1998). It enables farmers and researchers to obtain rapid and semi-quantitative results without requiring sophisticated laboratory equipment. However, while effective for screening purposes, the picrate paper method may lack the precision and sensitivity of laboratory-based techniques such as spectrophotometry or gas chromatography. However, the method is susceptible to environmental factors, such as humidity and temperature and these can influence the stability of picrate papers and the accuracy of colour readings (Bradbury *et al*. 1999). Additionally, the semi-quantitative nature of the method may not detect low cyanide levels accurately, potentially

underestimating the risk in sweet varieties. Despite these limitations, its easiness of usage and accessibility renders it a valuable tool for preliminary assessments of cyanide content in cassava on farms, particularly in rural parts where laboratory infrastructure is unavailable.

Despite the toxicity risks, farmers continue cultivating high-cyanide cassava varieties due to their unique properties and cultural significance. Communities have developed several processing methods to reduce cyanide levels in leaves, peels and pulp. Such methods constitute sun drying, fermentation and cooking (Akintonwa *et al*. 1994; Diallo *et al*. 2014; Ndubuisi and Chidiebere *et al*. 2018). Fermentation, a common traditional processing method, not only detoxifies cassava (Tumwesigye, 2014), but also enhances the texture and taste of flour used in preparing *ugali* or mealy and porridge. In contrast, improved cassava varieties such as 'Agriculture' contain low cyanide levels and require no fermentation. However, these improved varieties are often reported to produce tasteless porridge and *ugali*, reducing their preference among local consumers.

In Migori County, cassava tubers (pulp) with high cyanide content are frequently fermented to produce local brews such as "*busaa*" among the Luo community and "*busara*" among the Kuria. This practice highlights the cultural and economic significance of high-cyanide cassava varieties beyond direct consumption, further explaining their continued cultivation.

5.4.2.1 Cassava leaves

Fresh cassava leaves are important vegetables and are cooked fresh. Of interest in this study was the Wild cassavas-001 (Acc. 026) and Wild cassava (Acc. 029) (Table 4.12). These are perennial plants and are commonly believed to be of high cyanide

concentrations in the leaves and in the roots. In this study these plants had low cyanide contents in the leaves that is below the WHO maximum recommended threshold. The leaves are characteristically large (Plate 5.1) as compared to the common varieties grown such as *Adhiambo lera.* The wild cassava had relatively low cyanide levels in the foliage, making it a potential vegetable crop. When compared to other accessions, Wild cassava-001 exhibits the highest high biomass production, followed by Kasukali (Acc. 015) with medium-sized leaves and Selele-002 (Acc. (024) with the smallest leaves (Plate 5.1). Given their perennial growth habits, low cyanide concentration in the leaves, large leaf surface area, and in inference high yield as compared to all the other accessions, Wild cassava-001 and Wild cassava-002 (Table 4.12) could be promoted for cultivation as a vegetable crop. This would contribute to food security and dietary diversity.

Plate 5.1: From Left to Right: Leaves of Wild Cassava-001, Kasukali and Selele-002 Cassava Varieties

5.4.2.2 Cassava tubers (peeled or unpeeled)

Traders in Migori and Kisii counties prefer to buy unpeeled cassava chips. This gives them higher biomass than the peeled chips (Plate 5.2). The outer skin and fibrous layers account for a considerable portion of the tuber's total mass, which is lost when cassava is peeled. This weight reduction directly affects the market value and potential revenue farmers can earn from selling their produce. Consequently, most farmers prefer selling unpeeled cassava tubers to maximize their income, as buyers often purchase based on weight and or volume. The unpeeled cassava chips also reduce peeling labour cost to the farmers.

Plate 5.2: Peeled and unpeeled cassava tubers

When cassava is peeled (Plate 5.2), not only does it lose mass, but it also becomes more perishable, limiting the duration within which it can be stored or transported before spoilage sets in. In contrast, unpeeled cassava has a longer shelf life due to the protective outer layer, making it more convenient for transportation and storage. To the consumer, the unpeeled cassava chips contain more fibre in the flour which is beneficial for food

digestion. The wholistic nature of this study is that we can select cassava varieties with low cyanide concentrations in the peel and in the pulp. Examples of such varieties are Nyatanga -002 (Accs. 036), Agriculture -012 (Accs. 067), Mtwapa -009 (Accs. 089) and Adhiambo lera (Accs. 090) (Table 4.12).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

There was phenotypic variability within the cassava germplasm collected. Despite the variability found within the germplasm, it is concluded that cassava phenotype base in Kenya is narrow as it was revealed that majority of the phenotypes were clustered in one group. The Principal Component analysis (PCA) is effective in elucidating the phenotypic diversity and genetic structure of cassava germplasm.

