EXPLORING THE POPULATION STRUCTURE AND GENETIC DIVERSITY OF *Moringa oleifera* USING DARTSEQ DERIVED SNP MARKERS

JANTOR NDALO

I56/CTY/PT/39330/2016

THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE (BIOTECHNOLOGY) IN THE SCHOOL OF PURE AND APPLIED SCIENCES OF KENYATTA UNIVERSITY

NOVEMBER, 2025

DECLARATION

I, Jantor K Ndalo, declare that the work presented in this thesis is my original work and has not been presented for a degree or any other award in any other university or any other institution.

Signature …. ………. Date …27/11/2025……

Jantor Ndalo (B.Sc.)

I56/CTY/PT/39330/2016

Department of Biochemistry, Microbiology, and Biotechnology

Supervisors:

We hereby confirm that the candidate conducted the work reported in this thesis under our supervision.

Signature ………………………………. Date …17-11-2025……………………………….

Prof. Steven Runo
Department of Biochemistry, Microbiology, and Biotechnology
Kenyatta University

Signature ………………………………. Date …17-11-2025………………………………..

Dr. Alice Muchugi
Theme Leader - Biodiversity and Trees Genetic Resources
World Agroforestry

DEDICATION

To Dr. Alice Muchugi, my parents; Mr and Mrs Enock Ndalo, my siblings and to Victor Kirui for their support encouragement and prayer.

ACKNOWLEDGEMENTS

I acknowledge The Almighty God, who gave me the will, focus, strength health and guidance to conduct this research. I appreciate my family and friends for consistent encouragement, prayers and support during this study. I acknowledge World Agroforestry Tree's Theme (GRU and AOCC unit) for the space, germplasm, materials, and expertise for this study. Special thanks go to Prof. Steven Runo, Dr. Alice Muchugi and Dr. Prasad Hendre and the entire molecular laboratory team for their critique, guidance, and supervision throughout the study, Zakayo Kinyanjui, Simon Kang'ethe, Ludy Keino, Agnes Were and the entire ICRAF's Gene bank team for facilitating the germplasm collection. Added gratitude to KU's Department of Biochemistry, Microbiology and Biotechnology for providing the expertise and critiques during the entire study.

TABLE OF CONTENTS

DECLARATION ... ii

DEDICATION ... iii

ACKNOWLEDGEMENTS ... iv

TABLE OF CONTENTS ... v

LIST OF FIGURES ... viii

LIST OF TABLES ... ix

ABBREVIATIONS AND ACRONYMS ... x

ABSTRACT ... xi

CHAPTER ONE: INTRODUCTION ... 1

1.1 Background information ... 1

1.2. Statement of the Problem ... 3

1.3 Hypothesis. ... 4

1.4 Objectives ... 5

1.4.1 General Objective ... 5

1.4.2 Specific Objectives ... 5

1.5 Justification ... 5

CHAPTER TWO: LITERATURE REVIEW ... 7

2.1 Botanical and Phenotypic Description of Moringa ... 7

2.2 Origin and Distribution of Moringa ... 9

2.3 Ecology of Moringa ... 10

2.4 Moringa Establishment and Mating Systems ... 11

2.5 Uses Of Moringa. ... 12

2.5.1 Moringa as a Food Source ... 13

2.5.2 Medical Uses 16

2.5.3 Food Fortification...18

2.5.4 Agroforestry 19

2.5.5 Other Uses of Moringa 19

2.6 Genetic Diversity and Characterization of Moringa..20

2.7 DArTseq Technology 22

CHAPTER THREE: MATERIALS AND METHODS 27

3.1 Plant Materials 27

3.2 DNA Isolation and Genotyping by Sequensing 28

3.3 DNA Quality Analysis 30

3.4 SNP Discovery By DArTseq GBS Platform. 30

3.5 SNP Calling 32

3.6 Genetic Diversity Analysis 32

3.6.1 Moringa Phylogeny 32

3.6.2 Analysis of Molecular Variance...32

3.7 *Moringa oleifera* Structure Analysis 33

3.7.1 Structure Analysis 33

3.4.4 Principal Coordinates Analysis (PCOA)...34

CHAPTER FOUR: RESULTS 35

4.1 DNA Quality Analysis 35

4.2 Moringa Diversity Analysis 35

4.2.1 Moringa Phylogeny 35

4.2.2 Analysis of Molecular Variance...37

4.3 Population Structure Analysis 39

4.3.1 Principal Coordinate Analysis (PCoA) 39

4.3.2 Cluster Analysis in Structure 39

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS..41

5.1 Discussion..41

5.2 Conclusions 46

5.3 Recommendations 46

5.4 Suggestions for Future Studies and Suggestions..47

5.5 Challenges of the Study..48

REFERENCES..49

APPENDICES..57

Appendix I: Map Showing Moringa Distribution Across the Tropical Zones of The World. 57

Appendix II: Details Of the Plant Materials Used for This Study..58

Appendix III: Reagents, Instruments, And Their Suppliers 62

Appendix IV: Research Approval 63

Appendix V: Research Authorization..64

LIST OF FIGURES

Figure 2. 1 Images showing Moringa a) trunks b), leave c) seeds d) roots and e) flowers... 9
Figure 2. 2 Maps of moringa prevalence verses region infested by malnutrition..... 14
Figure 2. 3 A schematic diagram of Moringa parts and their medical applications . 18
Figure 2.4 Diagram showing DNA library preparation workflow 25
Figure 2.5 A schematic representation of DArTSeq workflows............................... 26
Figure 3.1 a map showing sample collection sites with colored sections indicating countries where samples were collected. 28
Figure 3. 2 schematic diagrams of DNA isolation workflows................................. 30
Figure 4. 1 Neighbor joining population tree (19 provenances) calculated from Bootstrap values of one hundred replicates and viewed in Figtree (Rambaut, 2012) showing four distinct clades of the 19 provenances used in the study .. 36
Figure 4. 2 Neighbor joining individual tree (330 accessions)................................ 37
Figure 4. 3 Pie chart illustrating the proportion of molecular variance among and within populations of *Moringa oleifera* in GeneAlex (6.503) (Peakall & Smouse 2012). Pops = population 38
Figure 4. 4 showing four cluster groups of the PCoA by provenance in GeneAlex. The color codes reflect Moringa sample country source while the circled regions represent the four population clusters. 39
Figure 4.5 **A** Graphical plot showing delta-K=2, **B** Population structure used to cluster the 95 Moringa accessions into two (K2), three(K3) or four(K4) groups. Admixed genomes of accessions are represented by bars with multiple colors. 40

LIST OF TABLES

Table 2. 1 2025 Global Moringa Products Market Overview (2025) 12

Table 2. 2 Nutrient Composition of *Moringa oleifera* Leaves and Their Functional Benefits (Mohai Ud Din et al., 2025) 16

Table 3. 1 Analysis of molecular variance among and within *M. oleifera* provenances showing 77% molecular variance within population and 23% among population. 38

ABBREVIATIONS AND ACRONYMS

AFLPs	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular
DArT	Diversity Arrays Technology.
DNA	Deoxyribonucleic Acid
GBS	Genotyping by Sequencing
HWE	Hardy Weinberg Equilibrium
ICRAF	International Centre for Research in Agroforestry
ISSRs	Inter-simple Sequence Repeat Markers
MSA	Microsatellite Analyzer
NGs	Next Generation Sequencing
PCR	Polymerase Chain Reaction
QC	Quality Check
QTL	Quantitative Trait Loci
RAPDs	Radom Amplified Polymorphism DNA
RFLPs	Restriction Fragment Length Polymorphism
SNPs	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats

ABSTRACT

Moringa oleifera is a versatile tree native to the foothills of the Himalayas and now naturalized in the tropics. *Moringa* is nutritionally significant owing to its high nutrient and antioxidant content. Despite its increasing use as a fodder crop, wood fuel, medicine, an anticoagulant and a potential source of biodiesel, the genetic diversity of *Moringa* across the tropics remains a subject of intense investigation. Previous studies have used hybridization-based methods such as microsatellites which have not fully resolved its genetic diversity. In this study, genetic diversity and structure of 95 accessions of *Moringa* from tropical regions were analyzed using single nucleotide polymorphisms. Genomic DNA was extracted from 19 provenances collected from Africa, Caribbean and Southeast Asia. 3968 SNP markers were identified using DArTSEQ technology, which combines complexity reduction methods with next-generation sequencing. These were culled to a final set of 1913 informative markers that were then used for population structure and genetic diversity analysis. Unweighted neighbour joining phylogeny and principal coordinate analysis revealed four distinct clusters related to the geographic origin: Caribbean (Haiti/Jamaica) and East African (Kenya/Tanzania) were identified to be a subset of the West African (Mali/Ghana) population while the Southern African (Malawi) segregated distinctly. Philippines samples clustered separately and farthest as expected. Analysis of molecular variance revealed high gene flow within populations (77 %) compared to among populations (23 %). Bayesian modelling in structure with best k being two still distinctly segregated the Southern African (Malawi) population from the other African regions suggesting a distinct introduction. The significant admixture of individuals noted in structure is typical of unnatural introductions possibly through anthropogenic events. This study highlights the power of SNP markers from DArTSEQ technology in elucidating the genetic structure and molecular diversity of *Moringa oleifera*. Malawi and Philippines being the most diverse should be considered as candidates for conservation improvement and utilization since they have higher ecosystem functioning. The discovered SNPs enable genome-wide association studies to accelerate marker-assisted breeding. These results too have implications for germplasm collection, improvement, conservation, utilization strategies and policies. Further research utilizing advanced genomic tools will enhance our knowledge of *Moringa oleifera* and support its sustainable utilization for various applications.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Moringa oleifera (Family *Moringaceae*) is a multipurpose fast-growing deciduous tree naturalized in tropical and subtropical regions of Asia, Africa, South and Central America, and the Caribbean. Appendix 1 is a map showing *Moringa* distribution across the tropical zones of the world.

Moringa answers to the nickname "the natural nutrition of the tropics" because all its parts are harnessed for nutrients such as protein, vitamin C, β-carotene, mineral salts, and natural antioxidants. *Moringa* has more vitamins, calcium, potassium, iron, and protein than carrots, oranges, milk, bananas, spinach and yoghurt respectively (Rockwood et al., 2013). Thus, *Moringa* is an ideal source species for the food fortification industry since these nutrient constituents are deficient in most household diets in developing countries (Hendre et al. 2019). In agriculture, *Moringa* is utilized as a fodder plant due to its high nutritional value. Pharmacologically, *Moringa* is used in the treatment of various conditions such as diabetes, high blood pressure, arthritis, and anemia due to its prominent level of antioxidants. In biotechnology, it is a potential raw material for biodiesel and anticoagulants for water purification *(*Popoola and Obembe 2013*;* Shahzad *et al.,* 2013; Leone *et al.,* 2015;Tian *et al.,* 2015 ;Adebayo *et al.,* 2015)

Moringa is resilient to harsh environments such as arid regions (Daba 2016; Thakur & Bajagain 2020). Therefore, it is an economically important plant for arid areas where people face hunger and nutrient related deficiencies (Gandji et al. 2018).

Despite these benefits, *Moringa* remains underutilized (Kumssa et al.2017;Gandji et al. 2018) and is listed among the 101 neglected crops by the African Orphaned Crop Consortium (AOCC) (K. Dawson et al. 2019; Hendre et al. 2019). However, genome assemblies and diversity study of *Moringa* can help accelerate it's current breeding, domestication and conservation efforts (Dawson et al. 2018).

Previously, the genetic diversity of *Moringa* was determined using amplified fragment length polymorphism (AFLP), random amplification of polymorphic DNA (RAPD), and Cytochrome P450 gene sequence and microsatellite marker analyses. As these are all nucleic acid amplification techniques, they are laborious and throughput limited (Kusolwa & Rwegasira 2018, Kumar et al, 2009; Barilli et al. 2018). DArTSeq technology is a robust, cost effective, reproducible and high-resolution marker technique, which can evaluate hundreds to thousands of markers across the entire *Moringa* genome (Garavito *et al.* 2016; Nemli *et al.,* 2017; Barilli *et al.,* 2018; Borzęcka *et al.,* 2018). It combines DArT complexity reduction techniques with next generation sequencing platforms to generate genome-wide SNPs at quick and high resolution and low cost without prior knowledge of the species' genome sequence (Barilli et al. 2018). DArT generates two types of markers: SilicoDArT and SNP markers (*Kilian*, 2015). SilicoDArT are dominant markers that score for the presence or absence of a single allele whereas DArTseq-based SNPs are co-dominant markers (Alam, et al . 2018). This marker platform has been used to study genetic diversity, genetic mapping, and population structure in multiple crop species.

This study aimed to determine the genetic diversity and population structure of *Moringa* accessions from Kenya, Mali, Malawi, Nigeria, Tanzania, Ghana, Jamaica, Haiti, and Philippines using the DArTSeq technique. Our findings demonstrate how

modern genotyping technologies are applicable in the breeding, improvement, identification of threatened populations of *Moringa*, for conservation and management of gene banks.

1.2. Statement of the Problem

Moringa oleifera is a fast-growing, drought-tolerant tree widely recognized for its nutritional, medicinal, and environmental benefits. Its leaves, seeds, and pods are rich in essential nutrients and bioactive compounds, making it a valuable resource for food security, health, and agroecological restoration. These attributes have led to its widespread cultivation across tropical and subtropical regions, with farmers and industries increasingly adopting it for diverse applications (Busani et al., 2011).

Despite its growing popularity, the genetic diversity and population structure of *Moringa oleifera* remain poorly characterized, especially across its expanded geographic range. As the species spreads through farmer-led initiatives and informal seed systems, there is limited understanding of how much genetic heterogeneity is retained or lost across different countries and provenances. This lack of molecular data poses a challenge for conservation planning, breeding program design, and sustainable utilization.

While *Moringa oleifera* is known for its wide adaptability and genetic richness, studies reveal that genetic erosion has occurred in cultivated populations, particularly in Africa and parts of the Caribbean. This is primarily due to: Founder effects (Muluvi et al., 1999). Limited breeding and selection pressure: (Alavilli et al., 2022). Geographic bottlenecks: (Rockwood et al., 2013; Ondieki et al., 2024). Anthropogenic mixing: (Shahzad et al., 2013; Mgendi et al., 2010).

Current breeding efforts largely rely on conventional methods such as phenotypic selection and cross-pollination, which are constrained by environmental variability and limited genetic insight (Salunkhe et al., 1977). These approaches have not yielded consistent improvements in yield, resilience, or quality traits, partly due to the absence of robust genomic tools and baseline diversity information. Without clear knowledge of genetic variation, efforts to enhance *Moringa's* applications, whether nutritional, industrial, or ecological—remain speculative and inefficient.

Moreover, the absence of curated genomic resources hinders the development of improved cultivars and the identification of climate-resilient genotypes. As global interest in *Moringa* intensifies, there is an urgent need to apply advanced molecular technologies such as DArTSeq SNP genotyping to uncover its genetic architecture. Marker-Assisted Selection (MAS), which depends on well-characterized genetic diversity and population structure, offers a promising pathway to accelerate breeding, conservation, and domestication efforts.

This study seeks to fill the existing knowledge gap by generating high-resolution molecular data on *Moringa oleifera* across multiple countries and provenances. The findings will inform strategic conservation, guide breeding interventions, and support the sustainable scaling of *Moringa* as a multipurpose agroforestry species in tropical landscapes.

1.3 Hypothesis.

1. The 330 *Moringa oleifera* samples from nineteen provenances across eight countries and three regions exhibit minimal or no molecular diversity.

2. Populations of *Moringa oleifera* across the eight countries are genetically homogeneous.

1.4 Objectives

1.4.1 General Objective

To assess the molecular diversity and genetic structure of *Moringa oleifera* populations across multiple countries and provenances, in order to inform conservation and utilization strategies and guide future breeding programs.

