DIVERSITY STUDY OF DRUMSTICK (*MORINGA OLEIFERA* LAM.) USING MORPHOLOGICAL CHARACTERS AND MOLECULAR MARKERS

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JANUARY, 2018

DECLARATION

I hereby declare that the work in this thesis entitled Diversity study of Drumstick (*Moringa oleifera* Lam.) using morphological traits and molecular markers has been carried out by me in the Department of Plant Science Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

AMAO Abiola Oluwafunke …….. ……..
Student Signature Date

The above declaration is confirmed.

…….
Prof. C.A. Echekwu
Chairman Supervisory Committee. Date …….

CERTIFICATION

This thesis entitled DIVERSITY STUDY OF DRUMSTICK (*MORINGA OLEIFERA* LAM.) USING MORPHOLOGICAL CHARACTERS AND MOLECULAR MARKERS by Abiola Oluwafunke AMAOmeets the regulations governing the award of the degree of doctor of philosophy of Ahmadu Bello University Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this thesis to all determined minds willing to pursue their desired goals and ambition in life.

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ABSTRACT

Germplasm diversity analysis and characterization provides the foundation for crop improvement programme. This work has used morphologicaland molecular tools to study diversityand characterize*Moringa oleifera*. The study was undertaken at the Forestry Research Institute of Nigeria (FRIN), Jericho Ibadan arboretum from 2013 to 2016 with the aim of determining accessions for future genetic improvement program. The experiment was laid out in a randomized complete block design with three replications. The morphological data were subjected to Principal Component Analysis (PCA), Rank Summation Index (RSI) to determinetheir pattern of variation, identify major traits responsible for the variation and suggest appropriate accessions for breeding.Significant ($P < 0.05$) variation were observed among the accessions for surviving ability, pod diameter and number of seed/pod and highly significant ($P < 0.01$) differences for days to flowering, days to fruiting, pod length, plant height and secondary branches. Phenotypic variances and phenotypic coefficients of variation were slightly higher than genotypic variances and genotypic coefficients of variation for all the traits. Positive significant ($P<0.05$) correlations were observed between survival ability and vegetative characters, as well as between survival ability and yield related characters. Negative significant correlations were observed between early flowering and yield related characters; likewise between early flowering and vegetative characters.From the principal component analysis, the first five principal components out of the seventeen PCextracted had eigenvalues >1 and altogether captured 87.5% of the variation. Principal components 1, 2, 3, 4 and 5 exhibited 50.1%, 11.3%, 10.2%, 9.0% and 6.8% variability respectively.Cluster analysis grouped the 31 accessions into three main clusters with four sub clusters.The accessions were grouped into clusters with reference to their overall performance in the evaluated traits.Based on RSI,Accessions FRINMOR12-17, FRINMOR12-22 and

FRINMOR12-6 were the best materials in terms of total number of pods, total number of seeds, the seed yield, 100seed weight, pod weight, number of seeds per pod, secondary branches and flowering branches. These accessions are therefore considered the best potential materials for the improvement of *Moringa* in Nigeria.None of the 10 Random Amplified Polymorphic DNA primers showed amplification. However, five micro-satellites amplified and produced clear bands which were selected for the characterization analysis. The mean polymorphic information content was 0.48, gene diversity was 0.52 while the allele frequency was 0.57, and these results indicated there was actually gene diversity existing among the collections.High polymorphism and gene diversity were observed from the primers MO41 and MO1indicating the deduced information from the primers can be used with high level of certainty in subsequent analysis. The cluster analysis grouped the 31 accessions into two main clusters with three sub clusters. Fifteen out of the thirty one accessions were having 0.00 genetic distances between them and were grouped into six clusters. Both the morphologicalanalysis and molecular marker evaluations were not able to discern a clear cut geographical clustering of the accessions.

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ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
ANOVA	Analysis of Variance
BUK	Bayero University Kano
CV	Coefficient of Variation
DNA	Deoxyribonucleic Acid
ECHO	Educational Concerns for Hunger Organization
FRIN	Forestry Research Institute of Nigeria
GCV	Genotypic Coefficient of Variation
IITA	International Institute of Tropical Agriculture
MAS	Marker Assisted Selection
NACGRAB	National Centre for Genetic Resources and Biotechnology
NILST	Nigerian Institute of Science Laboratory Technology
NTSYS	Numerical Taxonomy and Multivariate Analysis System
PCA	Principal Component Analysis
PC	Principal Component
PCV	Phenotypic Coefficient of Variation
PCR	Polymerase Chain Reaction
PIC	Polymorphic Information Content
RAPD	Random Amplified Polymorphic DNA
RFLPs	Restriction Fragment Length Polymorphisms
RIS	Rank index summation
SSR	Simple Sequence Repeat

SFRS	Savanna Forest Research Station
UPGMA	Unweighted pair group method with arithmetic means

CHAPTER ONE

1 INTRODUCTION

Background

*Moringa oleifera*Lam commonly known as drumstick is the most widely cultivated species of the monogenetic family, Moringaceae (Fuglie, 2013). Drumstick is a fast growing deciduous perennial tree,the bark is whitish grey in colour and it is surrounded by a thick cork (Parotta, 1993).It is native to India in Asia and was introduced in 1920s to lower part of China, south east to the Philippines, westward to Africa and America (Pandey *et al.,* 2010). A total of 13 tropical and subtropical species of the its genus are known out of which some species such as *M. arborea*, *M. borziana, M. longituba, M. rivae, M. ruspoliana,* and *M. stenopetala* are endangered (Stephenson and Fahey, 2004). *Moringa oleifera* Lam. is the only cultivated species in the Moringa genus (Sanchez *et al*., 2006).Thetree comprises of four different edible parts: leaves, pod, stem and root (Morton, 1991).It is also referred to as 'Mother's best friend' as it is consumed to increase a woman's milk production, and has sometimes been prescribed for treating anaemia (Siddhuraju and Becker, 2003; Estrella *et al*., 2000). It is referred to as the 'Natural nutrition of the tropics. Different parts of Moringa tree such as the leaves, fruits, flowers, and immature pods are highly nutritious, and have been used as a source of food in many countries like India, Pakistan, Philippines, Hawaii and several countries in Africa (Anwar and Bhanger,2003).*M. oleifera* leaves have been reported to be a rich sources of β-carotene, protein, vitamin C, calcium and potassium. In the Philippines, in addition to food uses, *M. oleifera* is used for animal feed (Sanchez *et al*., 2006). When supplemented in the diet of dairy animals, the leaves improve dry matter intake, digestion, and milk production without affecting

the smell, taste or colour of milk produced by the animal (Sanchez *et al.*,2006). Akinbamijo *et al*. (2004) and ITC (2004) reported inclusion of 20- 40% as supplementary food to groundnut hay based diet for ruminant animals having a high nutritional value on the animals.

It has also been reported as a natural coagulant for treatment of turbid water (Suarez *et al*.,2003; Bhatia, 2007) as well as a source of phytomedical compounds (Anwar *et al*.,2007). The medicinal uses, safety and efficacy of *M. oleifera* have been widely reported by several authors (Anwar *et al*., 2007; Popoola and Obembe, 2013; Hussain *et al*., 2014; Stohs and Hartman, 2015). The seed oil use as raw material for production of biodiesel is gaining attention globally as possible replacement for petrol and diesel fuel in unmodified engines (Fernandes *et al*., 2015). Information on diversity and population structure is expected to assist plant breeders in the selection of parents for crossing, thereby providing a more rational basis for expanding the gene pool and for identifying material that harbours alleles of value for plant improvement (Muluvi*et al.*, 2004).

M. oleifera is considered neglected and underutilized species (NUS) in some parts of the world (Padulosi *et al*., 2013; Rudebjer *et al*., 2013). The neglect may be probably due to little or no research attention from agronomic researchers and policy makers, loss of local knowledge and lack of established varieties (Padulosi *et al*., 2011; Padulosi *et al*., 2013). Trees require a lot of land and time to breed and test, and desirable traits are not always easily measured. Most tree speciespossess a lot of genetic and morphological diversity for most traits of interest due to their out-crossing. Presently in Nigeria, *M. oleifera* is beingunderutilized, as available germplasms are scarcely represented in *ex situ* collections and *in situ* collections and are poorly managed and conserved (Popoola and Obembe, 2013; Popoola *et al*., 2014). According to Azeez and

Morakinyo (2011), analysis of the association between various plant traits would help in identifying traits that are useful for production and breeding. This study therefore aimed at evaluating diversity among 31 accessions of *M. oleifera* using morphological and molecular means with minimal descriptors for drumstick (Santhoshkumar *et al*., 2013) and descriptors for legumes (IPGRI, 2006) as used and referenced by Popoola and Obembe (2013).

Justification for the Study

Drumstick has great potential in serving as a high-value crop for food, medicinal products, as well as fodder for animals, particularly in the developing tropical regions of the world.However,according to National Research Council (NRC) (2006), the species is still considered as an underutilized species and therefore a need for genetic improvement to widen its gene pool. It is therefore crucial to characterize any available accessions using agro-morphological characters. Although, morphometric traits are highly influenced by environmental factors, they have nonetheless, provided the basis for characterizing and evaluating intraspecific relationships among several plants (Amoatey *et al*., 2012; Amponsah *et al*., 2013).Great genetic variability is present in the natural and cultivated accessions of tree species. Presently, there is paucity in collection of cultivated and wild accessions of *M.oleifera*. A repository encompassing the genetic variability present in drumstick is needed to facilitate breeding programmes and develop elite varieties adapted to local conditions. Although the plant shows high morphological variability, which may be a resource for its improvement, the major factors that limit productivity are the absence of elite varieties adapted to local conditions and the use of seeds obtained through open pollination from plants in the planted area(Muluvi *et al.*,2004). Characterization and identification of cultivars has been performed using morphological and

physiological characters however such are not always available for analysis and most times do not remain independent of the changing environmental conditions.