Thus, relying on morphological traits alone for cassava germplasm classification is limited, as genetically distinct accessions may appear similar phenotypically. Environmental factors and growth stages can also affect the accuracy of morphological clustering.

There was a moderate genetic variability among the 40 cassava gerplasm analysed. Cluster analysis separated the accessions into two major clusters irrespective of collection zones, suggesting some genetic overlap across regions. The study confirmed that phenotypically similar accessions could be genetically distinct and vice versa, emphasizing the necessity of integrating molecular data with morphological traits for accurate genetic classification. The identified polymorphic markers serve as valuable molecular descriptors for cassava fingerprinting and breeding improvement.

Molecular characterization, using more precise genetic data, presented different clustering patterns in comparison with phenotypic classification. Thus, integrating phenotypic and molecular characterization provides a more accurate and comprehensive

understanding of cassava genetic diversity, essential for effective breeding and conservation strategies.

Cassava varieties exhibited significant differences in cyanide concentration across leaves, peels, and pulp. This may variations may be influenced by genetic variations and enzymatic activity in the cyanogenesis pathway. Varieties with high cyanide levels pose potential health risks if not adequately processed, highlighting the importance of detoxification methods to ensure safety. The cyanide concentration in cassava varieties generally increased from the outer tissues (peels) to the inner tissues (pulp). Both spectrophotometric and picrate paper test methods confirmed this trend, with the pulp showing higher cyanide levels than the peels. Variations among cassava accessions emphasize the need for careful variety selection to reduce cyanide exposure. Despite the risks, farmers continue to grow high-cyanide cassava varieties due to desirable traits like taste, texture, and suitability for specific products (e.g., local brews).

Wild cassava varieties generally displayed moderate cyanide levels, likely linked to their genetic adaptations. It shows potential as a vegetable crop due to its low cyanide levels in the foliage and high biomass production make it a promising option for improving food security and dietary diversity. The observed variability in cyanide levels across different cassava accessions affirms the importance of characterizing varieties based on cyanogenic potential to guide breeding programs and consumer recommendations. Unpeeled cassava has advantages for farmers since it maintains higher biomass (and therefore market value), has a longer shelf life, and reduces labor costs associated with peeling. The correlation coefficient (r) measures the strength and direction of linear relationships between variables. Moderate positive correlations in leaves and peels

suggested noticeable but not strong consistency between cyanide measurements from the color chart and spectrophotometer. In pulps, a strong positive correlation indicated high consistency, supporting the color chart as a reliable spectrophotometer proxy.

6.2 Recommendations

The following recommendations are suggested

1. Future studies on phenotypic characterization in Kenya should focus on the 11 phenotypic traits that accounted for 71% cumulative variation. The application of phenotypic descriptors in identification of cassava germplasm should be backed by utilization of molecular markers (genetic characterization), since the former alone does not reveal much diversity due to the effects of the environment on quantitative traits.
2. Through focusing on the eleven principal components identified, which explain a substantial portion of the variation, breeders can target specific traits such as leaf shape, root characteristics, and stem color to develop superior genotypes. Characterization of cassava into four major clusters provides a strategic framework for selecting parent plants, ensuring that breeding efforts are more efficient and tailored to enhance desirable traits. This technique will also support the conservation of genetic resources, fostering the development of resilient and high-yielding cassava varieties.
3. It can also be recommended that cassava breeding programs should integrate principal component analysis (PCA) into their selection processes to exploit the phenotypic diversity and genetic structure of cassava germplasm effectively.