1.4.2 Specific Objectives

1. To evaluate the level of molecular diversity among 330 samples collected from 19 provenances across 8 countries using DArTSeq SNP markers.
2. To determine the genetic structure and degree of population differentiation among *Moringa oleifera* populations from the eight countries.

1.5 Justification

There is a high rate of sensitization on the usefulness of moringa across the World. For instance, bodies like Moringa fund, Trees for Life, Global Moringa Network and Moringa News are set to educate on its production and usefulness. Governmental initiatives have been rolled out to market the usefulness of *Moringa* in countries like Ghana and Benin (Gandji *et al*., 2018). *Moringa* has become highly regarded around the world due to its multiple nutritional, pharmacological and environmental benefits. This array of benefits has attracted both agricultural and industrial attention so that massive cultivation of this plant is being done (Tian et al., 2015). The plant is also well adopted to arid and semiarid environment; a trait that makes it widespread within

the tropics (Tian *et al*., 2015). Increase in demand and consumption of *Moringa* therefore necessitates a well-defined breeding program to ensure its sustainability.

Molecular Characterization of *Moringa* germplasm is ideal in determining detailed genetic similarities and differences between and within *Moringa* populations (Olawaele *et al*., 2012, Mashood Alay *et al*., 2012), which will reduce chances of duplications in conservation blocks and breeding programs. These will maximize the use of genetic diversity between genetic populations to breed superior varieties and to identify *Moringa* populations with narrow genetic base and adoption to appropriate conservation. In doing this, there will be sustained and improved *Moringa* genetic resources and products.

CHAPTER TWO

LITERATURE REVIEW

2.1 Botanical and Phenotypic Description of Moringa

Moringa.oleifera (Lam) belongs to the order Brassicales, Family Moringaceae, and Genus Moringa (Raja *et al*., 2016). The monogeneric Moringa (Chukwuebuka, 2015) comprises 14 described species: *M. arborea*, native to Kenya; *M. rivae* indigenous to Kenya and Ethiopia; *M. borziana*, endemic to Somalia and Kenya; *M. pygmaea* native to Somalia; *M. longituba* indigenous to Kenya, Ethiopia and Somalia; *M. stenopetala* indigenous to Kenya and Ethiopia; *M ruspoliana* indigenous to Ethiopia; *M. ovalifolia* indigenous to Namibia and Angola; *M. drouhardii*, *M. hildebrandi* endemic to Madagascar; *M. peregrine* indigenous to Red sea and Horn of Africa, *M. Concanensis.* There are potentially more undescribed species, particularly from Africa (Olsona 2017). Notably, *M. oleifera* is most widely studied and cultivated due to its high adaptability to various biotic and abiotic conditions (Leone *et al*., 2015a, Chukwuebuka, 2015).

Moringa is an authentic diploid (2n=28) (Rufai *et al*., 2013). It is considered one of the plants with the smallest genome size(350mb) hence ideal as a model for functional genomic research in characterization of woody plants.(Tian et al., 2015). According to Muluvi (2004) Moringa breeding patterns estimates are: 26% selfing and 74% outcrossing. The plant is pollinated by birds, bees and other insects. (World Agroforestry Centre database; Morton, 1991; Jyothi *et al*., 2004).

Moringa is a deciduous tree with scant foliage. It has a resemblance of leguminous plants from a distance but so distinct in fruiting stage (World Agroforestry Centre database). It grows to a height range of between 8m to 12M (Raja *et al*., 2016).

Moringa leaves are bi-pinnate and most commonly tripinnate leaves whose growth size is 45-60cm.Single leaflets are ovate, elliptic, or oblong in shape, 10-24mm in length and 5-18mm in width with a slightly larger and obovate shaped terminal leaflet on each individual petiole. Moringa leaves alternate and spirally arranged themselves on the twigs. They are dark green on the interface and pale on the underside The leave petiole and leaflets bear tiny pore at the base which exude amber colored liquid (Csurhes & Navie, 2016).

Moringa has a broadly spreading slackened auxiliary panicle five pale green sepals approximately 8-10cm.long. Flowers are aromatic, androgynous with 5 petals that are thin veined yellow or cream with yellow midrib in some cases. The flower stamen has a waxy yellow anther which possesses hairy filaments, and a hairy oblong ovary containing a single locule comprising ovules. Flowering sets off with the first 6 months of planting and would only occur once a year for seasonally cool regions unlike for regions with more constant seasonal temperature and rainfall where lowering can occur throughout the year (World Agroforestry Centre database, Chukwuebuka, 2015).

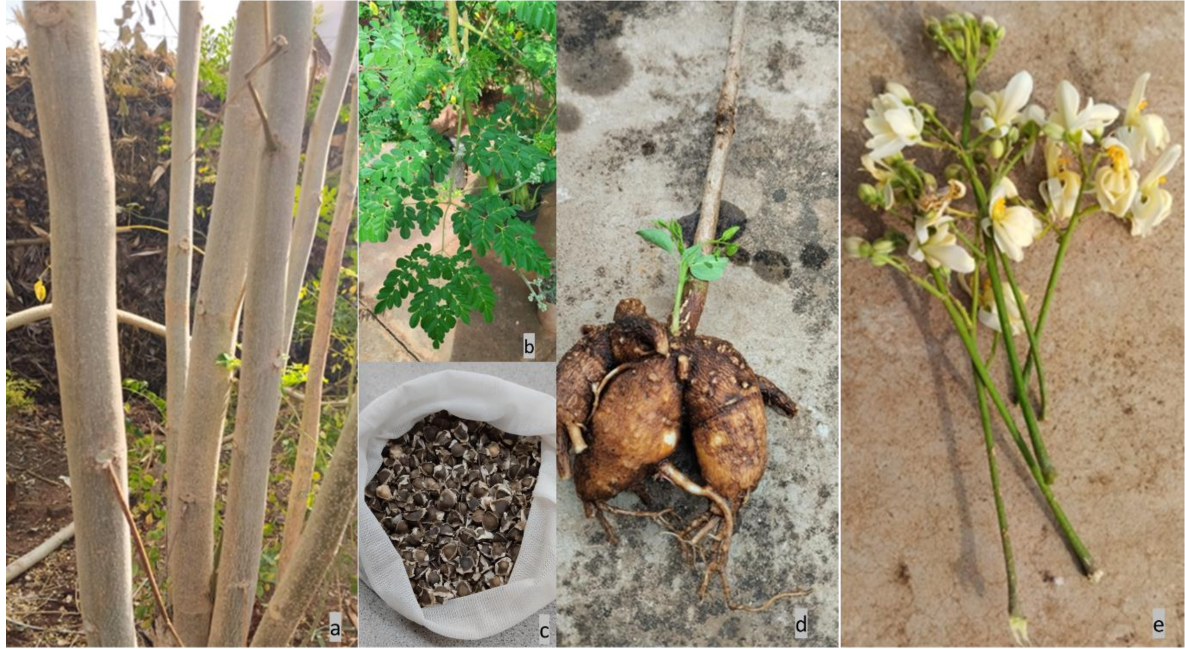

Figure 2. 1 Images showing Moringa a) trunks b), leave c) seeds d) roots and e) flowers.

The pendulous linear, three-sided fruits have nine longitudinal ridges. The pods which are dark green during flowering take 3 months to mature after flowering. Normally, each pod carries up to twenty-six seeds. An average sized Moringa tree is known to produce thousands of pods with numerous seeds. The pod is dehiscent.

The tree has a deep stout taproot, a wide spreading system of lateral roots and a single main trunk. Its wood is relatively soft, but the back is tenacious which exudes whitish to reddish gum under damage (Csurhes & Navie, 2016)

2.2 Origin and Distribution of Moringa

Moringa is native to north western India but currently naturalized in other tropical tracts of the world (Saini *et al*., 2013). It is extensively cultivated in Sub Saharan Africa and in some other parts of Africa (Verdcourt, 1985; GBIF, 2007). It is also highly naturalized in Southern and Eastern Asia (Verdcourt, 1985; Lu and Olson,

2001). Moringa is also naturalized in tropical zones of north and South America (ICRAF, 2001). Moringa cultivation is also done in multiple pacific Islands (Hancock and Henderson, 1988; ICRAF, 2001; PIER, 2007). Moringa is now well established in the following specific countries: India, Malaysia, Oman, Qatar, Saudi Arabia, United Arab Emirates, Yemen, Republic of Afghanistan, Bangladesh, Benin, Burkina Faso, Cameroon, Chad, Eritrea, Ethiopia, Gambia, Ghana, Guinea, Haiti, Indonesia, Iran, Kenya, Kiribati, Liberia, Mali, Marshall Islands, Mauritania, Myanmar, Nepal, Niger, Nigeria, Northern Mariana Islands, Pakistan, Philippines, Senegal, Sierra Leone, Sudan, Tanzania, Thailand, Togo, Uganda, Vietnam, Zanzibar.

2.3 Ecology of Moringa

Moringa establishes well around stream banks, and savanna zones though it is known to be highly adoptive to harsh semiarid environments with annual rainfall of as low as 500mm (Bosch, 2004; Ebert, 2014) and high temperature to the heights of 48°C (Csurhes & Navie, 2016). It has a strong antioxidant system which aids it in adapting to average saline conditions hence a mild reduction in its mineral quality (Elbert, 2014). Moringa optimum growth is recorded in lowlands but can still thrive in altitudes above 2000m (Elbert, 2014; Gandji *et al.*, 2018). The tree requires well drained neutral pH ranged soils which could be clay loam or sandy soil (Paliwal *et al*, 2011, Norman *et al* 2014). Moringa is not well acclimatized to cold freezing where in such condition it dies back or shades off the leaves and quickly sprouts forth in a favorable environment. This explains its rampant dissemination across the tropical regions of the world.

2.4 Moringa Establishment and Mating Systems

Genetic and environmental factors influence plant mating systems which are sensitive to plant density and population size (Schaal et al., 1998). Moringa breeding programs can be efficiently designed when Proper estimates of outcrossing rates are available (Birger et al., 2011). Moringa typically exhibit a self-pollinating mating system, where each flower contains both male and female reproductive organs. In addition to self-pollination, some species of Moringa may also exhibit outcrossing, where pollen is transferred between different plants (Muluvi et al., 2004) . Outcrossing can introduce genetic variation within the population, potentially enhancing adaptability to changing environmental conditions and increasing genetic diversity (Rodger et al., 2024). This genetic diversity resulting from Moringa outcrossing can be beneficial for resilience to diseases, pests, and other environmental stresses. Overall, the mating system of Moringa plants, which includes both self-pollination and outcrossing, plays a crucial role in their reproductive success, genetic diversity, and overall adaptability in different habitats.

Planting Moringa can be achieved by three methods: Direct sowing, sowing in germinators and transplanting of young seedlings and stem cutting. Moringa seeds develop and grow stronger when planted directly. They require little or no pretreatment for germination and have high germination rates of 80% but deteriorate to 50% after a year. Once planted, germination can occur after 5-12 days or take as long as after 30 days depending on seed age and pretreatment modes (Fuglie, 1999). Growth from stem cutting is faster but results to a shallow root system making the tree predisposed to moisture stress and wind damage (Church World Service, 2000)

2.5 Uses of Moringa.

Moringa plant is highly regarded due to its multiple commercial, medical, and nutritional importance. It has been labelled different names by different communities on the basis of its benefits as follows: natural nutrition of the tropics (Shahzad *et al*., 2013), because all its parts are useful, 'mother's best friend' because of its utility in increasing milk production in mothers,(Shahzad *et al*., 2013) a 'wonder tree' due to its economic importance (Rufai *et al*., 2013). Basically, all the parts of this tree are utile (Gandji *et al*., 2018). Table 2.1 below shows global supply and demand of Moringa products based on (Fortune Business Insights, 2025)

Table 2. 1 2025 Global Moringa Products Market Overview (2025)

Category	**Details**	**source**
Market Size (2025)	USD 9.3 billion	Future Market Insights
Forecast Market Size (2035)	USD 23 billion	Custom Market Insights
CAGR (2025–2035)	9.6%	Fortune Business Insights
Top Product Types	Leaf powder, seeds, oil, capsules, tablets	Business Research Insights
Major Applications	Food & beverages, personal care, pharmaceuticals	Research and Markets
Leading Supply Regions	India, Kenya, Philippines, Nigeria, Sri Lanka	Spherical Insights
Key Demand Regions	North America (largest), Asia-Pacific (fastest growing)	Market Data Forecast
Distribution Channels	Online retail, specialty stores, supermarkets	LinkedIn Market Overview
Supply Chain Challenges	Tariff shifts, logistics, regulatory compliance, geopolitical uncertainty	OG Analysis

2.5.1 Moringa as a Food Source

Moringa oleifera is widely recognized as a potent nutritional resource, with nearly every part of the plant contributing to dietary and health benefits—making it a strategic tool in combating malnutrition across the developing world.

Building on its reputation as "the natural nutrition of the tropics," Moringa's leaves, immature pods, flowers, and fruits are rich in essential nutrients and bioactive compounds. Beyond the impressive comparisons—such as having 4× more vitamin A than carrots, 7× more vitamin C than oranges, and 2× more protein than yogurt—Moringa also contains over 90 bioactive compounds, including flavonoids, polyphenols, and glucosinolates, which contribute to its antioxidant, anti-inflammatory, and antimicrobial properties.

Its leaves are especially nutrient-dense, offering high levels of calcium, potassium, iron, magnesium, and zinc, along with all essential amino acids. Immature pods, often consumed as vegetables, are rich in fiber and vitamin C, while flowers are edible and contain calcium and potassium. Seeds yield Moringa oil, which is high in oleic acid and used both nutritionally and cosmetically.

In traditional medicine, Moringa has been used to support digestive health, blood sugar regulation, and cardiovascular function, and recent studies suggest its potential in managing cholesterol levels, liver health, and oxidative stress. Its versatility and resilience in arid climates make it a valuable crop for food security and sustainable agriculture.s (Hendre *et al*. 2019) as reflected in figure 2.2 below.

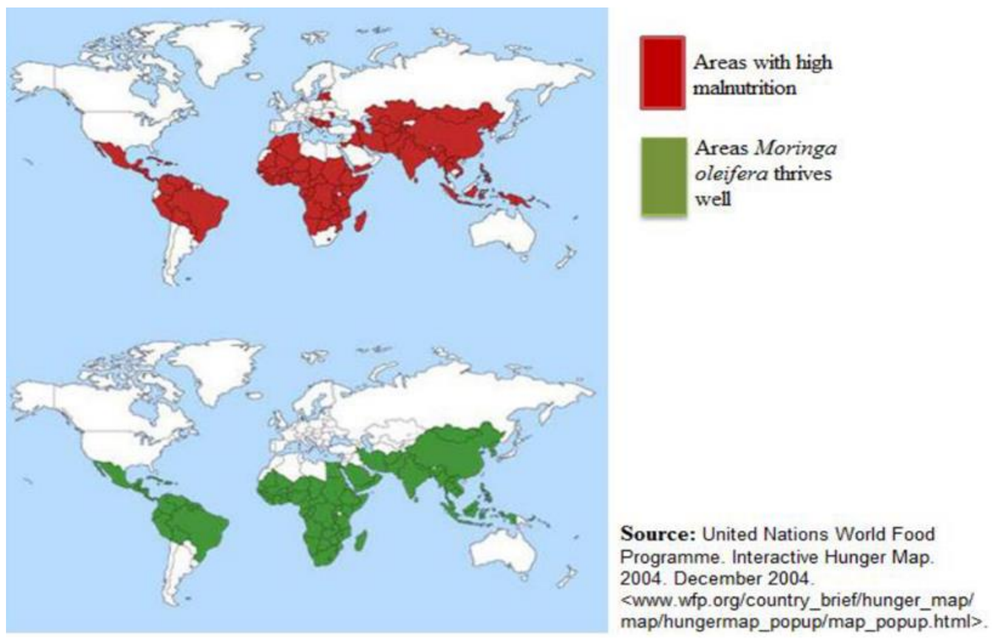

Figure 2. 2 Maps of moringa prevalence verses region infested by malnutrition.