Tree classification based on morphological characters can be more expensive to maintain than the use of molecular/ DNA markers looking at the expanse area of land for establishment and field maintenance of tree species. Morphological study is also more time demanding before a reasonable result is presented. Thus a more reliable method for assessing genetic variability in germplasm collections or pedigree is by the use of molecular markers. Molecular markers have been reported to be independent of effects or influence of environment (Mondini *et al.*, 2009) and it allows for identification of cultivars at early stage of the plant development (Kameswara, 2004). Furthermore, despite the various uses of *M. oleifera* and its morphological differentiation, the number of accessions in collections to create an active germplasm banks are only partly in existence across the world (Popoola *et al.*, 2013). However there are limited numbers of a well arranged repository with known family tree management. The problem has been how to collect, cultivate and characterise Nigerian drumstick accessions. This work has been able to collect some accessions from Nigeria and three from outside namely Mali, Israel and India to establish a drumstick plantation. This is going to be a repository which is intended to be referenced for any development of a collaborative network among National and International Research Centres and farmers that are already cultivating drumstick trees in Nigeria. This study is thus targeted towards:studying the genetic diversity existing in the established repository of *M. oleifera* in Forestry Research Institute of Nigeria (FRIN), Ibadan to determine the genetic components of variability using morphological attributes and the molecular marker tools.

Since classifying solely based on morphological attributes might not be reliable, Crouch(2001) made known the importance of marker assisted selection stating that it will offer great scope for increasing the efficiency of conventional plant breeding. Molecular markers are especially advantageous for traits with low heritability where traditional selection is difficult, expensive or lack accuracy or precision (Crouch, 2001).Added to this is the fact that identification of genetic variation is necessary for effective management and use of genetic resource. It is very important to subject these collections of drumstick accessions to genetic characterisation using molecular markers to understand the extent of variation available in the repository and to design strategies to eliminate duplicates and nearly similar accessions in the FRIN collection.Another focus is also to be able to improve the adaptability potential of the species for future genetic diversity studies on *Moringa oleifera* species in Nigeria.

Objectives

The main objective of this study is to assess the morphological and molecular variations existingamong thirty one accessions of *M. oleifera* collected withinand outside Nigeria with a view to creating a repository of provenances, defining the genetic and environmental components of phenotypic variability, for future geneticimprovement.

Specific objectives of this study were to:

1. Evaluate diversity existing among thirty one accessions of *Moringa*using morphological traits and molecular markers.
2. Determinethe pattern of association among the thirty one accessions and the morphological traits.
3. Determine major traits responsible for the expressed variation among the accessions.
4. Suggest superior accessions with potentialsfor future breeding programmes.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Origin and Geographical Distribution

In the monogeneric genus, Moringa of Moringaceae family are 13 species namely, *M. arborea*, indigenous to Kenya; *M. rivae* indigenous to Kenya and Ethiopia; *M. borziana*, indigenous to Somalia and Kenya; *M. pygmaea* indigenous 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* indigenous to Madagascar; *M. peregrine* indigenous to Red sea and Horn of Africa, *M. concanensis*, *Moringa oleifera* Lam. is indigenous to sub-Himalayan tracts of Northern India (Paliwal and Sharma, 2011). Among all these species in the genus Moringa, *M. oleifera* has so far become the most used and studied. The major *M. oleifera* producing country is India with an annual production of 1.1 to 1.3 million tons of tender fruits from an area of about 380 km^2 (Rajangam *et al.,* 2001). Most likely, British colonialists introduced *M. oleifera* in early twentieth century to Africa from India, while in the 1784 an Englishman took *M. oleifera* over to Jamaica (Shahzad, 2013). The export pathway was restricted to Indian coastal regions, where most movement of goods and people took place, and involved a relatively small number of accessions that belonged to a common or few populations. Information about the production in other countries is scarce or not available and cultivation is mainly in developing countries.

A study on local uses and geographical distribution of *M. Oleifera*(Popoola and Obembe, 2013) that covers the major agro-ecological region in Nigeria, clearly established that "though considered a non-indigenous species, *M. oleifera* has found wide acceptance among various ethnicities in Nigeria, who have exploit different uses (e.g., food, medicine, fodder, etc.). Palada

(1996); Nouman *et al*. (2014) reported that *Moringa oleifera* grows in any tropical and subtropical country with peculiar environmental features ranging from dry to moist condition having an annual precipitation of 760 to 2500 mm.Irrigation application requirement should not be more than 800 mm . The plant thrives well in any type of soil except heavy clay and waterlogged soil with pH ranging between 4.5 and 8, at an altitude of up to 2000 m.

2.2 Botanical Description of *M. oleifera*Lam

Moringa oleifera is a fast-growing, deciduous tree that can reach up to 12 m in height and the trunk can be as wide as 45 cm in diameter (Parotta, 1993). The bark of a matured tree has a whitish-grey colour and is surrounded by thick cork while young shoots have purplish or greenish-white hairy bark. The tree has an open crown of drooping, fragile branches and the leaves build up feathery foliage of tripinnate leaves. The flowers are bisexual, surrounded by five unequal, tiny veined, yellowish-white petals. The flowers are about 1.0 – 1.5 cm long and 2.0 cm broad. They grow on slender, hairy stalks in spreading or drooping manner and later flower in clusters which have a length of 10 – 25 cm (Parotta, 1993). Flowering begins within the first six months after planting. In seasonally cool regions, flowering only occurs once a year between April and June; but in more constant seasonal temperatures and with constant rainfall, flowering occurs twice or all year round (Parotta, 1993).The fruit is a hanging, three-sided brown capsule of 20 – 45 cm size which holds dark brown, globular seeds of one centimetre in diameter. The seeds have three whitish papery wings and are dispersed by wind and water. In cultivation, the stem is often cut annually to 1 – 2 m above the ground and allowed to re-grow so the pods and leaves remain within arm's reach.

2.3 Uses of*Moringa oleifera*Lam.

It is used for different purposes ranging from nutritional to medicinal and industrial uses.Anwar and Bhanger (2003) gave a report on different general uses to include allplant parts: the flower, fruits, the immature pods and leaves. All the parts are highly nutritious and have beenconsumed widely as vegetables in many parts of the world especially in developing countrieslike Africa, India, Phillipines and India (Fahey,2005).Its multiple uses and potentials attracted the attention of farmers and researchers in past historical eras. *Ayurvedi*c an Indian traditional medicine producer says that drumstick can prevent 300 diseases and its leaves have been exploited both for preventive and curative purposes (Ganguly, 2013). Ancient Egyptians used oil extracted from the seed in cosmetics preparation (Mahmood *et al.*, 2010).

The discovery of the nutritional and medicinal properties of drumstick had led to the spread of its cultivation in most tropical countries (Alessandro *et al*., 2015). In 2001,the first international conference on drumstickwas held in Tanzania and since then the number of congresses and studies has increased in disseminating the information about the incredible properties of *Moringa oleifera* Alessandro *et al.* (2015). Also in 2012, the first summit on *Moringa oleifera* was held in Ilorin, Nigeria in which a paper was presented to create awareness on the importance of *M. oleifera* and the need to introduce it into meals by the South West people because of its nutritional benefits according to Amao *et al.*(2012). All these inherent qualities of *M. oleifera*, has led to the species being named Miracle tree, Natural gift, and Mother's best friend (Fahey, 2005). The plant is well known almost all over the country due to the different medicinal and nutritional facts people have enjoyed from it both by the rural dwellers and the elites. In Southern Nigeria it is now cultivated by many household as a garden plant.

All the parts of *M. oleifera* plant are traditionally used for different purposes, but the leaves are usually the most used (Popoola and Obembe, 2013; Sivasankari *et al.,* 2014). Leaves are rich in protein, mineral, beta-carotene and antioxidant compounds, which are often lacking in diets of underdeveloped and developing countries. The leaves are added to food preparations as diet integrators. In traditional medicine, these leaves are used to treat several ailments including malaria, typhoid fever, parasitic diseases, arthritis, swellings, cuts, diseases of the skin, genito-urinary ailments, hypertension and diabetes. They are also used to induce lactation and boost the immune system against HIV/AIDS related symptoms (Popoola and Obembe, 2013; Kasolo, 2010; Yabesh, 2014), as well as cardiac stimulants and contraceptive remedy. It can be eaten raw, dried or as extracted aqueous infusion.

The seeds of drumstick are used for both human nutrition and traditional medicine. Root and stem barks are boiled in water and soaked in alcohol to obtain drinks and infusions that can be used to treat stomach ailments such as stomach pain, ulcer and indigestion; poor vision, joint pain, diabetes, anaemia and hypertension (Popoola and Obembe, 2013; Abe and Ohtani, 2013). It has also been reported to cure toothache, haemorrhoids and uterine disorder (Popoola and Obembe, 2013; Yabesh, 2014). Roots are soaked in water or alcohol and boiled with other herbs to obtain drinks and infusions as anti-helmintic and anti-paralytic drugs (Anwar *et al*., 2007) and as sexual stimulant. The flowers are used to produce aphrodisiac substances and to treat inflammations, muscle diseases, hysteria, tumours and enlargement of the spleen (Anwar *et al*., 2007, Yabesh, 2014).