4. Through utilization of the genetic variability identified through SCoT markers, breeders can create cassava cultivars with a broader genetic base, which is essential for resilience against biotic and abiotic stresses.
5. Cassava breeding programs should prioritize the integration of molecular characterization alongside phenotypic methods to ensure the selection of genetically diverse parents, as relying solely on observable traits can lead to the unintended selection of genetically similar accessions. The use of molecular tools like DNA markers will enhance allele diversity and heterosis, improving production of resilient cassava cultivars. Additionally, incorporating Bayesian clustering techniques in germplasm conservation efforts will help breeders identify distinct populations, ensuring that genetic diversity is preserved for long-term breeding success and sustainability.
6. There is a need for continued breeding programs aimed at developing cassava varieties with lower cyanogenic potential. Varieties such as Mbale-001 and Agriculture-012, which exhibited low cyanide concentrations, should be prioritized for cultivation to minimize the risk of cyanide toxicity in consumers.
7. Exploring wild cassava as a vegetable crop and for breeding low-cyanide varieties can enhance food security. Additionally, farmers should balance market demands by optimizing sales between peeled and unpeeled cassava.
8. The picrate paper method with the color chart can be used as an alternative method for measuring cyanide concentration, particularly in resource-limited settings. While it demonstrated moderate reliability for leaves and peels, its strong

correlation in pulps (r=0.936) suggested that it is highly consistent and suitable for routine assessments.

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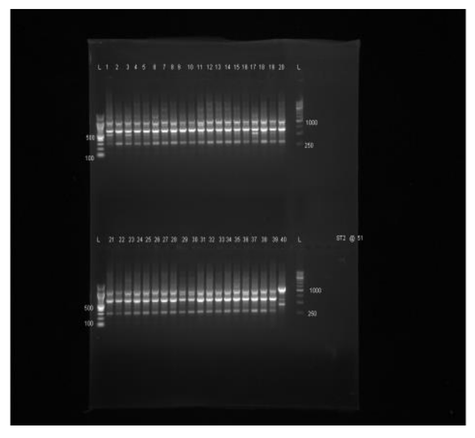
APPENDICES

Appendix I: Appendix I: SCoT Markers and Agarose Gel Electrophoresis with Amplification Profile



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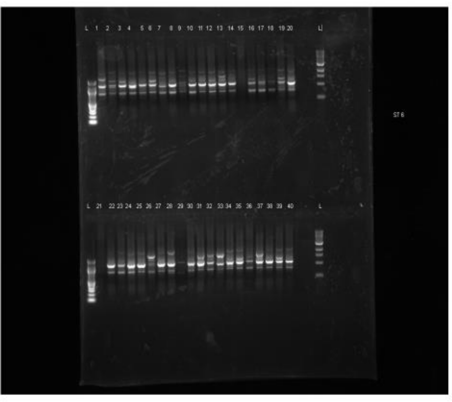

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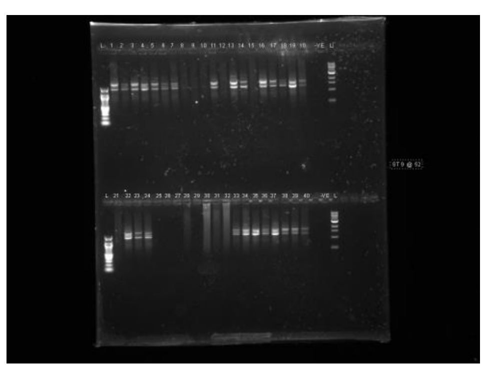

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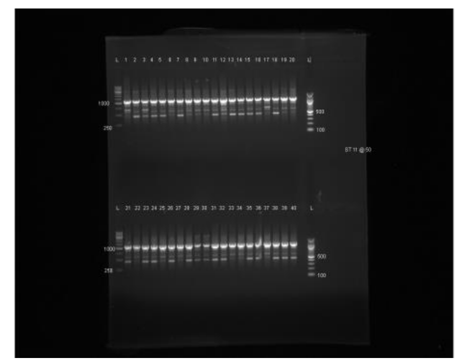

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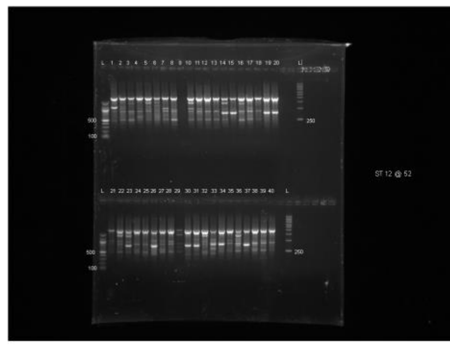