Moringa can therefore be a good supplement to address stunting, malnutrition, and hunger. According to Leone et al, (2015) these nutrient constituents make moringa a good supplement for counties facing malnutrition challenges.

The oil extricated from moringa seeds is exceptionally nutritious with high oxidative properties due to its lack of polyunsaturated fatty acids. The anti-oxidants: flavonoids, ascorbic acid, carotenoids and phenolics present in Moringa are significant in improving fatty food shelf life (Dillard & Bruce German, 2000; Siddhuraju & Becker, 2003; Mohdaly *et al*., 2010). Moringa is utilized by lactating mothers to increase milk production hence tagged 'mother's best friend' in Philippines (Shahzad *et al*., 2013)..

Moringa leaves are consumed as cooked vegetables or dried and crushed into a powder for culinary use which include preparation of stews, soups, omelets, classical chicken dish, pasta sauce when mixed with olive oils and salt, candies etc. Moringa

powder can also be incorporated in any type of food to supplement for proteins vitamins and mineral salts (Oyeyinka & Oyeyinka, 2018).

Moringa also serves as animal feed and fodder (Sanchez *et al.*2006a; Raja *et al* 2016; Oyeyinka & Oyeyinka, 2018). This is because of its high proteins, mineral salts and vitamin A, B, C. *M. oleifera* leaves in animal feed enhance digestibility and dry matter sequestration besides increasing milk quality and quantity (Sanchez *et al.,*2006a.). The table below shows nutrient composition of *Moringa oleifera* leaves and their functional benefits.

Table 2.2 Nutrient Composition of *Moringa oleifera* Leaves and Their Functional Benefits (Mohai Ud Din et al., 2025)

Nutrient/Compound	Approximate Content	Functional Benefits
Protein	25–30% (dry weight)	Builds and repairs tissues; supports immune function
Vitamin A (β-carotene)	6.8 mg/100g (fresh leaves)	Enhances vision, skin health, and immune response
Vitamin C	220 mg/100g (fresh leaves)	Boosts immunity, acts as antioxidant, improves iron absorption
Calcium	440 mg/100g (fresh leaves)	Strengthens bones and teeth; supports nerve and muscle function
Iron	7 mg/100g (fresh leaves)	Prevents anemia; supports oxygen transport in blood
Potassium	259 mg/100g (fresh leaves)	Regulates blood pressure and fluid balance
Magnesium	42 mg/100g (fresh leaves)	Supports enzyme activity, muscle function, and bone health
Zinc	0.6 mg/100g (fresh leaves)	Enhances immune response and wound healing
Fiber	2–3% (dry weight)	Aids digestion, regulates blood sugar, promotes gut health
Phenolic compounds	High levels	Provide antioxidant, anti-inflammatory, and antimicrobial effects
Flavonoids	Rich content	Protect against oxidative stress; support cardiovascular health
Essential amino acids	Present in balanced profile	Support growth, metabolism

2.5.2 Medical Uses

Medically, the plant is utilized by traditional healers, doctors, and nutritionists to treat multiple ailments and cub malnutrition. It’s seed extracts function as antipyretics, antimicrobials, antifungal and antitumor remedy (Chukwuebuka, 2015). Methanolic

extracts from roots serve as analgesics and have anti-convulsive properties (Leone et al., 2015; Hassanein, 2017). Flowers have aphrodisiac, abortifacient, anti-inflammatory, anti-tumor properties(Leone et al., 2015). Gum produced by the tree is applied in treating dental caries and treating headaches, fevers, intestinal complaints, asthma, syphilis and rheumatism when mixed with sesame (Chukwuebuka, 2015). The plants stem and stem back serve to treat cancer, ulcers, and earaches and prevent spleen enlargement and formation of glands in the neck. Its roots function as purgatives, treatment of eye and fever infections, glandular swelling, and diabetes (Anwar et al., 2007). The Schematic diagram figure 2.3 on the next page is a summary gives a summary of Moringa medical applications.

LEAVES: laxative, applied to sores as sinapism, treating headaches, piles, fevers, sore throat, bronchitis, eye and ear infections, scurvy and catarrh; leaf juice is believed to be anti-hypergylysemic (Razis, Ibrahim, & Kntayya, 2014), serves as an inti-inflamatory to glands.It also acts as an anti-fibrotic/ulcer and antihyperlipidemic agent (The Wealth of India, 1962; Dahot, 1988; Morton, 1991; Pal et al., 1995; Makonnen et al., 1997; Ghasi et al., 2000; Fuglie, 2001; Patel et al., 2010; Razis, Ibrahim, & Kntayya, 2014,)

ROOTS: treatment of gastric ulcers. and mucosal leisons,treating against kidney stones(Antilithic), applied as a rubefacient, vesicant, carminative, antifertility, anti-inflammatory,cardiac/circulatory tonic, used as a laxative, abortifacient, rheumatism treatment, inflammations, articular pains, lower back or kidney pain and constipation (The Wealth of India, 1962; Dahot, 1988; Padmarao et al., 1996; Ruckmani et al., 1998; Anwar et al., 2007, Razis, Ibrahim, & Kntayya, 2014)

STEM BACK:Rubefacient, vesicant and used to cure eye diseases and for the treatment of delirious patients, prevents enlargement of the spleen and formation of tuberculous glands of the neck, to destroy tumors and to heal ulcers. The juice from the root bark is put into ears to relieve earaches and also placed in a tooth cavity as a pain killer, and has anti- tubercular activity (Bhatnagar et al., 1961; Pal et al., 1995; Siddhuraju and Becker, 2003; Anwar et al., 2007; Razis, Ibrahim, & Kntayya, 2014)

GUM: treating dental caries, and is astringent and rubefacient; the gum in combination with sesame oil, relieves headaches, fevers, intestinal complaints, dysentery, asthma and and can serve as an abortifacient, andin treating syphilis and rheumatism (Fuglie, 2001; Razis, Ibrahim, & Kntayya, 2014).

Medicinally applied as a stimulant, aphrodisiac, abortifacient, cholagogue; that cure inflammations, muscle diseases, hysteria, tumors, and enlargement of the spleen; lower the serum cholesterol, phospholipid, triglyceride, VLDL, LDL cholesterol to phospholipid ratio and atherogenic index; decrease lipid profile of liver, heart and aorta in hypercholesterolaemic rabbits and increased the excretion of faecal cholesterol (Bhattacharya et al., 1982; Dahot, 1998; Mehta et al., 2003; Siddhuraju and Becker, 2003; Razis, Ibrahim, & Kntayya, 2014)

Seed extracts are hepatoprotective: decreasing liver lipid peroxides, antiasmatic, antiathritic, thiocarbamate and isothiocyanate glycosids have been isolated from the acetate phase of the ethanolic extract of Moringa pods. other useful seed properties are: anti-inflammatory, antioxidant, antmicrobial and anticancer properties (Caceres et al., 1992; Faizi et al., 1998; Lalas and Tsaknis, 2002; Patel et al., 2010;Razis, Ibrahim, & Kntayya, 2014)

Figure 2. 3 A schematic diagram of Moringa parts and their medical applications

2.5.3 Food Fortification

Moringa is increasingly applied as a food fortification in many parts of the globe including Africa (Oyeyinka & Oyeyinka, 2018). For instance, in Nigeria moringa is applied in fortifying food formulations of corn soy and peanuts, amalla: stiff dough from yam, herbal biscuits, bread cake and yoghurt (Shiriki *et al*., 2015, Oyeyinka &

Oyeyinka, 2018).Moringa seeds and leaves powder are added to different foods commonly weaning foods (Tsutsumi & Onouchi, 2016).

2.5.4 Agroforestry

Agroforestry is the integration of trees on farm who's biological and economic interactions with other crops or animals lead to escalated productivity reduced risk, ensure sustainable income while impacting positively on the environment (Kumssa *et al*., 2017). Moringa economic projects generate economic benefits while contributing to building resilient landscapes in the following ways: controlling soil erosion in areas affected by strong wind and long dry spells, its green leaves can be used as a suitable mulch and soil fertilizer (Thakur & Bajagain, 2020). The tree is also useful as a border plant, and it offers aesthetic value to the environment. Moringa is also key as a semi shade to shield other crops from damaging direct sunlight in intercropping. (Kumssa *et al*., 2017).

2.5.5 Other Uses of Moringa

As a natural coagulant/ flocculant, *M. oleifera* is applied in water and sewage treatment plants in eliminating turbidity in drinking water, (Suarez *et al.*, 2003; Bhatia *et al* 2007).

The many other uses of moringa include production of biomass for biodiesel, fertilizing in alley cropping, production of biogas from leaves (Leone *et al*., 2015; Popoola *et al* 2016;), domestic cleaning agent from crushed leaves, in fencing, production of fertilizer from seed cakes, blue dye production from wood, juiced expressed from leaves used as foliar, preparation of green manure from leaves. The tree is also useful in gum production from tree trunks, clarifier of honey and sugarcane

juice using powders seeds, supply nectar for honey production, (Fuglie,1999; Adebayo *et al*., 2011), as a biopesticide that prevents seed dumping off when its leaves are incorporated in the soil, production of pulp from its wood, rope production from its back, its gum provide tannins for tanning hides. (fuglie,1999; Adebayo *et al*., 2011; Popoola and Obembe; 2013; Leone *et al*., 2015)

2.6 Genetic Diversity and Characterization of Moringa

Genetic resources conservation entails acquisition of the right germplasm, assessing for functional traits and preservation using appropriate procedures (Guimaraes et al., 2007). To achieve all this, the use of molecular marker technology is pivotal. The advent of molecular marker technology has made it easy to deduce sound genetic information from crops required in decision making for sustentation activities (Raza et al., 2015).

Moringa characterization is key for effective conservation, improvement, protection, and utilization of it's germplasm for breeding programs. Genetic markers variations in moringa genome are crucial in monitoring inter and intra species DNA sequence variation and in creating new sources of genetic variations by inculcating new and favorable traits within related species. (Varshney *et al.*, 2005). It is also helpful in the identification of rare alleles and genotypes that can be targeted for sustainable utilization. Key Moringa conservation decisions such as collections, identification of core collections and their distribution will be guided by this data.

Moringa Diversity and structure have previously been analyzed using various molecular markers. For instance, Muluvi *et al.,* (1999), using AFLP markers for 140 Indian, South Malawi and Kenya accessions deduced that the Indian genotype had

high genetic diversity. Investigating the genetic diversity of cultivated and wild *Moringa* genotypes along the coastal region of Tanzania, using RAPD markers, Mgendi *et al.,* (2010) proved that wild genotypes were more diverse than the cultivated ones. Using ISSR, RAPD and *cyt*

P450 as markers for eight Indian cultivars, Saini *et al.,* (2013) discovered that Indian cultivars were diverse and ISSR markers were the most effective marker for genetic diversity assessment. In their study on the intraspecific relationships of six populations of Nigerian *M. oleifera* species using 20 SSR markers, Popoola *et al.,* (2016) demonstrated that Nigerian accessions were diverse. Shahzad *et al.,* (2013) studied genetic diversity of 161 Moringa accessions from across continents by 19 SSR markers and chloroplast *atpB* gene. They demonstrated a high genetic diversity in wild collections of *M. oleifera,* corroborating previous findings (Mgendi *et al.,* 2010). Ondieki & Hunja, 2024 in their study on 164 *Moringa oleifera* genotypes from 17 coastal Kenyan provenances, revealed high genetic similarity across populations using DArTseq SNP markers, with key findings showing low within-population variation (2.55%).

Despite these studies, the genetic diversity of *Moringa* across the tropics is still poorly understood. This is possibly due limited regional representation, poor marker resolution, the excessive cost of PCR marker-based techniques. Use of PCR -based techniques is laborious and time consuming for large numbers of samples and only probe a few *Moringa* genome loci (Gill-Langarica et al., 2011; De La Fuente et al., 2013). Gandji *et al.,* (2018) reiterated the high morphological and molecular diversity within and among *Moringa* germplasm that is to be farther discovered through use of next generation techniques.

High resolution analysis can be used by combining these markers, but this would be very costly, time demanding and laborious. Ideal genetic markers should score up to a range of hundreds to thousands of markers across the entire genome, be cost-effective and have simple and reliable procedures (Kumar *et al.*, 2009). Though informative in depiction of moringa's genetic diversity, the markers above are gel-based, have low reproducibility, are dominant, labor intensive and they demand prior knowledge of sequence information before analysis (Jaccoud 2001; Wittenberg, 2007). SSR and AFLPs have high throughput, are efficient and can be assayed on highly parallel genotypic platforms, they however pause a challenge of high cost of assaying. (Stodart, *et al.*, 2005) Gandji *et al*, 2018 in his review on status and use of *Moringa oleifera* recommends the use of next generation sequencing (NGS) platform to discover genome-wide genetic markers for building cost effective and time saving dense genetic maps. DArTseq technology; a most resent genotyping by sequencing NGS platform has subjugated most of these challenges incurred by other genetic markers.

2.7 DArTseq Technology

As highlighted, studies on Molecular diversity of *M. oleifera* using gel-based markers like RAPD, SSR and AFLP have already been done. These gel-based markers are not fast in assaying many marker loci. These limitations can be averted by use of specialized technology like high throughput capillary electrophoresis equipment which can better allelic variability, reproducibility, and rapidity. However, they are still restricted by sequential nature related limitations and are costly. There is need to develop marker techniques that produce highly polymorphic markers which extended

vastly through the genome giving high throughput molecular information at low cost. (Cortés *et al*., 2011; NEMLİ *et al*., 2017)

Single Nucleotide polymorphism (SNP) Marker system is a technique highly utilized in genetic diversity studies because SNP markers are widespread throughout the genome of crops (Genal *et al.*,2009). SNP detection can be achieved by different methods which have both advantages and disadvantages in terms of cost, throughput and time factor (Cortés et al., 2011). A most recent genotyping by sequencing technology; DArTSeq, combines DArT complexity reduction techniques with next generation sequencing platforms to generate genome-wide SNPs at low cost, high speed and resolution without prior knowledge of crop sequence information (Barilli *et al*., 2018).

DArTseq technology has been applied in molecular diversity study on many plant species including wildtype by directly using SNPs and silico DArT markers. DArTseq platform can develop immense polymorphic markers for generating dense genetic maps at nominal costs. It's high throughput and high-density genetic maps have accelerated the dynamism of QTL detection (Tudi *et al.,* 2011; Raman *et al*., 2012). The technology is feasible on polyploids and can be developed rapidly for any genome de novo. DArTseq has been applied on more than 400 species popularly on novel species (http://www.diversityarrays.com/) including *Triticum* spp. (Stodart et al. 2005), *Secale cereale* (Bolibok-Bragoszewska et al. 2009) , *Citrullus lanatus* (Ren & Ray 2016) and *Sorghum bicolor* (Mace et al. 2008). GBS DArTseq technology allows extensive discovery of SNPs in diverse non-model organisms. Application of GBS and DArT technologies on thousands of plants genotypes yield high genetic discrepancy and variation on their germplasm *(Garavito et al., 2016).* DArTseq

technology has been massively utilized in genome profiling and diversity analysis, Monitoring composition of intricate DNA samples, genome selection, genetic and physical mapping, simultaneous marker assisted selection for several traits, rapid introgression of genome regions in selected backcrossing programs among many others (Wittenberg et al., 2005).The principle behind DArTSeq is that, a genomic representation has two fragments type; constant fragment which is common in DNA sample of individuals of the same species and random variable segment also called molecular marker which is not repetitive in all (Raman *et al*., 2012). The technology entails, isolating Genomic DNA, complexity reduction of DNA sample, amplification, sequencing, scanning, data manipulation and Data analysis (Curtolo *et al*., 2017). DArTSeq genotyping is used to reduce genome complexity by digesting genomic DNA with two restriction enzymes: *PstI* and *MseI*. After digestion, barcoded adapters are ligated to the DNA fragments, enabling sample identification and multiplexing. These adapter-ligated fragments are then amplified through PCR to enrich the library. Sequencing is carried out on the Illumina HiSeq 2500 platform, generating single-end reads of 77 base pairs. This read length capture the informative regions adjacent to the restriction sites.