Beyond the uses of *M. oleifera* as food and for human health, the leaves have been used as a natural plant growth enhancer. The leaves are rich in zeatin, a plant hormone belonging to the

cytokinin group. Leaf extracts can stimulate plant growth and increase crop yield. Ashfaq *et al*.(2012) had reported the beneficial effects on crops like wheat, maize and rice. Leaf and seed extracts show bio-pesticide activity and has been effective in the control of the larvae and adults of *Trigoderma granarium* and reduced the incidence of fungi on groundnut seeds (Ashfaq *et al.*,2012). *M. oleifera* seeds had also been widely utilized as biomass for biodiesel production. Due to the increasing energy demand and environmental problems associated with fossil fuels, the improvement of alternative fuels and renewable sources of energy is required. Biodiesel can replace petroleum-derived oil (petrol and diesel), without any sulphur or aromatic compound and with lower emission of monoxides, hydrocarbons and particulates. Furthermore, biodiesel can reduce dependence on imported fuels: a crucial problem in developing countries (Karmaka *et al*., 2010).The seeds have an oil content of 30–40% by weight yield. The oil is known as Ben oil, it is sweet, non-sticking, non-drying oil anddoes not go rancid. The oil has been used for fine machine lubrication, and in the manufacture of perfume and hair care products (Tsaknis *et al*., 1999) with a high-quality fatty acid composition i.e., high oleic acid (>70%) (Rashid*et al*.,2009). In addition they possess significant resistance to oxidative degradation. Since biodiesel production with *M. oleifera* seed oil is not in direct competition with existing farmland and with food crops and as *M. oleifera* can grow on degraded land. Studies suggest that *M. oleifera* biodiesel is an acceptable substitute to fossil fuels, even when compared against biodiesel derived from vegetable oil of other species.Zaku *et al*. (2015) reporting (Berger *et al.,* 1984) said one of the best known uses for *M. oleifera* is the use of powdered seeds to flocculate contaminants and purify drinking water. Popoola and Obembe, (2013) also mentioned the usefulness of the seed powder for water purification, replacing dangerous and expensive chemicals such as aluminiumsulphate.

Fresh leaves of drumstick are reported to contain 11,300–23,000 IU of vitamin A (Ferreira *et al*., 2008). Vitamin A plays key roles in many physiological processes such as vision, reproduction, embryonic growth and development, immune competence and brain function. Its deficiency is still prevalent in many developing countries and considered responsible for child and maternal mortality (Alvarez *et al*., 2014). The fresh leaves of are also a good source of carotenoids with pro-vitaminA action. They contain 6.6–6.8mg/100g of β-carotene (Price, 1985; Kidmose *et al*., 2006), comparable to Carrots (6.9 mg/100g) but greater than Pumpkin and Apricots which contain 3.6 and 2.2mg/ 100g, respectively (Alessandro *et al.,* 2015). β-carotene is more concentrated in the dried leaves, with amounts ranging from 17.6 to 39.6mg/100 g of dry weight (Price, 1985;Joshi and Mehta, 2010; Moyo *et al.* 2011). This wide range may be explained by the different environmental conditions existing among different origin countries, genetics of the plant, drying (Joshi and Mehta, 2010), extraction and analysis methods employed.

It is a rich source of vitamin C, because the fresh leaves contain approximately 200mg/100g (Ramachandran *et al.*, 1980) greater than orange (Alessandro*et al.,* 2015). These amounts are of particular interest, because the vitamin C intervenes in the synthesis and metabolism of many compounds, like tyrosine, folic acid and tryptophan, hydroxylation of glycine, proline, lysine carnitine and catecholamine. It facilitates the conversion of cholesterol into bile acids and hence lowers blood cholesterol levels and increases the absorption of iron in the gut by reducing ferric to ferrous state. Finally, it acts as an antioxidant, protecting the body from various deleterious effects of free radicals, pollutants and toxins (Chambial *et al.*, 2013). However, vitamin C is sensitive to heat and oxygen, this causes rapid oxidization, so much so that its concentration in the *M. oleifera* dried leaves is lower than in the fresh leaves, dropping to 18.7-140 mg/100 g of DW (Price, 1985; Joshi and Mehta, 2010; Iqbal and Bhanger, 2006).Its fresh leaves are a good source of vitamin E (in particular α-tocopherol) and contain approximately 9.0mg/ 100g

(Alessandro*et al.*, 2015) of this compound, similar to nuts. Vitamin E acts mainly as liposoluble antioxidants, but it is also involved in the modulation of gene expression, inhibition of cell proliferation, platelet aggregation and regulation of bone mass (Borel *et al.*, 2013).Drying procedure determines the concentration of vitamin E up to values of 74.45–122.16 mg/100g of dry weight (Price, 1985; Sanchez-Machado *et al.*, 2006). Among vitamins of group B, only thiamine, riboflavin and niacin are present in *M. oleifera* leaves. The amount of riboflavin in dried leaves seem very high compared to that of fresh leaves.

2.4 Tree Improvement

Forest tree improvement also known as genetic improvement usually refers to the applied use of forest genetics to improve the quality of trees. "Tree breeding" is often used as a synonym for tree improvement, but it may also refer to specific activities such as controlled pollination. Zobel and Talbert (1984) defined forest tree breeding as "activities geared to solve some specific problem or to produce a specifically desired product. Tree improvement is an integral part of silviculture. The improvement activities provide the raw material for artificial regeneration used by the Silviculturist. In a report by Robbins andDitlevsen (1988),tree improvement has been described as means of providing direct avenue to introduce genetically improved seedlings (or cuttings) into the reforestation system. The improvement can be viewed as an investment in future to increase productivity accrued in terms of increased growth, better form and wood quality, and improved insect and disease resistance.

The planting of genetically improved seedlings on new sites likewise enriches the local gene pool of that species. Future populations of these trees can be expected to be more heterogeneous

than the local stands as well as more productive (Namkoong, 1974). During the selection and breeding of forest trees, a tremendous volume of data is generated regarding species—site interactions, growth and tree quality information, and other physiological relationships.Robbins and Ditlevsen (1988) reported that the fundamental concepts of gene conservation are an integral part of the tree improvement process. Genes are passed from parents to offspring (inherited) and therefore the more a characteristic is under genetic control, the more it will be expressedby the offspring. However, if the environment is the major cause ofvariation, then differences will be less expressed. Genetic variation is responsible for many of the differences between species, separate populations of the same species (provenances) and individual trees.The most basic form of improvement is based on this assumption by using superior stands of trees as sources of seeds. Stands of an appropriate origin are selected and managed for seed collection from the better individuals within the stand. Use of seed stands quickly results in well-adapted seed but is not likely to be associated with high levels of improvement. The tree breeder can achieve higher gains, however, by concentrating on the selection of superior individuals which are referred to as plus trees. The degree of superiority which must be shown by a plus tree with respect to the mean of the population from which it is selected is a reflection of the selection intensity used, with a higher threshold reflecting a higher intensity. The collection of plus-trees identified at the beginning of a breeding programme is referred to as the base population.

Two different methods of improvement are generally used:

- Provenance studies – to identify the best wild populations
- Tree breeding programmes – to select and breed from the best individuals within the best populations

The impact of the tree improvement on forest productivity has been reported to be substantial through the breeding, testing and selection process (Li *et al*., 1999).

2.5 GeneticImprovements of*M. oleifera*Lam

It had been planted around the world and is naturalized in almost the entire tropical belt; this to a great extent has increased the variability of the species. It is a cross-pollinated tree, having high heterogeneity in form and yield. As a cross pollinating species it has higher gene diversity compared to self-pollinating species (Alvarez *et al*., 2001). The expression of the large gene pool is possibly as a result of widespread pollen and seed (Schierenbeck *et al.,* 1997).Study on its cytology as reported by Ramachandran *et al*. (1980) revealed that it is a true diploid species with 2n = 28.Muluvi *et al.* (2004) reported that *Moringa* is adapted to a mixed mating system and the proportion was recorded as 26% of selfing. This evidence had a strong impact on the breeding program, as inbreed lines and hybridization allowed improvement of the species.The 26% selfing observed can lead tooverestimation of the proportion of additive genetic variance and appropriate adjustments are thereforerequired. However, the presence of selfing as well as early sexual maturity (6 months to 1 year) provides an opportunity for developing inbred lines and hybridisation(Muluvi *et al*., 2004).Variability has been reported in flowering time (Ramachandran *et al.,* 1980;Raja *et al*., 2013) (from annual type to perennial type), tree nature (from deciduous to evergreen), tree shape (from semi spread to upright) and resistance to hairy caterpillar (Rajangam *et al*., 2001; Mgendi *et al*., 2011). At Kerala Agricultural University in India, several varieties have been developed and available (Saint Sauveur, 2001). Alessandro *et al*. (2015) reported other research centres in a few other countries apart from India that focused on *M. oleifera* improvement, they are: Asian Vegetable Research Development Centre (AVRDC) (Taiwan), Rural Development Initiative (Zambia), Moringa Philippines Foundation (Philippines) and Moringa Community (Zambia). In spite of the great variability of *M. oleifera,* there are limited institutions with a germplasm bank or data base with either cultivated or

spontaneous accessions. The divergence between genetic variability inherent to the species and poor variability reflected in germplasm banks should be fixed since it represents an obstacle for the progress of breeding programmes. Within the broad differences attributable to origin, considerable variation exists among individual trees and the more detailed techniques of tree breeding to exploit variations at this level. Breeding work seeks to establish whether the observed superiority of an individual is passed on through seed to its progeny in the next generation.