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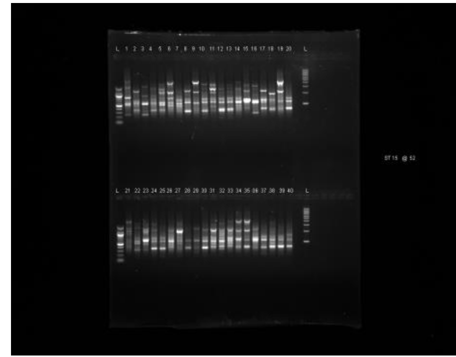

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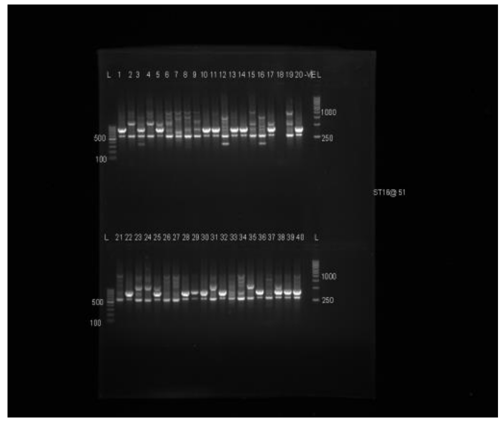

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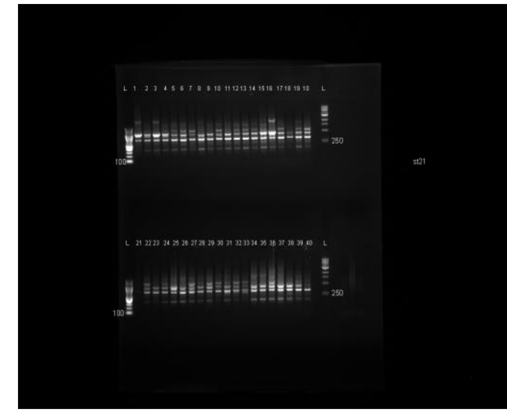

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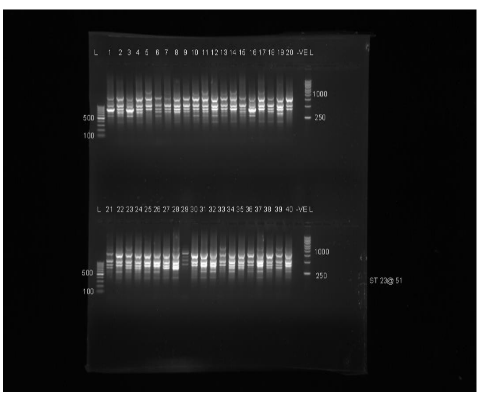

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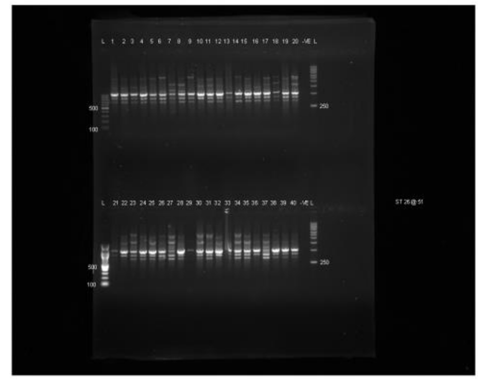

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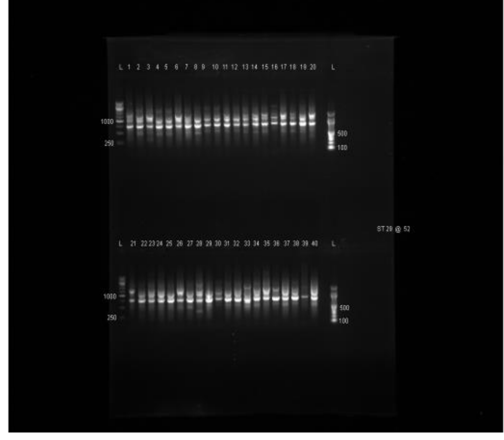