Marker scoring is performed using DArTsoft14, a proprietary bioinformatics pipeline. Two types of markers are identified and scored:

- **SilicoDArT markers**, which are scored based on the presence (1) or absence (0) of specific restriction fragments.
- **SNP markers**, which are detected within the sequenced fragments and similarly scored in binary format.

Both marker types are aligned to the reference genome to determine their chromosomal positions. This alignment supports downstream applications such as genetic diversity analysis, linkage mapping, and trait association.

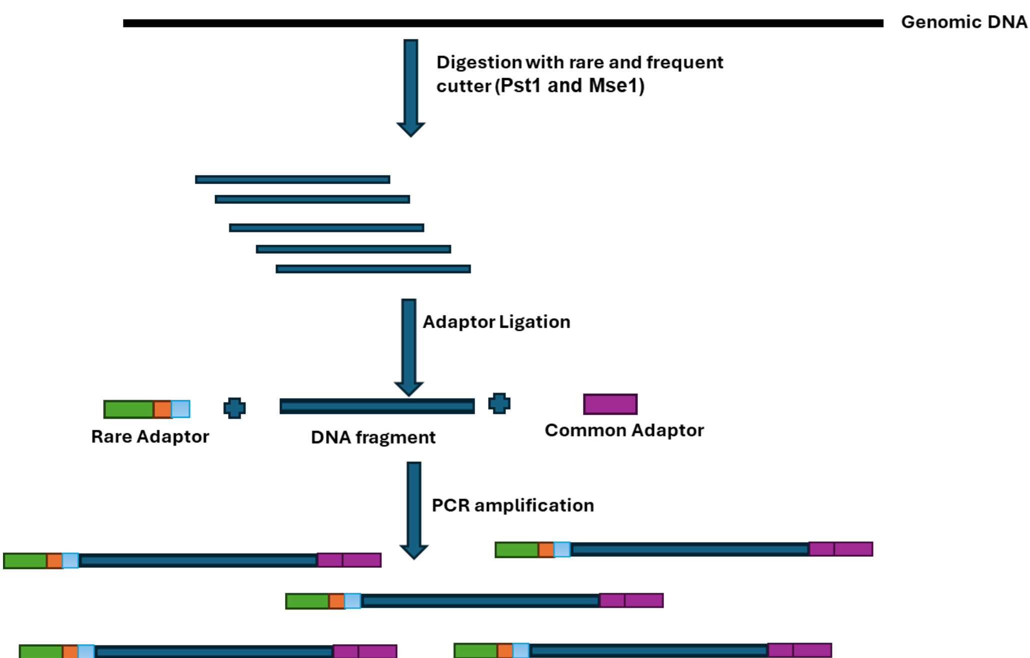

Figure 2.4 Diagram showing DNA library preparation workflow

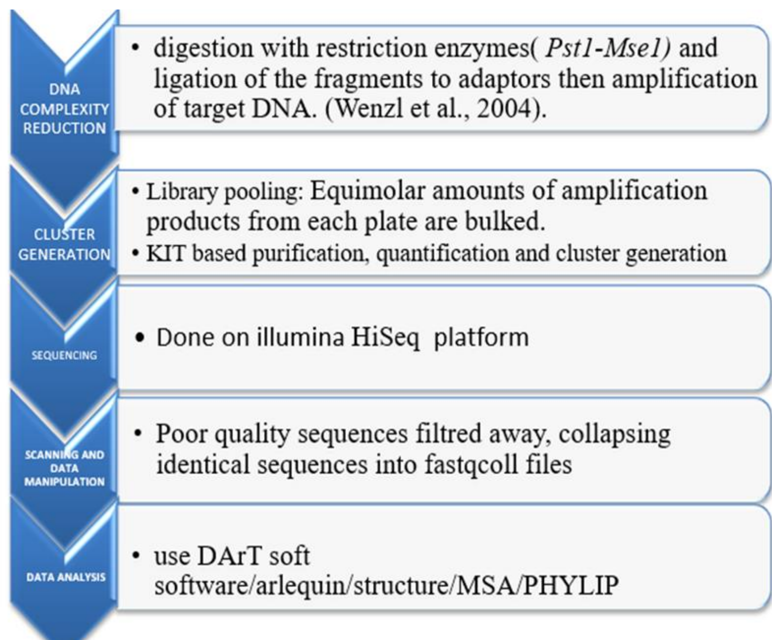

Figure 2.5 A schematic representation of DArTSeq workflows

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant Materials

A total of 330 plant samples—individual specimens collected for laboratory analysis—were obtained from 95 accessions, each representing a genetically distinct source of M. oleifera maintained in the World Agroforestry (ICRAF) gene bank and three samples from a field collection (T5, T6, and T7). The samples had been collected from 19 field sites: Kenya (Mbololo-30, Meru-26, Machakos-one, Nairobi-one Busia-one, Samburu-one, Rongo-one, and Bondo-22), Mali (Niamakoro-one and Segou-one), Malawi (Mangochi-two, Makanjira-one, Nthiwatiwa-one) Philippine (Pangasinan-one, Caloocan-one) Tanzania (Singida-one), Haiti-one, Jamaica-one, and Ghana-one. Philippine samples were considered as reference samples owing to its geographic proximity to India since there was no Indian accession in the gene bank during the period of study. The table in appendix 2 is a list of *Moringa* samples and their provenances with their population distribution.

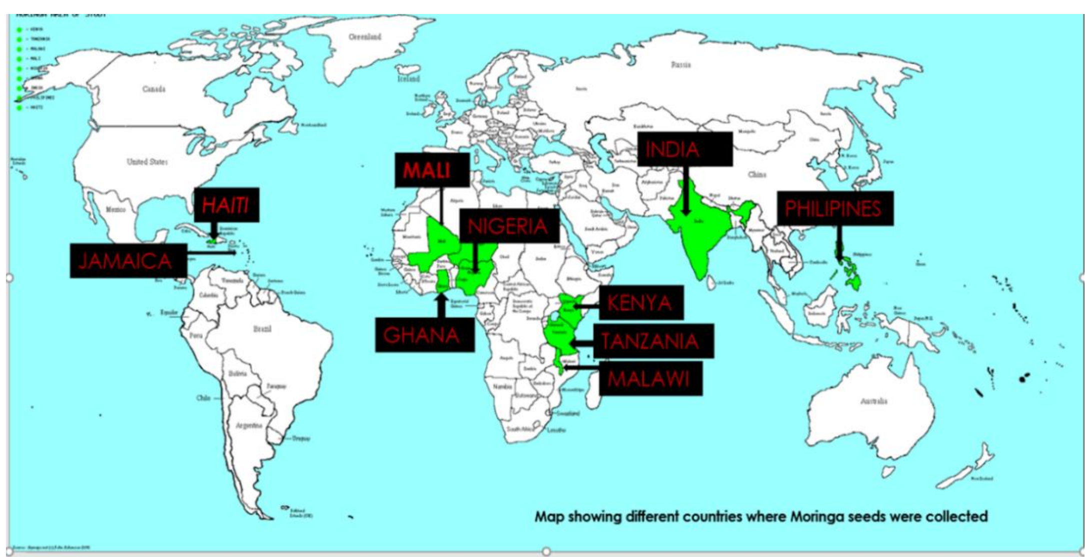

Figure 3.1 a map showing sample collection sites with colored sections indicating countries where samples were collected.

3.2 DNA Isolation and Genotyping by Sequensing

Genomic DNA was extracted from plant leaf tissue using the ISOLATE II Plant DNA Kit (Bioline, BIO-52070), following the manufacturer's protocol. Approximately 100 mg of fresh leaf tissue or 50 mg of dried leaf material was homogenized using liquid nitrogen and a pre-chilled mortar and pestle. The resulting powder was transferred to a 2 mL microcentrifuge tube for lysis.

For cell 400 µL of Lysis Buffer PA1 was added to the homogenized sample, followed by 10 µL of RNase A solution. The mixture was vortexed thoroughly and incubated at PA for 10 minutes. In cases where the sample absorbed excessive buffer, additional Lysis Buffer PA1 was added, and the volumes of RNase A and Binding Buffer PB were adjusted proportionally

The crude lysate was filtered by loading it onto an ISOLATE II Filter (violet) placed in a 2 mL Collection Tube and centrifuged at 11,000 × g for 2 minutes. The clear

flow-through was collected, and if a pellet was visible, the supernatant was carefully transferred to a new 1.5 mL microcentrifuge tube without disturbing the pellet. To adjust DNA binding conditions, 450 µL of Binding Buffer PB was added and mixed thoroughly.

The sample was then loaded onto an ISOLATE II Plant DNA Spin Column (green) placed in a new 2 mL Collection Tube and centrifuged at 11,000 × g for 1 minute. The flow-through was discarded. For sample volumes exceeding 700 µL, the loading and centrifugation steps were repeated.

To wash and dry the silica membrane, 400 µL of Wash Buffer PAW1 was added to the column and centrifuged for 1 minute at 11,000 × g. This was followed by two washes with Wash Buffer PAW2: first with 700 µL and then with 200 µL, each centrifuged at 11,000 × g. The final centrifugation lasted 3 minutes to ensure complete drying of the membrane.

DNA was eluted by placing the spin column into a new 1.5 mL microcentrifuge tube and adding 50 µL of pre-warmed Elution Buffer PG (PA) directly onto the membrane. After a 5-minute incubation at PA, the column was centrifuged at 11,000 × g for 1 minute. A second elution was performed using an additional 50 µL of Elution Buffer PG into the same tube. The eluted DNA was stored at–20°C until further use.

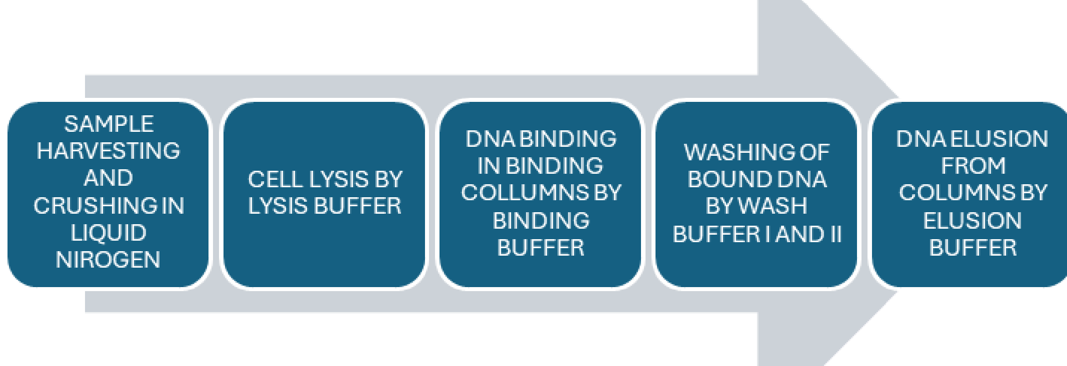

Figure 3. 2 schematic diagrams of DNA isolation workflows

3.3 DNA Quality Analysis

The quality, quantity and integrity of DNA were estimated using the NanoDrop ND2000c spectrophotometer (Thermo Fisher Scientific), Qubit ® 2.0 fluorimeter (Thermo Fisher Scientific) and 0.8%(w/v) agarose (Sigma-Aldrich: A9539) gel autoradiographs stained with 0.3X GelRed (Biotium: Cat No. 41001) respectively. Details of all reagents and instruments used are captured in the table in appendix 3. For all samples, DNA concentrations were standardized to final concentrations of 50 ng/µl. Samples were then used in DArTseq analysis, which was outsourced to DArT Pty. Ltd., Canberra, Australia (Alam et al. 2018).

3.4 **SNP Discovery By DArTseq GBS Platform.**

DArTseq, a proprietary genotyping by sequencing NGS technology developed by diversity array Technology Pty Ltd, Canberra, Australia, was applied in Moringa SNP marker discovery (https://www.diversityarrays.com/about-us/). Concisely, the process was sectioned into complexity reduction by endonucleases to enrich genome representations, cluster generation and sequencing by Illumina HiSeq 2500.

Genomic representation of the samples was prepared as follows: ninety-six plex DArTseq libraries for the 330 moringa samples was prepared using 50ng of DNA for each sample. Each sample was digested separately by P*st*1-M*se*1 restriction enzymes to augment the samples' genomic representations with single copy sequences; a process labeled complexity reduction. Complexity reduction products were ligated to site specific barcode adaptors and common adapters and amplification done according to Roman *et al*. (2014). Library pulling of the PCR product was done by spooling equimolar quantities of amplification products from each sample of the ninety-six wells of microtiter plate. The pooled samples were introduced to the c-Bot for bridge amplification where molecules of each library were attached to the flow cell surface and amplified to form clonal clusters.

The flow cell having the clusters generated during bridge amplification was transferred to HiSeq 2500 Illumina sequencer (Illumina, USA) for sequencing. Sequencing the single reads was done for seventy-seven cycles to generate base calling files (*.bcl files) which were the main output files for downstream data conversions. The *.bcl files generated underwent the following steps: first they, were collapsed into fastqcall files by Illumina bcl2fastq software embedded in primary workflow (Barilli *et al*., 2018). Secondly the sequencing reads were split according to the barcode sequence by DArTdb software, a process called de-multiplexing. The de-multiplexed samples were taken through two filtrations: more stringent for barcode sequence and less stringent for remaining part of the sequencing read as described by Elgea *et al* (2014). The sequence tags were copied to DArTdb software for permanent storage. Compressed sequence tags were extracted from DArtdb and loaded to DArTsoft14 for marker data extraction.

3.5 SNP Calling

The SNPs were assigned ‘0’ for reference allele homozygous,’1’ for SNP allele homozygote ‘2’ for heterozygous and ‘-‘null allele homozygous. The SNP data was filtered for a minimum of 0.05% heterozygosity per marker and a maximum of 5% missing data per sample whereas 20% missing data per marker in excel worksheet.

The DArTseq markers informativeness was vetted by calculating polymorphism information content (PIC), which gave a maximum PIC of 0.5 as described by (Kusolwa & Rwegasira, 2018). Only 1913 informative markers and all the 330 genotypes were selected after filtering.

3.6 Genetic Diversity Analysis

3.6.1 Moringa Phylogeny

The data was analyzed to estimate pairwise Nei’s chord genetic distance (Nei *et al*., 1983) using Microsatellite Analyzer (MSA) ver. 4.05 (Dieringer & Schlötterer, 2003). The genetic distance matrix was then used to draw Neighbor Joining tree using PHYLIP ver. 3.695 updated in April 2013 (Felsenstein, 1993). Two types of trees were drawn, one for population, based on the 19 provenances and individual tree based on the 330 accessions The trees were viewed using figTree ver 4.0 (Rambaut, 2012)

3.6.2 Analysis of Molecular Variance

Genetic variation within and among different *M. oleifera* provenances was partitioned by Analysis of molecular variance (AMOVA) in GenAlex version 6.503(Peakall & Smouse, 2012). An output pairwise PhiPt matrix was created at 999 permutations and

the PhiPT and P values determined. The PhiPT values were utilized in giving value to the variation estimates and giving a summary of the degree of diversity among and within the populations.

3.7 *Moringa oleifera* Structure Analysis

3.7.1 Structure Analysis

Hardy Weinberg Equilibrium (HWE) tests were conducted using Arlequin ver 3.5.2.2 *(Excoffier, et al*., 2010) using default parameters (chain length of 1,000,000 dimerization steps of 100000) to identify SNPS in HWE. The *p* -values were corrected for false discovery rate (FDR) as per Benjamini & Hochberg (1995). P-value (q-value $\leq$0.05) was used. The SNPs in HWE were applied in deciding moringa structure.