2.6 Importance of Provenance

Provenance in forestry refers to the particular place where trees are growing or the place of origin of seeds or trees. Provenance may refer either to native or to planted trees growing at that place, but its common use is in reference to native trees (Otegbeye and Samarawira, 1992). By definition and in some usage, provenance could also refer to an individual parent tree. However, provenance should be restricted in definition to the population of trees growing at a place and not to an individual tree. Hence, "selecting the proper provenance" is equivalent to selecting the proper locality. It has a biological meaning roughly equivalent to a local population or deme. A provenance will be some part of a race, ecotype, cline, subspecies or variety. The term provenance refers to the geographic source of seed or plant material or to the plants from such a source.

It is widely believed that tree species with a wide geographical distribution exhibit considerable provenance variation in anatomy, morphology and physiology. Provenance research aims at defining the genetic and environmental components of phenotypic variability between trees from different geographic origins. Lopez- Upton (2005) in his test on species/ provenance test to

assess growth performance in *Pinus* species said that determining the bestspecies for a site is important, but to identify the most productive provenance of aspecies can be critical to the financial success of a plantation project.Singh *et al*. (2010) in their study on *Quercus glauca* Thunb reportedthe usefulness of provenance evaluation trial in determining morphological variation in seed characteristics amongst different provenances of this species. The knowledge of seed morphology, germination and seedling growth of some provenances is an important step in helping identify which provenances could be proposed for plantation establishment. A study on provenance variation in *Faldherbia albida* seeds morphological characteristics, germination and early seedling growth by Fredrick *et al*. (2015)was used to recommend provenances to different sites.Information from provenance research has great practical use in the improvement of forest stands. The most obvious and most important use at present is to assure sources of seed that give well-adapted, productive trees in reforestation and afforestation programmes. Provenance research may take a long time to find the best seed source for any particular area, but it should define an acceptable seed source after one or two generations of testing.

2.7 Diversity within Species

Differences within species were apparent to taxonomists who collected them in various locations, and to foresters concerned with moving seed from place to place early in the nineteenth century. Subsequently, seed source studies have become more sophisticated in design and broader in scope. Variations in seed morphological characteristics, germination and seedling growth among provenances of the same species have been reported for many forest trees including *Faidherbia albida* (Dangasuk *et al*., 1997) and *Eucalyptus camaldulensis*(Otegbeye and Samarawira, 1992). Variation among the provenances might be attributed to genetic

differences caused by the adaptation of different provenances to diverse environmental conditions (Ginwal *et al*., 2005) and soil types (Elmagboul *et al.,* 2014). Genetic diversity has been described by Brown (1983) as the amount of genetic variability among individuals of a variety or population of species resulting from many genetic differences between individuals and may manifest in differences in DNA sequence, in biochemical characteristics like protein structure, in physiological properties like abiotic stress resistance or growth rate, or in morphological characters such as flower colour or plant form.Genetic variation in plant is generally accepted to be structured in space and time (Rao and Hodgkin, 2001). An essential prerequisite to determining what to conserve, where to conserve and how to conserve are gotten from the description of the extent and distribution of the different aspect of the genetic diversity in a species and the way it is structured. Genetic diversity is the basis for survival and adaptation. Survival and adaptation can be viewed in terms of time, space and fitness. Fitness includes adaptation, genetic stability and variability. Genetic diversity assessment has contributed widely to evolution, breeding and conservation of genetic resources (Wu *et al*., 1999). Extent of diversity is of significant importance for improvement and utilisation of genetic resources. Genetic diversity of species helps to formulate appropriate sampling strategies for both in situ and ex situ conservations, with the aim of protecting rate of establishment, survival and fecundity that is the ability to produce offspring in large numbers (Millar *et al*., 2000).

2.8 Studying Genetic Diversity within Species

The assessment of genetic diversity within and between populations of a particular species is routinely performed either at the Morphological, Molecular or Biochemical level. This study focuses on Morphological and Molecular characterization of *M. oleifera* accessions.

(i) Morphological characterization: This does not require expensive technology, but large area of land is often needed for these experiments, making it possibly more expensive than molecular assessment (Mondinni *et al*., 2009). These traits are often susceptible to phenotypic plasticity; conversely, this allows assessment of diversity in the presence of environmental variation.

(ii) Molecular characterization: This is based on a large variety of DNA molecular markers, which can be employed for analysis of variation (Agarwal *et al*., 2008). Different markers have different genetic qualities (they can be dominant or co-dominant, can amplify anonymous or characterized loci, can contain expressed or non-expressed sequences).

(iii) Biochemical analysis: This is based on the separation of proteins into specific banding patterns. It is a fast method which requires only small amounts of biological material (Volis*et al*., 2001) However, only a limited number of enzymes are available and thus, the resolution of diversity is limited.

2.8.1 Morphological characterization

Morphological techniques involve the following activities:

- Field assessment
- Analytical methods
- Multivariate analysis
- Principal component analysis
- Clustering analysis

2.8.1.1 Multivariate analysis

Multivariate analytical techniques simultaneously analyse multiple measurements on each individual under investigation. Itis widely used in analysis of genetic diversity irrespective of the dataset (Morphological, Molecular or Biochemical) (Mohammadi and Prasanna, 2003). Multivariate analysis can examine the variables simultaneously in order to access the key features of the process that produced them. The multivariate approach enables the exploration of the joint performance of the variables and determination of the effect of each variable in the presence of the others (Popoola*et al*., 2016) citing Palestina and Sosa, (2002). Mohammadi and Prasanna, (2003) stated that the use of established multivariate statistical algorithms is an important strategy for classifying germplasm, ordering variability for a large number of accessions, or analysing genetic relationships among breeding materials. Multivariate analysis provides both descriptive and inferential procedures; it can search for patterns in the data or test hypotheses about patterns of a priori interest. Multivariate descriptive techniques, can peer beneath the tangled web of variables on the surface and extract the essence of the system. Thus the goal of any multivariate approaches is simplification of the data to express what is going on in terms of a reduced set of dimensions.

2.8.1.2 Principal component analysis

Principal Component Analysis (PCA) is a multivariate tool that has been used in investigating multidimensional datasets with quantitative variables. Willey (1981) defined PCA as ‘a method of data reduction to clarify the relationships between two or more characters and to divide the total variance of the original characters into a limited number of uncorrelated new variables’. This allows visualization of the differences among the individuals and identifies possible groups.

The full method allows for a quick glance and analysis of correlations between the N variables collected in an experiment. The initial observations can also be quickly analysed and manipulated in different forms without any test for hypothesis. The PCA finds the principal components of the data that are the underlying structures of the data. Principal component is also defined as a linear combination of optimally- weighted observed variables basically, principal component analysis is a one-sample technique applied to data with no groupings among the observations and no partitioning of the variables into subsets **y** and **x (**Chatfield and Collins, 1980). In principal component analysis, maximization of the variance of a linear combination of the variables is sought. None of the variables is designated as dependent, and no grouping of observations is assumed. It follows a reduction procedure of a believed large number of variables in which some are seen as redundant that is not correlated with one another (Tabachnick and Fidel, 2007) maybe resulting from the fact that they measure same construct.The technique summarises patterns of correlations among observed variables and reduces a large number of observedvariables to a smaller number of components with several linear combinationscalled principal components (Tabachnick and Fidel, 2007) with each principlecomponent or eigenvalue being independent of other components.

Principal component analysis finds the direction where there is most variance with the use of the eigenvectors. The eigenvaluesare the number telling how much variance exists in that direction. The number of eigenvectors and eigenvalues is the number of the dimensions initially in the dataset. The eigenvector with the highest eigenvalue is the principal component. Correlation between principal components and the original variables is also expressed in PCA, this determines the variables varying together in a particular component and correlation between any two components will always be zero. The first PC summarizes most of the variability present in

the original data relative to all remaining PCs. The second PC explains most of the variability not summarized by the first PC and uncorrelated with the first PC and so on (Joliffe,1986). PC are orthogonal and independent of each other, thus each PC reveals different properties of the orthogonal data and may be interpreted independently (Mohammadi and Prasanna, 2003). It further enables plotting data in two dimensions to look at outliers, groups or clusters based on biological data (Chatfield and Collins, 1980).

2.8.1.3 Clusteranalysis

Cluster analysis searches for patterns in a data set by grouping the (multivariate) observations into clusters. Cluster analysis refers to a group of multivariate techniques whose primary purpose is to group individuals or objects based on the characteristics they possess, so that individuals with similar descriptions are mathematically gathered into same cluster (Hair *et al*., 1995).The goal is to find an optimal grouping for which the observations or objects within each cluster are similar, but the clusters are dissimilar to each other. Accessions in more distant groups provides the indicative of being dissimilar and may be considered suitable candidates for artificial crosses (Abreu *et al*., 1999) to generate superior genotypes.