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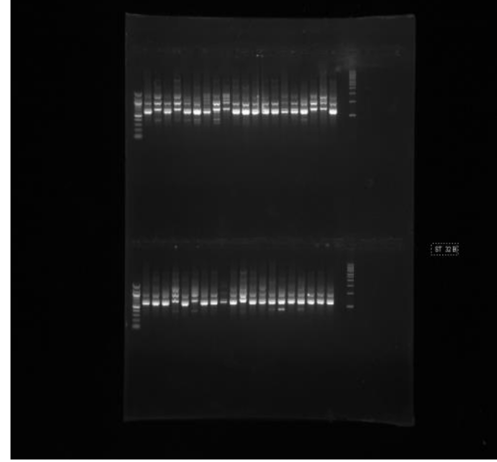

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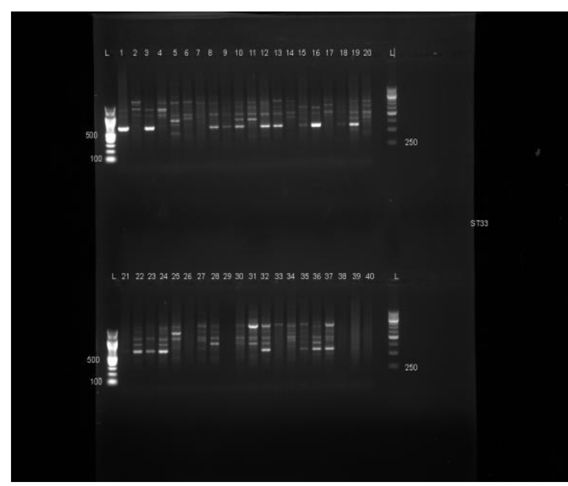

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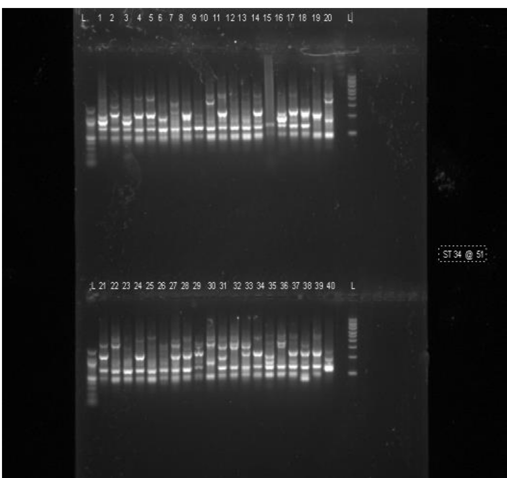

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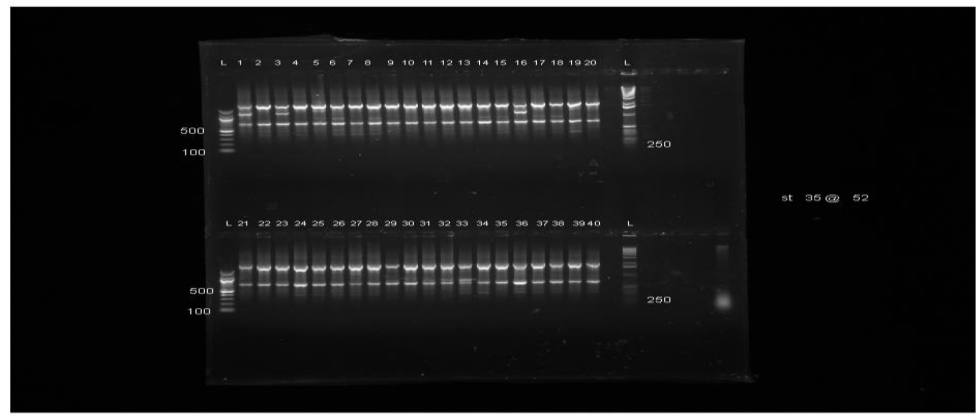

SCoT Marker 35

Appendix II: Appendix II: NACOSTI Research Permit

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