To determine the genetic structure among the 95 *M. oleifera* accessions, Bayesian clustering algorithm in STRUCTURE software v 2.3. (Pritchard *et al.,* 2000) was applied. The parameters used were as follows: Haploid data, an admixture model with correlated frequencies, number of clusters ,K, spurning between 2 and 10 with 10 runs per k value except for k=1 for which only one run was done, 100,000 Markov Chain Monte Carlos (MCMC) steps and 100,000 iterations , (Mogga *et al.,* 2018). Delta K, which is an ad hoc quantity analogous to the second order change of the log probability of data corresponding to the number of clusters inferred by structure was used as a criterion to determine the best K value in structure HARVESTER as per Evanno *et al.* (2005).

3.4.4 Principal Coordinates Analysis (PCOA)

To elucidate genetic relatedness and general assortment among the 95 *MORINGA.oleifera* accessions, Multivariate principle coordinate analysis (PCA) based on genetic similarity matrix was performed in GenAlex version 6.503 (Flanagan, 2014). Using genetic distance, the principal coordinates were generated through a covariance matrix with data. Individual populations were tagged codes for ease in visualization.

CHAPTER FOUR

RESULTS

4.1 DNA Quality Analysis

A total of 330 samples comprising ninety-two accessions yielded satisfactory quality and quantity of DNA and were applied for SNP discovery using Genotyping by Sequencing (GBS) approach as employed by DArTseq. A total of 3,968 SNPs were discovered through DArTseq. The data was deposited in World Agroforestry Dataverse. SNP data obtained was filtered as follows: maximum of 5% missing alleles per marker, minimum of 0.05 heterozygosity per marker, 1,913 SNPs were found to be suitable for further analysis.

4.2 Moringa Diversity Analysis

4.2.1 Moringa Phylogeny

Two phylogenetic trees were generated: one for population, based on the 19 provenances and individual tree based on the 330 accessions. Accessions clustered into four main clades, which were related to geographic origin. These were the Philippine clade, Malawi clade, West Africa & Caribbean clade (Ghana+Mali+Haiti+Jamaica) and East African clade (Kenya-Tanzania). This clustering was strongly supported by strong boot strap values (Figure 4.1). The Philippine and Kenya/Tanzania clade were the most distantly related. The greatest difference in genetic distance values was between Mbololo-Kenya accessions (0.177) and Pangasinan, Philippine (0.2692) implying that the two of them were the most distantly related. The two accessions from the Philippines which are close to Moringa center of origin were considerably distantly related from all the other populations.

The Ghanian, Malian, Haitian, and Jamaican accessions clustered into a larger clade inferring common ancestry. This was similarly observed for the Kenyan and Tanzanian accessions as reflected by both population (figure 4.1) and individual (figure 4.2) phylogenetic trees. Caribbean accession consistently grouped with Malian and Ghanian population in both population and individual phenograms suggesting a common origin. East Africa accessions were closer to the Caribbean and west Africa population than they were with the Philippines and Malawi accessions by population genetic distance insinuating a common entry point.

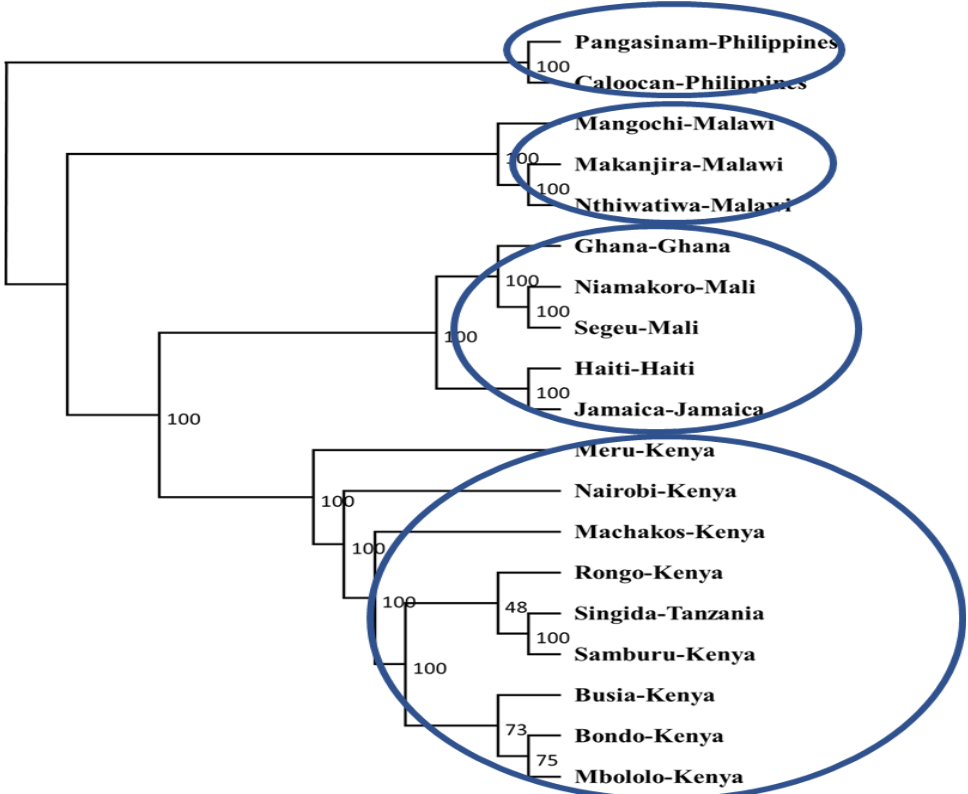

Figure 4. 1 Neighbor joining population tree (19 provenances) calculated from Bootstrap values of one hundred replicates and viewed in Figtree (Rambaut, 2012) showing four distinct clades of the 19 provenances used in the study

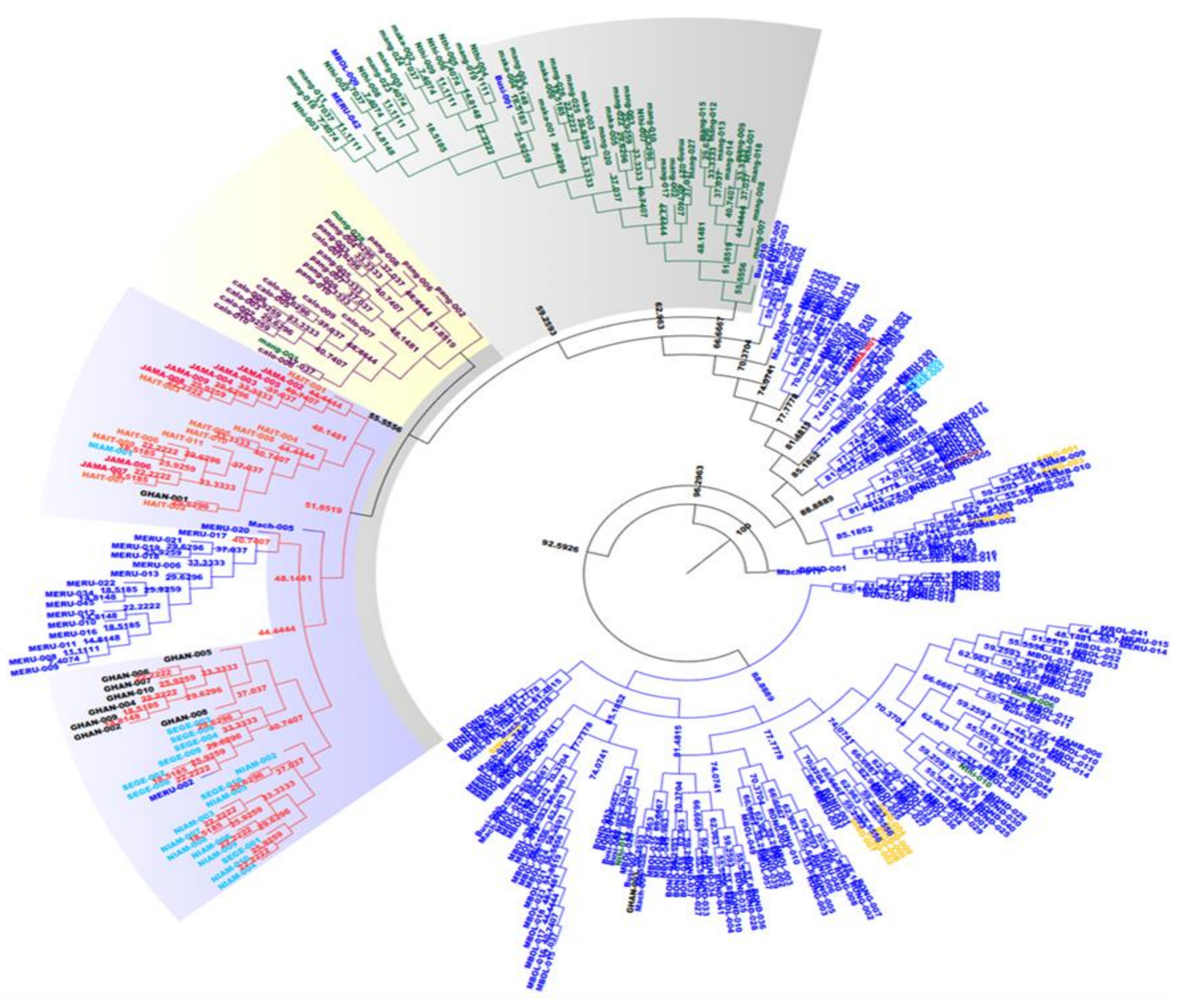

Figure 4. 2 Neighbor joining individual tree (330 accessions)

4.2.2 Analysis of Molecular Variance

Analysis of molecular variance (AMOVA) among and within populations indicated a notable intra and interpopulation differentiation (Table 1, Figure 5) with 23% and 77% of variation appropriated to among populations and within populations, respectively (*P* 0.019, 999 permutations and PhiPT of 0.17). This indicates high intraspecific divergence within the populations and a low divergence among the populations.

Table 3.1 Analysis of molecular variance among and within *M. oleifera* provenances showing 77% molecular **variance** within population and 23% among population.

Source	df	SS	MS	Est. Var.	%
Among Population	7	11077.125	1582.446	26.922	23%
Within Population	652	59632.160	91.460	91.460	77%
Total	659	70709.285		118.382	100%

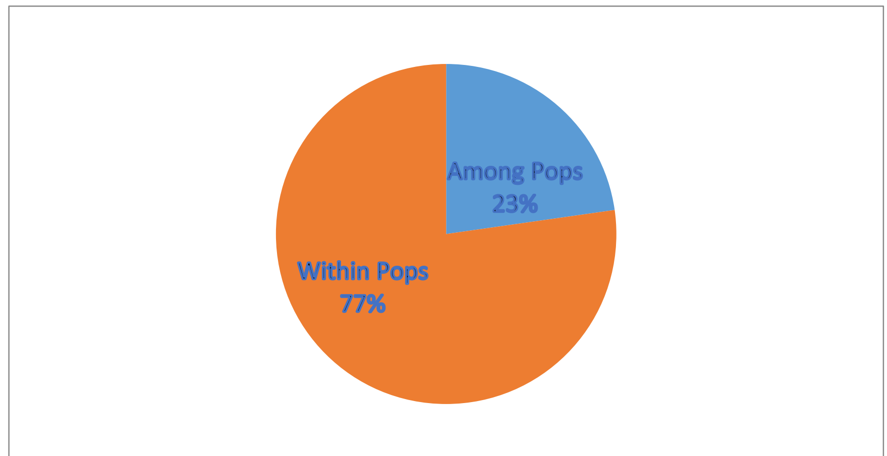

Figure 4. 3 Pie chart illustrating the proportion of molecular variance among and within populations of *Moringa oleifera* in GeneAlex (6.503) (Peakall & Smouse 2012). Pops = population

4.3 Population Structure Analysis

4.3.1 Principal Coordinate Analysis (PCoA)

The DArTSeq SNP data matrix was used to generate the principal coordinate analysis for the ninety-five accessions of *Moringa. oleifera* from eight different countries. PCOA grouped the ninety-five accessions into four main cluster groups: (a). 2 Philippine accessions, (b). 5 Malawi accessions(c). 1 Ghana+2 Mali+1 Haiti+1 Jamaica accessions and (d). 81 Kenya+Tanzania accessions. The cluster groups are in tandem with the NJ Bootstrap tree.

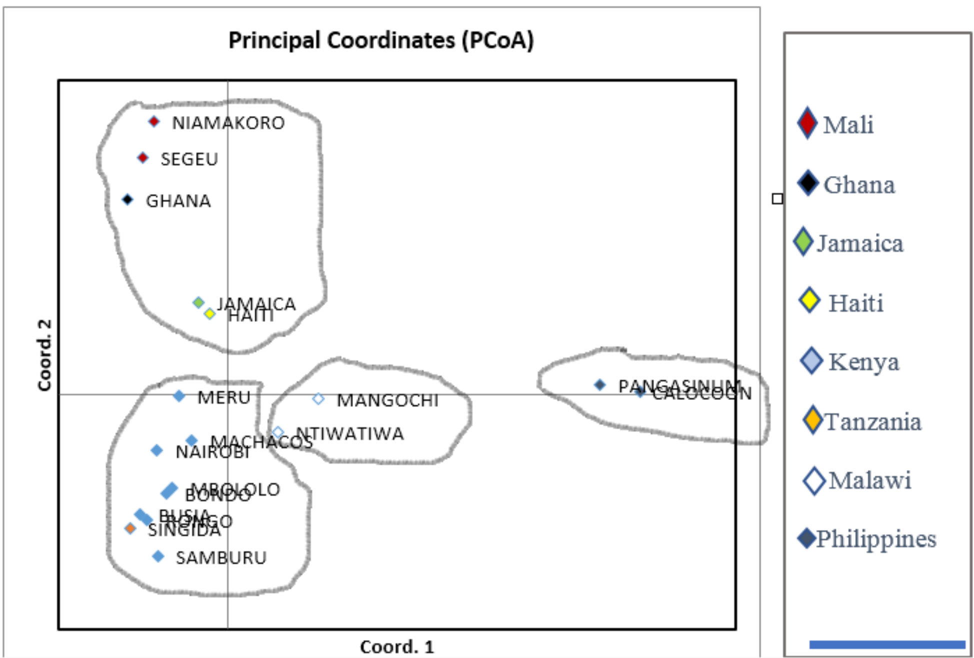

Figure 4. 4 showing four cluster groups of the PCoA by provenance in GeneAlex. The color codes reflect Moringa sample country source while the circled regions represent the four population clusters.

4.3.2 Cluster Analysis in Structure

Out of the 1913 SNPs run in Arlequin, 237 (245 after adjusted for FDR) were in HWE. Malawian population consistently segregated from the rest of the population at K2,

K3 and K4. Evanno et al., 2005 Structure Harvester, tool identified K=2 as optimal value of K as reflected in the graphical plot in figure 4.3a below.