Clustering analysis had been used in classifying same or more relatedindividuals into a group such that there is homogeneity within a group and heterogeneity between groups (Teodoro *et al.*, 2015).The aim is to find natural groupings in the data, the ones that make sense to the researcher. In cluster analysis, neither the number of groups nor the groups themselves are known in advance. To group the observations into clusters, many techniques begin with similarities between all pairs of observations. In many cases the similarities are based on some measure of

distance. Other cluster methods use a preliminary choice for cluster centres or a comparison of within- and between-cluster variability. It is also possible to cluster the variables, in which case the similarity could be a correlation; two common approaches to clustering the observation vectors are hierarchical clustering and partitioning. Hierarchical clustering starts with *n* clusters, one for each observation, and end with a single cluster containing all *n* observations. At each step, an observation or a cluster of observations is absorbed into another cluster. It can also be reversed and started with a single cluster containing all *n* observations and end with *n* clusters of a single item each. In Partitioning, the observations are simply divided into *g* clusters. This can be done by starting with an initial partitioning or with cluster centres and then reallocating the observations according to some optimality criterion. The result of a cluster analysis is a binary tree, or dendrogram, with *n*–1 nodes. The branches of this tree are cut at a level where there is a lot of 'space' to cut them, which is where the jump in levels of two consecutive nodes is large. Clustering analysis thus seeks to identify homogeneous subgroups of cases or variables based on some measure of distance and also identify a set of groups in which within-group variation is minimized.

2.8.2 Molecular assessment of genetic diversity

Molecular techniques have been applied to increase the understanding of the distribution and extent of genetic variation within and between species. Analyses of genetic diversity are usually based on assessing the diversity of an individual using either allozymes (i.e., variant forms of an enzyme that are coded for by different alleles at the same locus) or molecular markers, which tend to be selectively neutral (Mondini *et al.*,2009). Molecular markers are used to detect genetic variation within genotypes of interest at the DNA level. Variability studies helps in

identifying duplications within the collection, and the genetic linkage among the accessions which can be estimated and which are used in quantifying the genetic variability.Molecular markers may or may not correlate with phenotypic expression of a genomic trait. Molecular markers are phenotypically neutral and they occur throughout the genome. They are not influenced by environments, neither by pleiotrophism, or epistatic interactions (Kameswara, 2004). They offer numerous advantages over conventional, phenotype-based alternatives as they are stable and detectable in all tissues regardless of growth, differentiation, development, or defence status of the cell. They save time and cost (Tanksley *et al*., 1989).

Kameswara(2004) reported that molecular markers have been used in screening of germplasm, determining genetic diversity, identifying redundancies in collections, testing accessions stability and integrity.According to Mondini *et al*. (2009) an ideal molecular marker should possesses the following features: be polymorphic and evenly distributed throughout the genome; provide adequate resolution of genetic differences; generate multiple, independent and reliable markers; be simple, quick and inexpensive; need small amounts of tissue and DNA samples; link to distinct phenotypes; and, require no prior information about the genome of an organism. However, no molecular marker usually has all the listed characteristics.

The different methods of molecular assessment differ from each other with respect to important features such as genomic abundance, level of polymorphism detected, locus specificity, reproducibility, technical requirements and cost. Depending on the need, modifications in the techniques have been made, leading to a second generation of advanced molecular markers. Since 1999, molecular markers have become standards for the genetic characterization of drumstick.

The study began with co-dominant markers in 2010 when Wu *et al*. (2010) developed microsatellite markers. The first successful estimates of genetic diversity and population structure were obtained with Simple Sequence Repeat (SSR) in 2013 by Shahzad (Shahzad *et al*., 2013). These authors' evaluated accessions collected in different locations of Pakistan and accessions from different countries (India, Tanzania, Senegal, Mozambique, Zimbabwe, USA, Mexico, Haiti and Belize) obtained from Educational Concerns for Hunger Organization (ECHO). They found high genetic diversity in wild Pakistan accessions, and low genetic diversity in ECHO accessions. Moreover, ECHO accessions are more similar to those of a single province of Pakistan. Later on, a further investigation on twelve Indian populations, from northern and southern regions of India, was performed through SSR together with morphological markers (Ganesan *et al*., 2014). In their study, individuals from various geographical areas were not significantly different genetically, while a large variability existed in Indian populations.

Ever since then more research work has been carried out with the use of different molecular markers like Random Amplified Polymorphic DNA (RAPD) in Tanzania (Mgendi *et al*., 2010), different accessions in Nigeria (Abubakar *et al*., 2011; Popoola *et al*.,2014), commercially grown varieties in India (Saini*et al.,* 2013), new genotype developed in different countries (Thailand, USA, India and Malaysia, Tanzania, Taiwan) (Rufai *et al.,* 2013). Amplified Fragment Length Polymorphism (AFLP) markers has been used by (Muluvi *et al*., 1999) to investigate seven natural populations from India and introduced populations in Malawi and Kenya. They found a significant level of population differentiation and separation of genotypes based on geographical origin. This means that the genetic approach and its potential application in breeding programs have gained recognition in diversity studies.

The common aim of almost all the studies is the genetic diversification among different populations and/or accessions. These studies showed the higher level of genetic diversity in natural population compared to the cultivated ones. Cultivated accessions present in the considered germplasm banks are genetically close and need to be widened to promote increased diversity and use in breeding programs. Many studies disagree with Muluvi's conclusions that a significant level of population differentiation and separation of genotypes can be based on the geographical origin. Indeed no clusters were found according to geographical origins. This could be due to the planting spread that produced a high rate of gene flow through cross-pollination. Popoola *et al*. (2014) investigated morpho-metric characters along with molecular markers and showed a good correlation between 100 seed weight and pod length, pod weight, number of seeds per locule and Number of seed per pod. The diversity study on drumstick had been receiving an increased level of awareness from what it used to be some ten years back in Nigeria.

2.8.2.1 Random amplified polymorphic DNA (RAPD)

These were the first polymerase chain reaction (PCR) based molecular markers to be employed in genetic variation analyses (Welsh and McClelland, 1990; Williams *et al.,* 1991). The markers are generated through the random amplification of genomic DNA using short primers (decamers), separation of the obtained fragments on agarose gel in the presence of ethidium bromide and finally, visualization under ultraviolet light. The use of short primers is necessary to increase the probability that, although the sequences are random, they are able to find homologous sequences suitable for annealing. The DNA polymorphisms are then produced by rearrangements or deletions at or between oligonucleotide primer binding sites in the genome

(Williams*et al*., 1991). As this approach requires no prior knowledge of the genome analysed, it can be employed across species using universal primers. The major drawback of this method is that the profiling is dependent on reaction conditions which can vary between laboratories; even a difference of one degree in temperature is sufficient to produce a different pattern. Additionally, as several discrete loci are amplified by each primer, profiles are not able to distinguish heterozygous from homozygous individuals (Bardakci, 2001).

2.8.2.2 Microsatellitebased marker technique

Microsatellites or Simple Sequence Repeats (SSR) are sets of repeated sequences found within eukaryotic genomes (Bell and Ecker, 1994). These consist of sequences of repetitions, comprising basic short motifs generally between 2 and 6 base-pairs long. Polymorphisms associated with a specific locus are due to the variation in length of the microsatellite, which in turn depends on the number of repetitions of the basic motif Mondini *et al*. (2009). Microsatellite assays show extensive inter-individual length polymorphisms during PCR analysis of unique loci using discriminatory primers sets. Simple sequence repeats is used as a primer to amplify regions between the microsatellites (Shoba *et al*., 2010). Microsatellites are highly popular genetic markers as they possess: co-dominant inheritance, high abundance, enormous extent of allelic diversity, ease of assessing SSR size variation through PCR with pairs of flanking primers and high reproducibility.

Variation in the number of repeated core sequence of nucleotides at a SSR locus among different genotypes provides the basis for polymorphism that can be used in plant genetic studies. They are therefore excellent choice of DNA markers for genetic mapping in plants. Unlike RFLPs, for

instance SSR technology is PCR based, requires only nanograms of DNA, and is readily automatable. Unlike RAPDs, SSR markers have proven to be reliable and reproducible.It reveals larger number of fragments per primer than RAPD analysis (Bajpai *et al.*, 2008). Unlike AFLPs, they are co-dominant and species specific. Moreover, they are both size and sequence specific. They can be used in pedigree analysis to determine kinship among individuals, fingerprinting, forensics, genetic mapping, and phylogenetic analysis (Gupta *et al.*, 1996). However, the development of microsatellites requires extensive knowledge of DNA sequences, and sometimes they underestimate genetic structure measurements, hence they have been developed primarily for agricultural species, rather than wild species. Initial approacheswere principally based on hybridization techniques, whilst more recent techniques are based on PCR (Gupta and Varshney, 2000). Polymorphic Information Content (PIC) is a parameter that provides an estimate of the discriminatory power of molecular marker per primer.

CHAPTER THREE

3 MATERIALS AND METHODS

3.1 Materials Used for the Study and their Sources

Seeds were sourced from a field survey through collection of different accessions of *M. oleifera* from different locations within Nigeria. This was carried out with the help of the following institutions;

i. Department of Agronomy, Bayero University Kano, Kano-State (BUK).
ii. Department of Plant Science, Ahmadu Bello University, Zaria, Kaduna-State (ABU).
iii. Savanna Research Station, Samaru, Zaria (FRIN).

A total of 52 different sources of matured and dried pods were collected in different locations in March, 2013. The different lines collected were from some agro-ecological zones of Nigeria. Three out of the lines collected from BUK were from Mali, India and Israel (Table 3.1).

3.1.1 Nursery establishment

This study was conducted in Forestry Research Institute of Nigeria (FRIN), Ibadan (South west, Nigeria),located on Latitude $7^0 23^{'} 15''$ to 7^0 24'00''N and Longitude $3^0$51'00''N to$3^0$52'15''Eof the Greenwich Meridian,theclimate is West African monsoon with dry andwet season. The location has a meanannualrainfall of approximately 1548.9mm distributed within a period of 90 days. The mean maximum temperatureis 31.9^0C minimum 24.2^0C. Mean daily relative humidity is about 71.9% (FRIN, 2015).Seeds were sown in the nursery on April 19th, 2013. The potting media used wastop soil collected at a depth of 0 to 5cm from the ground level. In the nursery, seeds from each of the 52 locations were planted in blocks of sixty pots per provenance.

Table 3.1: List of provenances, their states and accession number

S/N	Provenance	State	Accession No
1	Ado	Ekiti	FRIN MOR12-1
2	Jos	Plateau	FRIN MOR12-2
3	Dutse	Jigawa	FRIN MOR12-3
4	Kano(Bayo)	Kano	FRIN MOR12-4
5	Markurdi	Benue	FRIN MOR12-5
6	UDUS	Sokoto	FRIN MOR12-6
7	Israel	Israel	FRIN MOR12-7
8	Ibadan	Oyo	FRIN MOR12-8
9	Mali	Mali	FRIN MOR12-9
10	BUK	Kano	FRIN MOR12-10
11	India	India	FRIN MOR12-11
12	Maiduguri	Borno	FRIN MOR12-12
13	Igumale	Benue	FRIN MOR12-13
14	Danje shanni	Borno	FRIN MOR12-14
15	Rumi	Kaduna	FRIN MOR12-15
16	Rano	Kano	FRIN MOR12-16
17	Danya	Kano	FRIN MOR12-17
18	Zaria	Kaduna	FRIN MOR12-18
19	Abeokuta	Ogun	FRIN MOR12-19
20	BT	Kano	FRIN MOR12-20
21	Gashua	Yobe	FRIN MOR12-21
22	Gombe	Gombe	FRIN MOR12-22
23	Sabua	Kastina	FRIN MOR 12-23
24	Lafia	Nasarawa	FRIN MOR12-24
25	Zaria Prof	Kaduna	FRIN MOR12-25
26	Kastina	Kastina	FRIN MOR12-26
27	Maigana	Kaduna	FRIN MOR12-27
28	Mokwa	Niger	FRIN MOR12-28
29	Bagauda	Kano	FRIN MOR12-29
30	Ilorin	Niger	FRIN MOR12-30
31	Abuja	FCT	FRIN MOR12-31

UDUS – Usman Dan Fodio University, Sokoto
BUK – Bayero University, kano
FCT –Federal Capital Territory

Watering of the pots was done as at when neededto ensure good germination which was monitored from date of sowing until no further germination was recorded. The seed emergence started from the 8th day after sowing and germination count was taken over a period of sixteen days after which no new seed germination was recorded. Germination was considered when the hypocotyl hook was evident above the soil surface adopting the work of Fandohan *et al*. (2010).Only 31 locations produced 30 or more healthy seedlings required for the field experimental design layout.

3.1.2 Field establishment

A total of thirty healthy seedlings were randomly selected from each accession in the nursery at six weeks after sowing (WAS) representing ten seedlings per accession in three replicates. The seedlings were subsequently transplanted on the 29th of May, 2013 to designed plots with an area of 10m by 2.5m double lines with inter and intra row spacing of 2.5 m x 2.5 m arranged in a Randomised Complete Block Design with three replicates in FRIN arboretum. The plants were allowed to get fully established on the field before taking any measurements on them. Spot weeding was done manually at interval of four weeks for a period of eight months when the plants were still at tender stage and later mechanically with field mower.

3.2 Morphological Variation Study

In order to identify the first three objectives of this study, the following data were collected on the established plantation. Data were collected from five plants randomly selected from within each plot or as required for the measured trait. Data collection runs through a period of three and a half years during which the plants were allowed to grow at will with no introduction of pruning, fertiliser application or leaf harvesting.

3.2.1 Data collection

Data on morphological traits were recorded as follows:-

Surviving ability: This is the number of plants that survived after three years of establishment in the field.

Regrowth potential: This is the number of new shoots that sprouted after abscission, counted at two weeks after first rain.

Stem diameter: this is the diameter of the stem at basal part of the plant taken six months after transplanting and it is measured in millimetres (mm) using a verneer Calliper.

Plant height: The height of the plant at six months after transplanting taken with a meter rule from the ground to the tip of the main stem, measured in centimetres (cm).

Number of primary branches: The total number of branches that emanate directly from the main stem at 24 months after transplanting.

Number of secondary branches: The total number of branches found growing on the primary branches 24 months after transplanting.

Number of flowering branches: The total number of branches that were flowering on a particular plant at 30 months after transplanting.

Number of days to first flowering: The number of days it takes a plot to have a tree that produced a flower after transplanting.

Number of days to first fruiting: The number of days it takes a particular plot to have a flower that produced a fruit after transplanting.

Number of pods per plot: The total number of pods harvested in a particular plot at harvesting.

Pod length: The distance between the base of a pod and the tip of the pod measured in centimetres using a meter rule.

Pod diameter: The diameter of the pod taken at the centre of the pod measured in millimetres with a veneer calliper.

Pod weight: The total weight of the harvested pods from each plot during harvest measured in grams using a weighing balance.

Number of seeds per plot: The total number of seeds produced from a plot during harvest.

Seed yield: The total weight of the harvested seeds per plot measured in grams using a weighing balance.

Hundred (100) seeds weight: The weight in grams of randomly selected 100 seeds from each accessions seed lot.

Number of seeds per pod: The total number of seeds in a pod from a plot.

3.2.2 Data analysis

The mean values of all data collected for each trait were analysed using Statistical Analytical System (SAS) (2002) package. Analysis of variance was determined while the Principal Component Analysis and correlation coefficient analysis were determined using XLSTAT 2007

programming package. The morphological character dendrogram was generated using XLSTAT 2007 and Numerical Taxonomy System of Statistics (NTSYS).

The model used for the analysis was:

$Y_{ijk} = \mu + \gamma_k + e_{ijk}$

Where; Y_{ijk} = the observed trait measured

μ = the grand mean of the character

γ_k = the genotypic effect

e_{ijk} = environmental effect.

The form of analysis of variance calculated to get the expected mean squares is shown below:

Table 3.2:Form of Analysis of Variance for Morphological Traits

Source of Variation		DF	MS	EMS
Replication	2	$(r-1)$	MS_r	
Provenance	30	$(p-1)$	MS_p	$\sigma_e^2 + r\sigma_p^2$
Error	60	$(r-1)(p-1)$	MS_e	σ_e^2

Mean separation was done by Least Significance Difference (LSD) where significance exists. Coefficient of variation was computed to compare the variability existing within each studied character.

3.2.3 Estimation of component of variance

The contribution of each component of variance to the total phenotypic variation was calculated by equating the appropriate mean squares to the expected mean squares (EMS) from the form of Analysis of Variance (ANOVA) in Table 3.3 using the method described by Singh and Chaudhary (1985) as follows:

$\sigma_e^2 = M_e$ $\sigma_g^2 = \frac{M_2 - M_1}{r}$

Where:

σ_e^2 = Error variance σ_g^2 = genotypic variance

M_1 = Error mean square M_2 = genotypic mean square

r = Number of replicate $\sigma_p^2 = M_e + \sigma_g^2$

The phenotypic coefficient of variation (PCV %) and genotypic coefficient of variation (GCV %) were estimated by method of Johnson *et al.*(1955) and used by Ajayi *et al.* (2014) as:

$$\text{PCV} = \frac{\sqrt{\sigma_p^2}}{\bar{X}} \times 100$$

$$\text{GCV} = \frac{\sqrt{\sigma_g^2}}{\bar{X}} \times 100$$

Where, σ_p^2 = Phenotypic variance, σ_g^2 = genotypic variance

$\bar{X}$ = Grand mean for character under consideration.

PCV and GCV were classified as follows: 0 – 10% = low; 10 -20% = moderate; >20% = high.

3.2.4 Heritability estimates

Heritability estimates how much of the phenotypic variation can be explained by genetic, or genetic-environmental effects. Broad-sense heritability (H_b^2) refers to the inclusion of all potential sources of genetic variation (additive, dominance, epistatic, maternal and paternal effects). Broad-sense heritability was estimated after Eckebi *et al.* (1977) as follows:

$$H_b^2 = \frac{\sigma_g^2}{\sigma_{ph}^2}$$

Where: $H_b^2 =$ Broad-sense heritability

$\sigma_g^2 =$ Genotypic variance

$\sigma_{ph}^2 =$ Phenotypic variance

The heritability was categorised as proposed by Robinson *et al.*, 1949 and used by Ajayi *et al.*(2014): 0-30% = low; 30-60% =moderate; >60% = high.