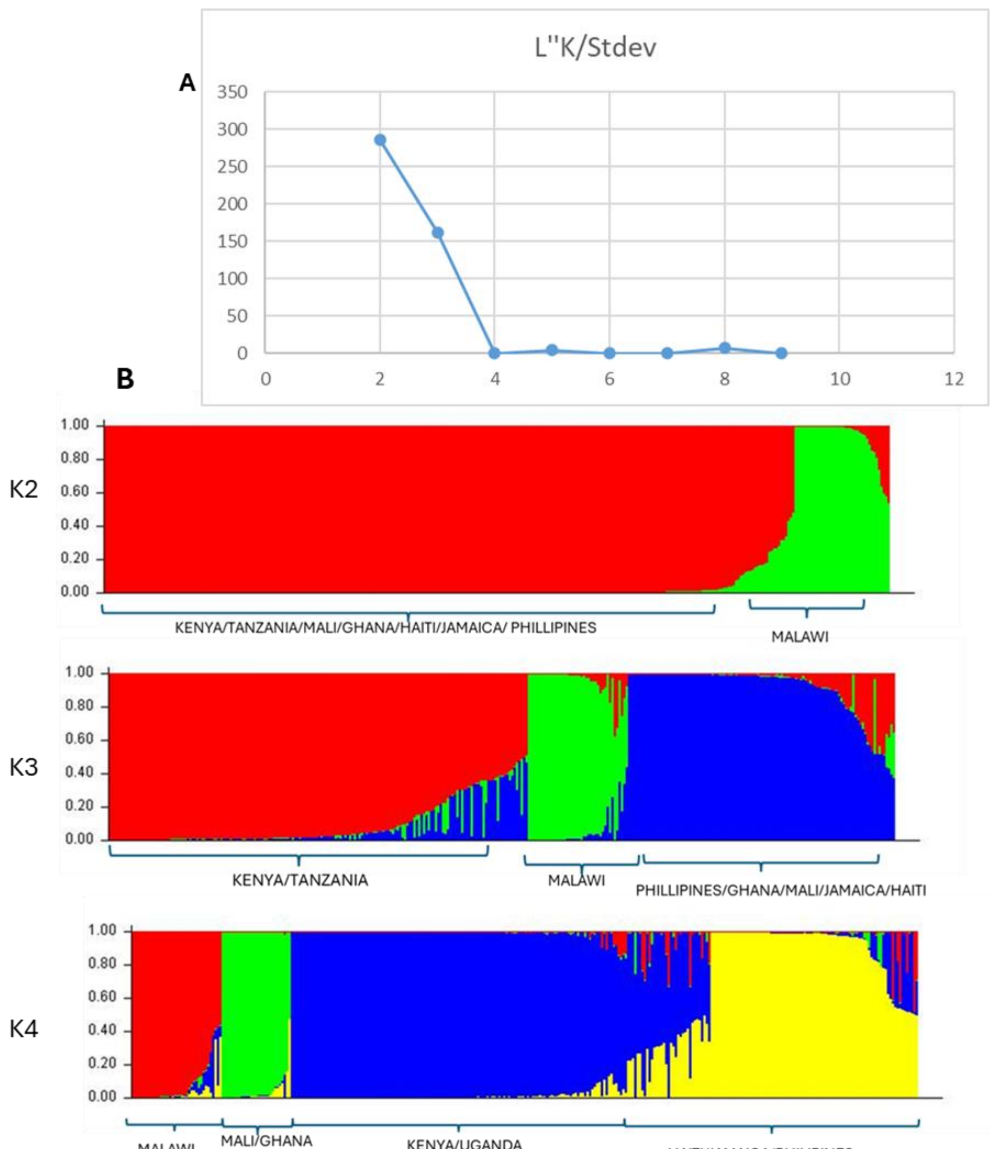

Figure 4.5 **A** Graphical plot showing delta-K=2, **B** Population structure used to cluster the 95 Moringa accessions into two (K2), three(K3) or four(K4) groups. Admixed genomes of accessions are represented by bars with multiple colors.

Malawian population distinct clustering corroborated with PCoA and neighbor joining trees. The clustering of Caribbean (Haiti/Jamaica), Southeast Asia (Philippines) and West Africa population (Ghana/Mali) as well as East African (Kenya/Tanzania) population was also common with PCOA and the phenetic trees.

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Analysis of genetic diversity and structure of *Moringa oleifera* is pivotal for effective identification, conservation, improvement, protection, and germplasm utilization for breeding programs (Saini et al. 2013). It is also important for monitoring inter and intra species DNA sequence variation and for the creation of new sources of genetic variations by inculcating new and favorable traits within germplasm (Varshney et al. 2005; Marone et al. 2012). Such information can also be helpful in the identification of rare alleles and genotypes that can be targeted for sustainable use. Relatedly, it is also important for making key decisions for the conservation of *Moringa* such as sample collection, identification of core collections and their distribution (Runo 2019).

In this study, DArTSeq (a high throughput next generation genotyping by synthesis (GBS) platform) generated SNP markers were used to ensure sufficient genome coverage among the 95 *Moringa* accessions, which were from: Philippines (2), Haiti (1), Jamaica (1), Ghana (1), Mali (2), Malawi (5), Tanzania (1), and Kenya (82). DNA samples were subjected to GBS by DArTSeq technology. A total of 3968 SNPs were generated with 98% average reproducibility and 91% minimum call rate. These were culled to 1,913 informative SNPs, which were further analyzed to determine the genetic diversity of *Moringa*.

There were elevated levels of genetic distinctness among and within *Moringa* populations across the continents as per comparisons using false discovery rates

(FDRs) at alpha levels of 0.05 which accounted for both richness and evenness of alleles. The expected heterozygosity *(H_e)* ranged between 0.05056 and 0.5008, while the observed (*Ho)* between *0.0541and 0.633*. *Ho* ranges being higher than *He* confirms high genetic diversity. This was supported by a P value of 0.019 and Fst value of 0.171313 which were strong evidence against the null hypothesis. The notable genetic variation demonstrated in this study is possibly due to adaptive differences, selection pressures and anthropogenic activities (Shahzad *et al.,* 2013).

A phenogram generated from SNP (Figure 4.1) indicated that Philippines populations were the farthest from the rest of the populations (0.2692) hence were the most diverse. Kenyan (0.2082) and Tanzanian (0.2092) populations were the least diverse inferring a common source of introduction. West Africa (Ghanaian, Malian) and Caribbean (Haitian, and Jamaican) populations formed a large clade. The Southeast Asia population: Philippines formed a clade of highly diverse members. The high diversity within the Philippine clade is likely due to multiple gene pools introduced as a resulted of the geographical proximity of its members to the *Moringa* center of origin: the foothills of the Himalayas. Ecologically, communities with dominant species of high genetic diversity have higher ecosystem functioning than those with low genetic diversity (Crutsinger *et al.*, 2006; Avolio *et al.,* 2012). Based on this study, Philippine population is likely to be more resilient and adaptive; it is thus a considerable core collection for conservation. East African population (Kenyan and Tanzanian) having similar geographic range possibly shared a common ancestor hence their high homogeneity. These East African populations appear to clutter together with the west African populations suggesting a common ancestry.

These findings support the hypothesis of the historical dissemination of *Moringa* as from the foothills of the Himalayas to North India then east to the lower parts of China, Southeast Asia, and Philippines. Subsequently, it was migrated westwards to Egypt, the horn of Africa, and the Mediterranean and finally to the West Indies. *Moringa* was introduced to Africa by Indian settlers via British imperialist's colonizers; hence most African populations phylogenetically cluster together (*Kantharajah and Dodd 1991;* Fahey 2016; Tsaknis et al. 1999; Tian et al. 2015) except for Malawi population which seem to have a unique introduction. Muluvi *et al.,* (1999) demonstrated in his study that Malawian *Moringa* populations clustered with Indian populations. Thus, *Moringa* was possibly directly introduced to Malawi by settlers from a specific region in India and at a unique time point (Prasuna *et al.,* 2009; Olsona 2017).

The West African and Caribbean populations were monophyletic. Specifically, clustering of these four populations supports the hypothesis that the transatlantic slave trade introduced a Malian population to Jamaica and Haiti.

Change in genetic structuring of Moringa plants has been influenced by: Patterns of genetic exchange and Patterns of historical relationship which include natural selection, geographic isolation, and human cultivation practices (Schaal et al., 1998). Natural selection plays a significant role in shaping the genetic diversity of Moringa populations, as certain genetic traits could have provided advantages in specific environmental conditions, leading to the adaptation and survival of certain individuals (Gerrish et al., 2020). Geographic isolation, such as mountain ranges, rivers, or other physical barriers, could also have had impact on the genetic structuring of Moringa populations by limiting gene flow between different groups of plants, resulting in distinct genetic clusters (Muluvi et al., 1999). Additionally, human cultivation

practices, such as selective breeding for desirable traits or the introduction of new varieties, could have influenced the genetic diversity and structure of Moringa plants (Mgendi et al., 2010). Overall, a combination of natural and human-driven forces has played a role in shaping the genetic structuring of Moringa populations with admixtures mostly resulting from human activities. The distinct clustering of Malawian population from the rest of the African populations in Structure at K=2 hypothesize that *Moringa* was introduced to the African continent via two major events, Malawi population having a unique introduction. Grouping Moringa into four populations in structure resulted into the clustering of west African and Caribbean population. It was therefore hypothesized that; the Caribbean and Eastern African populations are a subset of West African population. Parisod *et al.,* 2005 states that historical relationships of a plant population greatly contribute to their genetic structure; those with close common ancestry are more similar than those with distant common ancestry.

There was a notable admixture of entries not complying with geographical distribution which is typical to unnatural mixing conceivably through anthropogenic activities and migrations.

From the AMOVA analysis, the diversity structuring was 77% within populations and 23% among populations. The low genetic diversity among the cultivated collection was attributed to slowed genetic changes due to minimal competition and natural selection, since the plants were under ideal environmental conditions (Manoko *et al.*, 2008)

The small number of *Moringa* accessions introduced from India to Africa, have served as a core collection, resulting in minimal genetic diversity among most of the African populations due to founders' effect (Muluvi *et al.,* 1999). AMOVA output in this study is consistent with diversity structuring for outcrossing plant species where gene flow is attributed to pollen movement (Sheng *et al.,* 2005) since mating systems in plants contribute highly to patterns of genetic diversity within and among populations (Kithure *et al.,* 2015).

The findings of this study align with and expand upon previous research on the genetic diversity and structure of Moringa oleifera. Similar to the high within-population variation (77%) and moderate among-population differentiation (Fst = 0.171) observed here, Muluvi et al. (1999) reported limited genetic divergence among African populations, attributing it to founder effects and narrow introduction events. The clustering of East African and West African populations supports the hypothesis of a shared ancestry, consistent with Fahey (2016) and Kantharajah & Dodd (1991), who traced the crop's westward spread from the Indian subcontinent via colonial and trade routes. The distinctiveness of the Philippine population, as revealed in this study, echoes findings by Olson (2017), who emphasized Southeast Asia's proximity to the species' center of origin in the Himalayan foothills as a driver of its genetic richness. Moreover, the observed admixture and non-geographic clustering patterns reflect anthropogenic influences, such as seed exchange and cultivation practices, a phenomenon also noted by Shahzad et al. (2013) and Mgendi et al. (2010). These parallels reinforce the role of both natural evolutionary processes and human-mediated dispersal in shaping the global genetic landscape of Moringa oleifera.

5.2 Conclusions

This study revealed substantial genetic diversity and population structuring within Moringa oleifera across its global range, underscoring its evolutionary resilience and potential for targeted conservation and breeding. African populations, accessions from Malawi exhibited the highest levels of genetic uniqueness and intra-population diversity, suggesting the presence of locally adapted genotypes and possibly underutilized germplasm. This distinctiveness positions Malawi as a critical reservoir for future agroforestry improvement and climate-resilient breeding programs on the continent.

Globally, the Philippines emerged as the most genetically diverse region, reflecting both historical introductions and extensive cultivation across varied agroecological zones. The high allelic richness and high population differentiation observed in Philippine accessions highlight the region's role as a genetic melting pot for M. oleifera, offering valuable insights for international germplasm exchange and varietal development. The null hypotheses stating (the 330 *Moringa oleifera* samples from nineteen provenances across eight countries and three regions exhibit minimal or no molecular diversity and Populations of *Moringa oleifera* across the eight countries are genetically homogeneous) were therefore rejected since notable diversity was observed within and among *Moringa* populations.

5.3 Recommendations

1 Prioritize Malawi and Philippine accessions for inclusion in national and regional *Moringa* gene banks.

2 Use these diverse populations as founder material for breeding programs targeting resilience, yield, and nutritional traits.

3 Leverage SNP data from these populations to accelerate Marker-Assisted Selection (MAS).

4 Develop molecular profiles for desirable traits (e.g., drought tolerance, oil content, leaf biomass) using these genetically rich sources.

5 Enhance In Situ and Ex Situ Conservation by protect natural stands and farmer-managed populations in Malawi and the Philippines through community-based conservation. Also Support ex situ conservation in botanical gardens and research stations to safeguard genetic variability.

6 Facilitate International Germplasm Exchange by encouraging bilateral and multilateral sharing of diverse *Moringa* germplasm under fair and transparent access and benefit-sharing frameworks (e.g., Nagoya Protocol).

7 Use Malawi and Philippine lines to enrich genetically narrow populations in other regions.

8 Integrate Diversity Data into Policy and Extension

9 Train extension officers and farmers on the value of genetic diversity for climate adaptation and sustainable use.

10 Support Genomic Research and Trait Discovery

- Invest in genome-wide association studies (GWAS) using diverse populations to identify loci linked to key agronomic traits.
- Encourage collaboration between African and Asian research institutions for comparative genomics and trait mapping.

5.4 Suggestions for Future Studies and Suggestions

1. Grouping of Ghanaian, Malian, Haitian, and Jamaican populations came as a new finding and needs to be further investigated in future studies.

2. Future studies on a combination of genetic diversity, geographical distribution, the environment impact and genetics and specific phenotypic traits of *Moringa oleifera* will identify important attributes to be preserved and utilized for commercial selection and utilisation of the tree.
3. Future studies can be done using nanopore technology to improve on DArTSeq assembled Moringa SNPs.

5.5 Challenges of the Study

1 Limited Funding for Advanced Genomic Analysis: The study did not include Genome-Wide Association Studies (GWAS) due to financial constraints. This limited the ability to link specific SNP markers to agronomic traits such as drought tolerance, leaf yield, or nutrient content.

2 Seasonal Leaf Shading Effects: Sampling was affected by leaf shading during cold seasons, which may have influenced leaf quality and reduced the consistency of phenotypic observations. This could impact trait validation and marker-trait associations.

3 Restricted Geographic Sampling**:** While the study encompassed eight countries, it lacked reference samples from India—recognized as a centre of *Moringa oleifera* diversity—due to regulatory constraints that limited germplasm access. This omission may have

REFERENCES

Adebayo, S. A., Dzoyem, J. P., Shai, L. J., & Eloff, J. N. (2015). The anti-inflammatory and antioxidant activity of 25 plant species used traditionally to treat pain in southern African. *BMC Complementary and Alternative Medicine, 15*(1). https://doi.org/10.1186/s12906-015-0669-5

Alam, M., Neal, J., Connor, K. O., Kilian, A., & Topp, B. (2018). Ultra-high-throughput DArTseq-based silicoDArT and SNP markers for genomic studies in macadamia. *Unpublished manuscript*, 1–20.

Alavilli, H., Lee, H., Lee, E. S., & Park, S. U. (2022). Miracle tree *Moringa oleifera*: Status of genetic diversity, breeding, and propagation. Plants, 11(22), 3132. https://doi.org/10.3390/plants11223132

Anwar, F., Latif, S., Ashraf, M., & Gilani, A. H. (2007). *Moringa oleifera*: A food plant with multiple medicinal uses. *Phytotherapy Research*. https://doi.org/10.1002/ptr.2023

Avolio, M. L., Beaulieu, J. M., Lo, E. Y. Y., & Smith, M. D. (2012). Measuring genetic diversity in ecological studies. *Plant Ecology, 213*(7), 1105–1115. https://doi.org/10.1007/s11258-012-0069-6

Barilli, E., Cobos, M. J., Carrillo, E., Kilian, A., Carling, J., & Rubiales, D. (2018). A high-density integrated DArTseq SNP-based genetic map of *Pisum fulvum* and identification of QTLs controlling rust resistance. *Frontiers in Plant Science*. https://doi.org/10.3389/fpls.2018.00167

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, *57*(1), 289–300. https://doi.org/10.2307/2346101

Bhatia, S., Othman, Z., & Ahmad, A. L. (2007). Coagulation-flocculation process for POME treatment using *Moringa oleifera* seeds extract: Optimization studies. *Chemical Engineering Journal*. https://doi.org/10.1016/j.cej.2007.01.034

Birger, Y., Blumenfeld, O., Bartov, E., & Burgansky-Eliash, Z. (2011). Advances in production of Moringa. *Horticultural College and Research Institute*, *249*(10), 1587–1590. https://doi.org/10.1007/s00417-011-1716-7

Bolibok-Bragoszewska, H., Heller-Uszyńska, K., Wenzl, P., Uszyński, G., Kilian, A., & Rakoczy-Trojanowska, M. (2009). DArT markers for the rye genome—Genetic diversity and mapping. *BMC Genomics, 10*. https://doi.org/10.1186/1471-2164-10-578

Borzęcka, E., Hawliczek-Strulak, A., Bolibok, L., Gawroński, P., Tofil, K., Milczarski, P., Stojałowski, S., Myśków, B., Targońska-Karasek, M.,

Gradzielewska, A., Smolik, M., Kilian, A., & Bolibok-Bragoszewska, H. (2018). Effective BAC clone anchoring with genotyping-by-sequencing and Diversity Arrays Technology in a large genome cereal rye. *Scientific Reports*. https://doi.org/10.1038/s41598-018-26541-y

Chukwuebuka, E. (2015). *Moringa oleifera*: "The mother's best friend." *International Journal of Nutrition and Food Sciences, 4*(6), 624. https://doi.org/10.11648/j.ijnfs.20150406.14

Cortés, A. J., Chavarro, M. C., & Blair, M. W. (2011). SNP marker diversity in common bean (*Phaseolus vulgaris* L.). *Theoretical and Applied Genetics, 123*(5), 827–845. https://doi.org/10.1007/s00122-011-1630-8

Crutsinger, G. M., Collins, M. D., Fordyce, J. A., Gompert, Z., Nice, C. C., & Sanders, N. J. (2006). Plant genotypic diversity predicts community structure and governs an ecosystem process. *Science, 313*(5789), 966–968. https://doi.org/10.1126/science.1128326

Csurhes, S., & Navie, S. (2016). Horseradish tree. *National Nutrient Database for Standard Reference Legacy Release*. https://ndb.nal.usda.gov/ndb/foods/show/11222?n1=%7BQv%3D1%7D&fgcd=&man=&lfacet=&count=&max=25&sort=default&qlookup=moringa+leaves&offset=&format=Full&new=&measureby=&Qv=1&ds=SR&qt=&qp=&qa=&qn=&q=&ing=

Curtolo, M., Cristofani-Yaly, M., Gazaffi, R., Takita, M. A., Figueira, A., & Machado, M. A. (2017). QTL mapping for fruit quality in Citrus using DArTseq markers. *BMC Genomics, 18*(1), 1–16. https://doi.org/10.1186/s12864-017-3629-2

Daba, M. (2016). Miracle tree: A review on multi-purposes of *Moringa oleifera* and its implication for climate change mitigation. *Journal of Earth Science & Climatic Change, 7*(8). https://doi.org/10.4172/2157-7617.1000366

Dawson, I. K., Barnes, A., Jamnadass, R., Danquah, E., Mumm, R. H., Hoad, S., Burnett, F., Hale, I., Mausch, K., Hendre, P., Powell, W., & Revoredo-Giha, C. (2019). Breeders' views on the production of new and orphan crops in Africa: A survey of constraints and opportunities. *ICRAF Working Paper*. https://doi.org/10.5716/WP19007.PDF

Dawson, I. K., Hendre, P., Powell, W., Sila, D., McMullin, S., Revoredo, C., Odeny, D. A., Graudal, L., Roshetko, J. M., Hale, I. L., Van Deynze, A., Prabhu, R., Cheng, S., Xu, X., Muchugi, A., Jamnadass, R., Bonington, S., & others. (2018). Supporting human nutrition in Africa through the integration of new and orphan crops into food systems: Placing the work of the African Orphan Crops Consortium in context. *ICRAF Working Paper*. https://doi.org/10.5716/WP18003.PDF

De La Fuente, G. N., Frei, U. K., & Lübberstedt, T. (2013). Accelerating plant breeding. *Trends in Plant Science, 18*(12), 667–672. https://doi.org/10.1016/j.tplants.2013.09.001

Dieringer, D., & Schlötterer, C. (2003). MICROSATELLITE ANALYSER (MSA): A platform-independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes*. https://doi.org/10.1046/j.1471-8286.2003.00351.x

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology, 14*(8), 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin 3.5.2 manual. *Molecular Ecology Resources, 10*, 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Felsenstein, J. (1993). PHYLIP (Phylogenetic Inference Package, version 3.5c). *Department of Genetics, University of Washington, Seattle, WA*. https://doi.org/10.2987/10-6090.1

Fuglie, L. J. (1999). *The miracle tree: Moringa oleifera. Natural nutrition for the tropics*. Church World Service.

Gandji, K., Chadare, F. J., Idohou, R., Salako, V. K., Assogbadjo, A. E., & Kakaï, R. L. G. (2018). Status and utilisation of *Moringa oleifera* Lam: A review. *African Crop Science Journal, 26*(1), 137. https://doi.org/10.4314/acsj.v26i1.10

Garavito, A., Montagnon, C., Guyot, R., & Bertrand, B. (2016). Identification by the DArTseq method of the genetic origin of the *Coffea canephora* cultivated in Vietnam and Mexico. *BMC Plant Biology, 16*(1). https://doi.org/10.1186/s12870-016-0933-y

Gerrish, P. J., Galeota-Sprung, B., Cordero, F., Sniegowski, P., Colato, A.-D., Hengartner, N., Vejalla, V., Chevallier, J., & Ycart, B. (2020). Natural selection and the advantage of recombination. *bioRxiv*. https://doi.org/10.1101/2020.08.28.271486

Guimaraes, E. P., Ruane, J., Scherf, B. D., Sonnino, A., & Dargie, J. D. (2007). Marker-assisted selection: Current status and future perspectives in crops, livestock, forestry and fish. *Food and Agriculture Organization of the United Nations*. https://doi.org/10.1111/j.1439-0388.2009.00834.x

Hassanein, A. M. A. (2017). Nutritional, chemical and molecular characterisation of *Moringa oleifera* Lam. and *Moringa peregrina* (Forssk.) Fiori genotypes. *Journal of Horticultural Science and Biotechnology*, 1–10. https://doi.org/10.1080/14620316.2017.1399834

Hendre, P. S., Muthemba, S., Kariba, R., Muchugi, A., Fu, Y., Chang, Y., Song, B., Liu, H., Liu, M., Liao, X., Sahu, S. K., Wang, S., Li, L., Lu, H., Peng, S., Cheng, S., Xu, X., Yang, H., Wang, J., … Jamnadass, R. (2019). African Orphan Crops Consortium (AOCC): Status of developing genomic resources for African orphan crops. *Planta, 250*(3), 989–1003. https://doi.org/10.1007/s00425-019-03156-9

Kilian, A. (2015). Genotyping innovations for crop improvement. Canberra, Australia: Diversity Arrays Technology Pty Ltd.

Kithure, R. K., Muchugi, A., Jamnadass, R., Njoka, F. M., & Mwaura, L. (2015). Genetic diversity of *Faidherbia albida* (Del.) A. Chev accessions held at the World Agroforestry Centre. *Forests, Trees and Livelihoods, 24*(4), 219–230. https://doi.org/10.1080/14728028.2015.1054439

Kumar, P., Gupta, V. K., Misra, A. K., Modi, D. R., & Pandey, B. K. (2009). Potential of molecular markers in plant biotechnology. *Plant Omics Journal*, Southern Cross Journals.

Kumssa, D. B., Joy, E. J. M., Young, S. D., Odee, D. W., Ander, E. L., Magare, C., Gitu, J., & Broadley, M. R. (2017). Challenges and opportunities for Moringa growers in southern Ethiopia and Kenya. *PLoS ONE, 12*(11), 1–15. https://doi.org/10.1371/journal.pone.0187651

Kusolwa, P., & Rwegasira, G. M. (2018). Discovery of novel single nucleotide polymorphic (SNP) markers for genetic mapping of cashew (*Anacardium occidentale* L.). *Unpublished manuscript*.

Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J., & Bertoli, S. (2015). Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of *Moringa oleifera* leaves: An overview. *International Journal of Molecular Sciences, 16*(6), 12791–12835. https://doi.org/10.3390/ijms160612791

Mace, E. S., Xia, L., Jordan, D. R., Halloran, K., Parh, D. K., Huttner, E., Wenzl, P., & Kilian, A. (2008). DArT markers: Diversity analyses and mapping in *Sorghum bicolor*. *BMC Genomics, 9*. https://doi.org/10.1186/1471-2164-9-26

Manoko, M. L. K., Van Den Berg, R. G., Feron, R. M. C., Van Der Weerden, G. M., & Mariani, C. (2008). Genetic diversity of the African hexaploid species *Solanum scabrum* Mill. and *Solanum nigrum* L. (Solanaceae). *Genetic Resources and Crop Evolution, 55*(3), 409–418. https://doi.org/10.1007/s10722-007-9248-z

Marone, D., Panio, G., Ficco, D. B. M., Russo, M. A., De Vita, P., Papa, R., Rubiales, D., Cattivelli, L., & Mastrangelo, A. M. (2012). Characterization of wheat DArT markers: Genetic and functional features. *Molecular Genetics and Genomics, 287*(9), 741–753. https://doi.org/10.1007/s00438-012-0714-8

Mgendi, M. G., Manoko, M. K., & Nyomora, A. M. (2010). Genetic diversity between cultivated and non-cultivated *Moringa oleifera* Lam. provenances assessed by RAPD markers. *Journal of Cell and Molecular Biology, 8*(2), 95–102.

Mogga, M., Sibiya, J., Shimelis, H., Lamo, J., & Yao, N. (2018). Diversity analysis and genome-wide association studies of grain shape and eating quality traits in rice (*Oryza sativa* L.) using DArT markers. *PLoS ONE, 13*(6). https://doi.org/10.1371/journal.pone.0198012

Mohai Ud Din, R., Eman, S., Zafar, M. H., Chong, Z., Saleh, A. A., Husien, H. M., & Wang, M. (2025). *Moringa oleifera* as a multifunctional feed additive: Synergistic nutritional and immunomodulatory mechanisms in livestock production. *Frontiers in Nutrition, 12*(June), 1–15. https://doi.org/10.3389/fnut.2025.1615349

Mohdaly, A. A. A., Sarhan, M. A., Smetanska, I., & Mahmoud, A. (2010). Antioxidant properties of various solvent extracts of potato peel, sugar beet pulp and sesame cake. *Journal of the Science of Food and Agriculture, 90*(2), 218–226. https://doi.org/10.1002/jsfa.3796

Muluvi, G. M., Sprent, J. I., Odee, D., & Powell, W. (2004). Estimates of outcrossing rates in *Moringa oleifera* using amplified fragment length polymorphism (AFLP). *African Journal of Biotechnology, 3*(2), 146–151. https://doi.org/10.5897/ajb2004.000-2026

Muluvi, G. M., Sprent, J. I., Soranzo, N., Provan, J., Odee, D., Folkard, G., McNicol, J. W., & Powell, W. (1999). Amplified fragment length polymorphism (AFLP) analysis of genetic variation in *Moringa oleifera* Lam. *Molecular Ecology, 8*(3), 463–470. https://doi.org/10.1046/j.1365-294X.1999.00589.x

Nei, M., Tajima, F., & Tateno, Y. (1983). Accuracy of estimated phylogenetic trees from molecular data—II. Gene frequency data. *Journal of Molecular Evolution*. https://doi.org/10.1007/BF02300753

Olson, M. E. (2017). *Moringa* frequently asked questions. *Acta Horticulturae, 1158*, 19–32. https://doi.org/10.17660/ActaHortic.2017.1158.4

Ondieki, S. K., Korir, J. C., & Hunja, C. W. (2024). *Evaluation of population genetics of Moringa oleifera provenances from Coastal Kenya using SNP markers*

Oyeyinka, A. T., & Oyeyinka, S. A. (2018). *Moringa oleifera* as a food fortificant: Recent trends and prospects. *Journal of the Saudi Society of Agricultural Sciences, 17*(2), 127–136. https://doi.org/10.1016/j.jssas.2016.02.002

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—An update. *Bioinformatics, 28*(19), 2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Popoola, J. O., Bello, O. A., & Obembe, O. O. (2016). Phenotypic intraspecific variability among some accessions of drumstick (*Moringa oleifera* Lam.). *Canadian Journal of Pure and Applied Sciences, 10*(1), 3681–3693.

Prasuna, C. P. L., Chakradhar, R. P. S., Rao, J. L., & Gopal, N. O. (2009). EPR and IR spectral investigations on some leafy vegetables of Indian origin. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. https://doi.org/10.1016/j.saa.2009.05.020

Pritchard, J. K., & Wen, W. (2002). Documentation for structure software: Version 2. *In Practice, 29*.

Raja, R. R., A, M. S., B, S. V., Navyasri, D. M., C, G. S., & D, S. G. (2016). *Moringa oleifera*—An overview. *RA Journal of Applied Research*, October. https://doi.org/10.18535/rajar/v2i9.05

Raman, H., Raman, R., Nelson, M. N., Aslam, M. N., Rajasekaran, R., Wratten, N., Cowling, W. A., Kilian, A., Sharpe, A. G., & Schondelmaier, J. (2012). Diversity array technology markers: Genetic diversity analyses and linkage map construction in rapeseed (*Brassica napus* L.). *DNA Research, 19*(1), 51–65. https://doi.org/10.1093/dnares/dsr041

Rambaut, A. (2012). FigTree v1.4.0. Available from http://tree.bio.ed.ac.uk/software/figtree/

Raza, S., Farooqi, S., & Mubeen, H. (2015). Role of molecular markers and their significance. *American Journal of Pharmacy and Health Research, 3*(December).

Ren, R., & Ray, R. (2016). Genetic diversity and population structure of core watermelon (*Citrullus lanatus*) genotypes using DArTseq-based SNPs. *The Journal of Agricultural Science*. https://doi.org/10.1017/S1479262115000659

Rockwood, J. L., Anderson, B. G., & Casamatta, D. A. (2013). Potential uses of *Moringa oleifera* and an examination of antibiotic efficacy conferred by *M. oleifera* seed and leaf extracts using crude extraction techniques available to underserved indigenous populations. *International Journal of Phytotherapy Research, 3*(2), 61–71.