3.2.5 Correlations

Genotypic and phenotypic correlation coefficients were estimated for all possible pairs of characters examined from the corresponding variance and components of covariance. These were estimated using the standard procedure as used by Kashiani and Saleh (2010) from the equations:

$$r_{pxy} = \frac{\sigma_{p_{XY}}^2}{\sqrt{\sigma_{px}^2 X \sigma_{py}^2}} \qquad r_{gxy} = \frac{\sigma_{gxy}}{\sqrt{\sigma_{gx}^2 X \sigma_{gy}^2}}$$

Where:

$r_{pxy} =$ Phenotypic correlation coefficient between characters X and Y

$\sigma_{px}^2 =$ Phenotypic variance of character X

$\sigma^2_{py} =$ Phenotypic variance of character Y

$r_{gxy} =$ Genotypic correlation coefficient between characters X and Y

$\sigma^2_{gx} =$ Genotypic variance of character X

$\sigma^2_{gy} =$ Genotypic variance of characters Y

3.2.6 Rank summation index

To identify superior accessions, an index, Rank Summation Index (RSI) according to Malumba and Mock (1978) was generated from eight traits namely: total number of pods, total number of seeds, the seed yield, 100seed weight, pod weight, number of seeds per pod, secondary branches and flowering branches. Malumba and Mock (1978) described RSI as 'parameter free' index. The index was formed by ranking each trait in descending order and then the values attached to each trait are added to obtain the sum of the ranks; this indicates the overall classification of the accessions (Cruz and Ragazzi, 2001). Accessions with good total number of pods, total number of seeds, the seed yield, 100seed weight, pod weight, number of seeds per pod, secondary branches and flowering branches ranked first, while the least performing ones ranked last. RSI computation by Malumba and Mock (1978) is as follows:

$$RSI = \sum_{i-1}^{n} R_i = S$$

Where,

RSI = Aggregate performance of an accession using the ranking of each of the desired traits.

R_i= Rank of the meanof each of the desired traits.

3.2.7 Principal component analysis

In determining the plant traits responsible for the expressed variation and the relative contribution of the various traits contributed to the total variability among the provenances, principal component analysis (PCA) technique was employed. The XLSTAT (2007) data analysis solution package was used to run the PCA in the excel data sheet. The XLSTAT will display the Eigenvalues in a table and in a chart known as the scree plot. The eigenvalue shows the amount of information summarized in every dimension. The eigenvalue is the most commonly used factor in solving the number of components problem. The dimensions were arranged in ascending order with highest amount of information coming first, with this approach only eigenvalues that are greater than 1were retained and used in the interpretation.The eigenvectors gave the direction where there is most variance among the considered characters. Important traits that are most strongly correlated with each component and having magnitude farthest from zero either positive or negative are used in interpreting the components in each Principal components from other associated eigenvectors. The factor loading of characters on the components showed the characters contributing to the variability expressed. The loading showed the correlation between a specific component and the character, character values greater than 0.4 had meaningful contribution to the load in the components. The contributions of each accession to the components are seen in the accessions with higher values in percentage. These were considered as contributing most of the effects to each component.

3.2.8 Cluster analysis

Cluster analysis was used to organize the accessions into groups. The more similar accessions are grouped togetherwhile accessions in different clusters have dissimilar attributes compared to each other as described by Hair *et al*. (1995).

3.2.9 Euclidean similarity distance

Genetic distances were computed using Mean Euclidean Distance as described by Madhulatha (2012)

$$d_{ij} = \sqrt{\frac{1}{k}\sum_{1}^{k}(X_{ik} - X_{jk})^2}$$

Where d_{ij} =genetic distance between two accessions

K =Number of accessions

X_{ik} = accession 1

X_{jk} = accession 2

Accessions with smaller distances in between them are more similar while objects with larger distances are more dissimilar.Euclidean similarity index indicates the level of disparity between the accessions, thus the higher the values the wider the dissimilarities or dispersion between the accessions.

3.3 Materials and Methods for Molecular Markers Analysis

In order to achieve the fourth objective the following were carried out on the same established moringa plantation at six months after planting.

3.3.1 Plant materials

The plant materials used for the genetic diversity study were the 31 accessionsestablished in the FRIN arboretum. At six months after field establishment, sample collection was made on each of the 31 accessions randomly in the plot. This was done at the tip of freshly growing branch early in the morning and the samples were refrigeratedat 4.4^0C for four days till they were ready for use. The DNA extraction was carried out at the laboratory of the Nigerian Institute of Science Laboratory Technology (NISLT), Samonda, Ibadan, OyoState.

3.3.2 DNA extraction

Genomic DNA samples were extracted using a RPN-8510 illustra DNA extraction kit Phytopure for plant DNA extraction. (Buckinghamshire, U.K). 3g of young leaf tissue was ground with liquid Nitrogen and to this powder 15 ml of preheated CTAB buffer (65°C) was added. It was then incubated at 65°Cin a water bath for one hour. After bringing the tubes to room temperature, equal volume (15ml) of chloroform: Isoamyl alcohol (24:1) was added and the contents were mixed well for 10 minutes to form an emulsion. It was then centrifuged at 10,000 rpm for15 minutes at 15°C. The supernatant was transferred to a fresh tube and the chloroform: isoamyl alcohol step was again repeated. The aqueous phase was transferred to a new tube and equal volume of ice cold isopropanol was added and incubated in a freezer overnight. The contents were then centrifuged at 10,000 rpm for 20 minutes at 16°C.The pellet was now saved by discarding the solution. The pellet was washed with 70% ethanol by centrifuging the contents at 10,000 rpm for 10 minutes. The alcohol was discarded and the pellet was air dried. The pellet was then dissolved in 3 ml of double distilled water. Then 1 µl of RNase was added and incubated at37 °C for 30 minutes. DNA was precipitated by adding 50 µl of 3M sodium acetate and 7.5 ml of 100% ethanol and the contents were again centrifuged at 10,000 rpm f or 10

minutes.Supernatant was discarded. The pellet was washed with 70 % ethanol and air dried. It was finally dissolved in TE buffer (150 µl) and stored at - 20°C for long term use.

3.3.3 DNA quantification

The quantification of the DNA was carried out using a Nanodrop at the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan.

3.3.4 Genotyping using SSR markers

Ten Microsatellite SSR primers were selected based on the polymorphic information from the list prepared by Wu and Yang (2010) for *Moringa oleifera*. The details of the primers and their sequence are presented in Table 3.4. The PCR was carried out with an initial denaturing at 95^0C for 5 mins, followed by 30cycles of 94 ^{0}C for 30 seconds, primer-specific annealing temperature 55 to 61 ^{0}C for 30 seconds, 72 ^{0}C for 30 seconds and a final extension at 72 ^{0}C for 8 mins and a hold at 4 ^{0}C. For enrichment of the fragments containing SSRs, the PCR products, with a size range of 200 to 1000 bp, were denatured at 95 ^{0}Cfor 5 min and were then hybridized with 5^1biotinylatedprobe (AG) in a 250-mL solution(4.16. SSC and 0.07% SDS) at 48^0C for2 hours. The mixture was incubated at room temperature for 30 min with constant gentle agitation.

The amplified products were then electrophoresed in 8% polyacrylamide gels and the amplified fragments were visualized by silver staining as described by Bassam *et al.* (1991). Electrophoretic patterns were scored and checked with a 20-bp DNA ladder marker (Takara, Tokyo) used to estimate allele sizes. The gel pictures were recorded using Gel Documentation System in the Biosciences Laboratory, International Institute of Tropical Agriculture, Ibadan, Oyo, State.

Table 3.3: Characteristics of 10 RAPD primers used in the analysis

Primers	Sequence5'-3'	TF	PF%	PIC	MI	I	H
A18	AGG TGA CCG T	6	100	0.23	1.35	0.37	0.23
IDT02	TGA TCC CTG G	8	62	0.14	0.69	0.21	0.14
IDT15	GGT CGG AGA A	9	78	0.30	2.07	0.44	0.30
S01	CTA CTG GCG T	8	87	0.26	1.82	0.4	0.26
S18	CTG GCG AAC T	9	62	0.16	0.79	0.24	0.16
W02	ACC CCG CCA A	7	29	0.12	0.24	0.17	0.12
W19	CAA AGC GCT C	9	67	0.17	1.02	0.27	0.17
B02	AGT CAG CCA C	4	100	0.41	1.65	0.6	0.41
A3	AGT CAG CCA C	3	100	0.36	1.07	0.52	0.36
A8	GTG ACG TAG G	3	67	0.26	0.52	0.38	0.26

Totals (TF)
Polymorphic fragments (PF%)
Polymorphic information content (PIC)
Marker index (MI)
Shannon index (I)
Genetic diversity (H)

Table 3.4: Characteristics of 10 polymorphic microsatellite markersfor*Moringa oleifera* used in the study

Locus	Primer sequence ($5^{1}3^{1}$)	Repeat motif	Allele size (bp)	$Ta^{Z}(C^{0})$
MO1	F TTGTCTGCCTCCTTTTGTCA R AACTGTCACCCTCCTATCCA	(TC)17	150–158	58
MO6	F GCATAGCCACCTTTACTCCT R GACTTTTGAACTCCACCACC	(AG)7-(AG)6	497–520	61
MO10	F CTTTACACCTCAGTATCCCT R GTTCGGCTTATGTTCTCGTT	(CT)20-(CT)5	294–306	58
MO13	F TTTCGGGTTTTCTTTCACGG R AGCTCACTTTCCATCTCCAT	(CT)15	331–341	58
MO15	F CCCCTCTATTTCCATTTTCC R GCTCCATAAACCCTCTTGCT	(TC)10CCT(TC)6	143–149	59
MO18	F TTTTCCTCCCTTATTGTGCC R CCGTTGCCCTTTGTGGTTCA	(GA)6A(AG)16	240–259	58
MO41	F TGGGATTAGGGCATTAGAAA R TAGTGGGTCCAAGACAAAGC	(GA)10	147–153	55
MO48	F AGAAGAACCCAACAGAGGAT R CTTTTCACTAACCACCACCC	(TC)8C(CT)15A(AC)7	210–230	58
MO55	F ATTACAGAACGATGAAACCA R CTCTTTCCCTCCATTCAACC	(AG)8AA(AG)14	110–114	56
MO68	F TGCTTCGCTTCCTCTATTCT R ACCACAGGCTTGCTTCAGTA	(GA)12	240–250	56

Source: Wu et al., 2010

Ta^{Z}PCR annealing temperature.