Rodger, Y. S., Dillon, R., Monro, K., Pavlova, A., Coates, D. J., Byrne, M., & Sunnucks, P. (2024). Benefits of outcrossing and their implications for genetic management of an endangered species with mixed-mating system. *Restoration Ecology, 32*(1), 1–13. https://doi.org/10.1111/rec.14057

Rufai, S., Hanafi, M. M., Rafii, M. Y., Ahmad, S., Arolu, I. W., & Ferdous, J. (2013). Genetic dissection of new genotypes of drumstick tree (*Moringa oleifera* Lam.) using random amplified polymorphic DNA marker. *BioMed Research International*. https://doi.org/10.1155/2013/604598

Runo, S. (2019). Modern breeding approaches for durable resistance against the parasitic plant *Striga*. *Afrika Focus, 32*(2), 109–115. https://doi.org/10.21825/af.v32i2.15770

Saini, R. K., Saad, K. R., Ravishankar, G. A., Giridhar, P., & Shetty, N. P. (2013). Genetic diversity of commercially grown *Moringa oleifera* Lam. cultivars from India by RAPD, ISSR and cytochrome P450-based markers. *Plant Systematics and Evolution, 299*(7), 1205–1213. https://doi.org/10.1007/s00606-013-0789-7

Schaal, B. A., Hayworth, D. A., Olsen, K. M., Rauscher, J. T., & Smith, W. A. (1998). Phylogeographic studies in plants: Problems and prospects. *Molecular Ecology, 7*(4), 465–474. https://doi.org/10.1046/j.1365-294x.1998.00318.x

Shahzad, U., Khan, M. A., Jaskani, M. J., Khan, I. A., & Korban, S. S. (2013). Genetic diversity and population structure of *Moringa oleifera*. *Conservation Genetics, 14*(6), 1161–1172. https://doi.org/10.1007/s10592-013-0503-x

Sheng, Y., Zheng, W., Pei, K., & Ma, K. (2005). Genetic variation within and among populations of a dominant desert tree *Haloxylon ammodendron* (Amaranthaceae) in China. *Annals of Botany, 96*(2), 245–252. https://doi.org/10.1093/aob/mci171

Shiriki, D., Igyor, M. A., & Gernah, D. I. (2015). Nutritional evaluation of complementary food formulations from maize, soybean and peanut fortified with *Moringa oleifera* leaf powder. *Food and Nutrition Sciences, 6*(5), 494–500. https://doi.org/10.4236/fns.2015.65051

Stodart, B. J., Mackay, M., & Raman, H. (2005). AFLP and SSR analysis of genetic diversity among landraces of bread wheat (*Triticum aestivum* L. em. Thell) from different geographic regions. *Australian Journal of Agricultural Research, 56*(7), 691–697. https://doi.org/10.1071/AR05015

Suarez, M., Entenza, J. M., Doerries, C., Meyer, E., Bourquin, L., Sutherland, J., Marison, I., Moreillon, P., & Mermod, N. (2003). Expression of a plant-derived peptide harboring water-cleaning and antimicrobial activities. *Biotechnology and Bioengineering*. https://doi.org/10.1002/bit.10550

Thakur, S. B., & Bajagain, A. (2020). *Moringa*: Alternative for the food security, climate resilience and livelihood improvement in Nepal. *International Journal of Research – Granthaalayah, 8*(3), 190–200. https://doi.org/10.29121/granthaalayah.v8.i3.2020.142

Tian, Y., Zeng, Y., Zhang, J., Yang, C. G., Yan, L., Wang, X. J., Shi, C. Y., Xie, J., Dai, T. Y., Peng, L., Zeng Huan, Y., Xu, A. N., Huang, Y. W., Zhang, J. J., Ma, X., Dong, Y., Hao, S. M., & Sheng, J. (2015). High quality reference genome of drumstick tree (*Moringa oleifera* Lam.), a potential perennial crop. *Science China Life Sciences, 58*

Tsutsumi, Y., & Onouchi, T. (2016). Electron microscopic detection of pathogens and neutrophil extracellular traps. *Microscopy, 65*(11), i18. https://doi.org/10.1093/jmicro/dfw085

Varshney, R. K., Graner, A., & Sorrells, M. E. (2005). Genic microsatellite markers in plants: Features and applications. *Trends in Biotechnology, 23*(1), 48–55. https://doi.org/10.1016/j.tibtech.2004.11.005

Wittenberg, A. H. J., Van Der Lee, T., Cayla, C., Kilian, A., Visser, R. G. F., & Schouten, H. J. (2005). Validation of the high-throughput marker technology DArT using the model plant *Arabidopsis thaliana*. *Molecular Genetics and Genomics, 274*(1), 30–39. https://doi.org/10.1007/s00438-005-1145-6

APPENDICES

Appendix I: Map Showing Moringa Distribution Across the Tropical Zones of The World.

Appendix II: Details Of the Plant Materials Used for This Study.

Species	Accession no	Provenance	Country	Sample	Latitude	Longitude
Moringa oleifera	ICRAF 05536	Busia	Kenya	10	0.4565	34.115
Moringa oleifera	ICRAF 07229	Machakos	Kenya	16	0.4565	34.115
Moringa oleifera	ICRAF 07627	Machakos	Kenya	4	0.4565	34.115
Moringa oleifera	ICRAF 07817	Meru	Kenya	1	0.1033	37.655
Moringa oleifera	ICRAF 07818	Meru	Kenya	1	0.1033	37.655
Moringa oleifera	ICRAF 07819	Meru	Kenya	1	0.1033	37.655
Moringa oleifera	ICRAF 07820	Meru	Kenya	2	0.1033	37.655
Moringa oleifera	ICRAF 07821	Meru	Kenya	2	0.1033	37.655
Moringa oleifera	ICRAF 07822	Meru	Kenya	1	0.1033	37.655
Moringa oleifera	ICRAF 07823	Meru	Kenya	3	0.1033	37.655
Moringa oleifera	ICRAF 07824	Meru	Kenya	2	0.1033	37.655
Moringa oleifera	ICRAF 07825	Meru	Kenya	2	0.1033	37.655
Moringa oleifera	ICRAF 07826	Meru	Kenya	2	0.1033	37.655
Moringa oleifera	ICRAF 07827	Meru	Kenya	2	0.1033	37.655
Moringa oleifera	ICRAF 07828	Meru	Kenya	2	0.1033	37.655
Moringa oleifera	ICRAF 07829	Meru	Kenya	2	0.1033	37.655
Moringa oleifera	ICRAF 07830	Meru	Kenya	2	0.1033	37.655
Moringa oleifera	ICRAF 07831	Meru	Kenya	2	0.1033	37.655
Moringa oleifera	ICRAF 07832	Meru	Kenya	2	0.1033	37.655
Moringa oleifera	ICRAF 07833	Meru	Kenya	2	0.1033	37.655
Moringa oleifera	ICRAF 07834	Meru	Kenya	1	0.1033	37.655
Moringa oleifera	ICRAF 07835	Meru	Kenya	2	0.1033	37.655
Moringa oleifera	ICRAF 07836	Meru	Kenya	2	0.1033	37.655
Moringa oleifera	ICRAF 07837	Meru	Kenya	2	0.1033	37.655
Moringa oleifera	ICRAF 07850	Meru	Kenya	2	0.1033	37.655
Moringa oleifera	Tree7	Meru	Kenya	1	0.6228	37.622

Moringa oleifera	Tree6	Meru	Kenya	2	0.6226	37.622
Moringa oleifera	Tree5	Meru	Kenya	1	0.6225	37.622
Moringa oleifera	ICRAF 07842	Nairobi	Kenya	10	-1.2333	36.633
Moringa oleifera	ICRAF 07847	Samburu	Kenya	10	1.2014	36.96
Moringa oleifera	ICRAF 07849	Rongo	Kenya	10	-0.7525	34.597
Moringa oleifera	ICRAF 07888	Bondo	Kenya	2	-0.1033	34.279
Moringa oleifera	ICRAF 07889	Bondo	Kenya	2	-0.1033	34.279
Moringa oleifera	ICRAF 07890	Bondo	Kenya	2	-0.1033	34.279
Moringa oleifera	ICRAF 07891	Bondo	Kenya	2	-0.1033	34.279
Moringa oleifera	ICRAF 07892	Bondo	Kenya	2	-0.1033	34.279
Moringa oleifera	ICRAF 07893	Bondo	Kenya	2	-0.1033	34.279
Moringa oleifera	ICRAF 07894	Bondo	Kenya	2	-0.1033	34.279
Moringa oleifera	ICRAF 07895	Bondo	Kenya	2	-0.1033	34.279
Moringa oleifera	ICRAF 07896	Bondo	Kenya	2	-0.1033	34.279
Moringa oleifera	ICRAF 07897	Bondo	Kenya	2	-0.1033	34.279
Moringa oleifera	ICRAF 07898	Bondo	Kenya	2	-0.1033	34.279
Moringa oleifera	ICRAF 07899	Bondo	Kenya	1	-0.1033	34.279
Moringa oleifera	ICRAF 07900	Bondo	Kenya	2	-0.1033	34.279
Moringa oleifera	ICRAF 07901	Bondo	Kenya	2	-0.1033	34.279
Moringa oleifera	ICRAF 07902	Bondo	Kenya	2	-0.1033	34.279
Moringa oleifera	ICRAF 07903	Bondo	Kenya	2	-0.1033	34.279
Moringa oleifera	ICRAF 07904	Bondo	Kenya	2	-0.1033	34.279
Moringa oleifera	ICRAF 07905	Bondo	Kenya	2	-0.1033	34.279
Moringa oleifera	ICRAF 07906	Bondo	Kenya	2	-0.1033	34.279
Moringa oleifera	ICRAF 07907	Bondo	Kenya	2	-0.1033	34.279
Moringa oleifera	ICRAF 07908	Bondo	Kenya	2	-0.1033	34.279
Moringa oleifera	ICRAF 07909	Bondo	Kenya	2	-0.1033	34.279
Moringa oleifera	ICRAF 07910	Mbololo	Kenya	2	-3.2818	38.468
Moringa oleifera	ICRAF 07911	Mbololo	Kenya	2	-3.2818	38.468

Moringa oleifera	ICRAF 07912	Mbololo	Kenya	2	-3.2818	38.468
Moringa oleifera	ICRAF 07913	Mbololo	Kenya	2	-3.2818	38.468
Moringa oleifera	ICRAF 07914	Mbololo	Kenya	2	-3.2818	38.468
Moringa oleifera	ICRAF 07915	Mbololo	Kenya	2	-3.2818	38.468
Moringa oleifera	ICRAF 07916	Mbololo	Kenya	2	-3.2818	38.468
Moringa oleifera	ICRAF 07917	Mbololo	Kenya	2	-3.2818	38.468
Moringa oleifera	ICRAF 07918	Mbololo	Kenya	2	-3.2818	38.468
Moringa oleifera	ICRAF 07919	Mbololo	Kenya	2	-3.2818	38.468
Moringa oleifera	ICRAF 07920	Mbololo	Kenya	2	-3.2818	38.468
Moringa oleifera	ICRAF 07921	Mbololo	Kenya	2	-3.2818	38.468
Moringa oleifera	ICRAF 07923	Mbololo	Kenya	3	-3.2818	38.468
Moringa oleifera	ICRAF 07924	Mbololo	Kenya	2	-3.2818	38.468
Moringa oleifera	ICRAF 07925	Mbololo	Kenya	2	-3.2818	38.468
Moringa oleifera	ICRAF 07926	Mbololo	Kenya	1	-3.2818	38.468
Moringa oleifera	ICRAF 07927	Mbololo	Kenya	2	-3.2818	38.468
Moringa oleifera	ICRAF 07928	Mbololo	Kenya	2	-3.2818	38.468
Moringa oleifera	ICRAF 07929	Mbololo	Kenya	3	-3.2818	38.468
Moringa oleifera	ICRAF 07930	Mbololo	Kenya	1	-3.2818	38.468
Moringa oleifera	ICRAF 07931	Mbololo	Kenya	2	-3.2818	38.468
Moringa oleifera	ICRAF 07932	Mbololo	Kenya	2	-3.2818	38.468
Moringa oleifera	ICRAF 07933	Mbololo	Kenya	2	-3.2818	38.468
Moringa oleifera	ICRAF 07934	Mbololo	Kenya	2	-3.2818	38.468
Moringa oleifera	ICRAF 07935	Mbololo	Kenya	2	-3.2818	38.468
Moringa oleifera	ICRAF 07936	Mbololo	Kenya	2	-3.2818	38.468
Moringa oleifera	ICRAF 07937	Mbololo	Kenya	2	-3.2818	38.468
Moringa oleifera	ICRAF 07938	Mbololo	Kenya	2	-3.2818	38.468
Moringa oleifera	ICRAF 03295	Mbololo	Kenya	3	-3.2818	38.468
Moringa oleifera	ICRAF 07846	Ghana	Ghana	10	10.022	-1.5089

Moringa oleifera	ICRAF 07845	Haiti	Haiti	10	18.443	-72.295
Moringa oleifera	ICRAF 07843	Jamaica	Jamaica	9	17.941	-76.293
Moringa oleifera	ICRAF 08035	Mangochi	Malawi	5	-14.466	35.217
Moringa oleifera	ICRAF 08036	Makanjira	Malawi	6	-13.705	35.047
Moringa oleifera	ICRAF 08037	Mangochi	Malawi	12	-14.466	35.217
Moringa oleifera	ICRAF 08038	Mangochi	Malawi	11	-14.466	35.217
Moringa oleifera	ICRAF 08039	Thiwatiwa	Malawi	11	-13.507	33.837
Moringa oleifera	ICRAF 07632	Niamakoro	Malawi	10	12.594	-7.9606
Moringa oleifera	ICRAF 07633	Segou	Malawi	10	13.432	-6.2481
Moringa oleifera	ICRAF8030	Caloocan	Philippine	10		
Moringa oleifera	ICRAF 08029	Pangasinan	Philippine	10	15.894	120.29
Moringa oleifera	ICRAF 07848	Singida	Tanzania	10	-6.7541	34.156

Appendix III: Reagents, Instruments, And Their Suppliers

Item	**Company Source**	**Country/Brand Origin**
GelRed (Cat No. 41001)	Biotium	USA
Isolate II Plant DNA Isolation Kit	Bioline	UK (Meridian Bioscience)
Qubit Reagents (e.g., dsDNA HS Assay Kit)	Invitrogen	USA (Thermo Fisher Scientific)
Qubit® 2.0 Fluorimeter	Thermo Fisher Scientific	USA
NanoDrop ND2000c Spectrophotometer	Thermo Fisher Scientific	USA
Agarose (A9539, 0.8% w/v)	Sigma-Aldrich	USA/Germany
RNase A	New England Biolabs	USA
Lambda DNA	New England Biolabs	USA
Eppendorf Centrifuge	Eppendorf AG	Germany
DArTseq Genotyping Services	DArT Pty. Ltd.	Australia

Appendix IV: Research Approval

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 020-8704150

Internal Memo

FROM: Dean, Graduate School

DATE: 15th July, 2022

TO: Mr. Jantor Ndalo
C/o Department of Biochemistry,
Microbiology & Biotechnology

REF: I56/CTY/PT/39330/2016

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that Graduate School Board, at its meeting on **20th June, 2022**, approved your Research Proposal for the M.Sc. Degree entitled, **"Molecular Characterization of Moringa oleifera (Lam.) Using Diversity Arrays Technology (DArTSeq) Generated SNPs."**

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking and Progress Report Forms per semester. The Forms are available at the University's Website under Graduate School webpage downloads.

Also, please ensure that you publish article(s) from your thesis before submitting it to Graduate School for examination as per the Commission for University Education and Kenyatta University guidelines.

Thank you.

JULIA GITU
FOR: DEAN, GRADUATE SCHOOL

CC. Chairman, Department of Biochemistry, Microbiology & Biotechnology
Supervisors:

1. Prof. Steven Runo
   C/o Biochemistry, Microbiology & Biotechnology Dept.
   Kenyatta University

2. Dr. Alice Muchugi
   Genetic Resource Unit, World Agroforestry (ICRAF)
   C/o Biochemistry, Microbiology & Biotechnology Dept.
   Kenyatta University

Appendix V: Research Authorization

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 020-8704150

Our Ref: I56/CTY/PT/39330/2016 DATE: 15th July, 2022

Director General,
National Commission for Science, Technology
and Innovation
P.O. Box 30623-00100
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR MR. JANTOR NDALO – REG. NO. I56/CTY/PT/39330/16

I write to introduce Mr. Jantor Ndalo who is a Postgraduate Student of this University. He is registered for M.Sc. degree programme in the **Department of Biochemistry, Microbiology & Biotechnology.**

Mr. Ndalo intends to conduct research for a M.Sc. thesis Proposal entitled, **"Molecular Characterization of Moringa oleifera (Lam.) Using Diversity Arrays Technology (DArTSeq) Generated SNPs."**

Any assistance given will be highly appreciated.

Yours faithfully,

PROF. ELISHIBA KIMANI
DEAN, GRADUATE SCHOOL

JG/eww