3.3.5 Gel scoring

The SSR electrophoretic profile of each gel was transformed into a binary matrix of visible presence (1) and absence (0). The SSR data were subjected to analysis to determine the major allele Frequency, genetic diversity and Polymorphic Information Content (PIC) using power maker 3.5 (Liu and Muse, 2005). Genetic distances across the accessions and neighbour joining trees were calculated using power maker 3.5

CHAPTER FOUR

4 RESULTS

4.1 Morphological Analysis Results

4.1.1 Mean performance

The mean performances for the morphological characters of *M. oleifera*are presented in Table 4.1.Survival of the plants (Surv) in each accession for the period of 3years under study ranged from 1 to 7 plants. Sixteen accessions performed well above the mean value of 3.59 for Survival ability. Accessions FMOR12-2 and FMOR12-23 are the most stable in adaptation to the experimental area for the three years under study. The two accessions had overall surviving plants of 6 and 7 plants, respectively. Accession FMOR12-10 had the least adaptable potential with only 1 plant surviving for the period under study.

The regrowth ability after abscission (Reg) ranged from 1 to 5 shoot count with a mean of 2.33. Accessions FMOR12-31 had the best Regrowth potential with 5 shoots followed by accessions FMOR12-23, FMOR12-9, FMOR12-2 and FMOR12-3 having 4 shoots each. Eight of the accessions FMOR12-7, FMOR12-8, FMOR12-10, FMOR12-11, FMOR12-16, FMOR12-19, FMOR12-20 and FMOR12-27 had 1 shoot and are the least performing ones.

The plant Stem diameter (SD6M) at 6 months after field establishment ranged from 8.45mm to 29.37mm, with a mean value of 18.05mm. Accessions FMOR12-31 and FMOR12-6 had the thickest diameter of 29.37mm and 25.10mm, respectively. Accessions FMOR12-20 and FMOR12-21 had the lowest diameter with 9.09mm and 8.45mm, respectively.

Plant height (H6M) at 6 months after establishment (MAE) ranged from 59.83cm to 257.30cm, with a mean of 133.09cm. The accessions with the tallest plants are FMOR12-22 and FMOR 12-31 with values of 257.30cm and 231.43cm, respectively.Accessions FMOR12-21 and FMOR12-8 with 65.88cm and 59.83cm,respectively had the shortest plants.

Table 4.1: The mean performance for the morphological characters of *Moringa oleifera*

S/N	Acce No	Surv	Reg	SD6M (mm)	H6M (cm)	Pbr	Sbr	Flbr	DFF	DFFR
1	FMOR12-1	2.0	2.0	13.24	67.53	10.40	5.33	6.93	102.0	172.7
2	FMOR12-2	7.0	4.0	13.23	98.87	3.25	1.00	2.42	141.0	243.0
3	FMOR12-3	5.0	4.0	22.34	126.21	7.16	9.94	12.27	144.0	305.3
4	FMOR12-4	4.3	2.3	23.61	134.12	5.40	5.69	7.10	174.3	234.3
5	FMOR12-5	4.0	3.0	17.91	130.83	5.58	9.00	8.63	205.0	267.0
6	FMOR12-6	5.0	2.0	25.10	145.73	5.69	15.11	12.97	136.5	274.0
7	FMOR12-7	2.0	1.0	16.10	109.37	3.89	13.67	11.78	122.0	192.0
8	FMOR12-8	2.0	1.0	9.62	59.83	5.52	10.55	7.54	192.5	299.5
9	FMOR12-9	4.0	4.0	20.70	127.23	15.00	15.31	19.67	197.0	222.3
10	FMOR12-10	1.0	1.0	9.55	68.57	4.67	0.00	5.00	162.0	231.0
11	FMOR12-11	2.0	1.0	22.85	102.06	11.17	27.17	18.00	183.5	274.0
12	FMOR12-12	3.0	2.0	15.59	114.08	8.89	10.78	12.17	194.5	166.0
13	FMOR12-13	3.0	2.0	15.99	101.30	8.53	7.75	12.77	136.3	221.7
14	FMOR12-14	5.0	3.0	15.40	103.70	5.50	13.00	9.81	188.0	257.3
15	FMOR12-15	5.0	3.0	15.89	128.31	6.39	8.56	5.30	154.0	231.3
16	FMOR12-16	3.0	1.0	17.77	107.93	8.50	13.83	21.22	133.0	253.7
17	FMOR12-17	5.0	3.0	19.09	116.09	9.11	22.33	16.92	160.3	264.0
18	FMOR12-18	3.0	2.0	18.43	111.40	5.83	8.03	2.25	184.0	255.0
19	FMOR12-19	3.0	1.0	14.84	120.00	2.19	7.20	3.44	117.5	227.0
20	FMOR12-20	2.0	1.0	9.09	81.00	4.52	2.80	1.79	434.5	491.5
21	FMOR12-21	3.0	2.0	8.45	65.88	2.33	7.70	3.70	358.0	419.0
22	FMOR12-22	5.0	3.0	24.37	257.30	6.87	21.67	15.00	107.0	187.0
23	FMOR 12-23	6.0	4.0	21.15	204.47	7.92	7.58	10.56	113.7	195.0
24	FMOR12-24	4.0	3.0	23.44	224.84	4.77	10.80	14.20	129.3	207.0
25	FMOR12-25	4.0	3.0	22.81	186.10	10.43	10.28	13.80	152.0	229.7
26	FMOR 12-26	5.0	3.0	22.96	183.73	10.69	6.56	8.57	123.0	194.5
27	FMOR12-27	2.0	1.0	17.59	114.87	4.89	4.00	5.33	124.5	256.5
28	FMOR12-28	4.0	3.0	13.97	133.49	5.43	17.42	10.93	112.0	177.0
29	FMOR12-29	3.0	2.0	18.34	210.54	8.57	17.78	14.75	110.0	176.0
30	FMOR12- 30	2.0	2.0	11.58	113.89	5.33	10.70	10.20	105.0	179.0
31	FMOR12-31	5.0	5.0	29.37	231.43	8.72	12.33	9.85	132.5	200.0
	Std Error	1.18	0.94	4.29	33.54	2.62	4.27	4.76	16.11	25.01
	Mean	3.59	2.33	18.05	133.09	7.01	10.97	10.44	162.23	242.04
	C.V %	57.52	69.91	41.60	44.11	65.45	68.43	80.15	20.12	20.43
	LSD	3.30	2.66	11.51	93.00	6.81	11.83	12.96	52.48	77.92
	Significance	*	Ns	Ns	**	Ns	**	Ns	**	**

. ** = significant at 0.01 level of probability, *=significant at 0.05 level of probability, Ns= non-significant difference, Surv=surviving ability, Reg=regrowth potential, SD6M= stem diameter, H6M= plant height, Pbr= number of primary branches, Sbr= number of secondary branches, flbr=number of flowering branches, DFF= days to first flowering ,DFFR= days to first fruiting.

Table 4.1 contd.

S/N	Acce No	Tpod	PLt (cm)	PD (mm)	PW (grams)	Tseeds	Syield (grams)	HSW (grams)	seeds/p
1	FMOR12-1	34.00	13.11	5.58	4.42	51.00	13.67	9.00	6.38
2	FMOR12-2	7.33	27.14	13.34	7.43	58.67	11.33	0.00	14.66
3	FMOR12-3	64.00	34.48	13.37	9.67	602.00	130.00	21.33	16.10
4	FMOR12-4	37.33	33.69	13.70	8.20	413.00	82.00	15.33	15.73
5	FMOR12-5	20.00	43.91	15.08	12.40	354.00	90.33	22.27	17.10
6	FMOR12-6	89.67	34.12	14.57	11.37	548.00	116.33	16.19	14.92
7	FMOR12-7	44.33	20.70	4.95	4.90	284.33	56.33	6.67	6.10
8	FMOR12-8	11.00	9.98	5.37	2.60	46.33	12.00	9.33	4.10
9	FMOR12-9	56.67	20.48	10.39	6.33	66.67	21.00	17.27	9.13
10	FMOR12-10	6.67	8.50	3.87	2.20	53.67	10.00	6.00	6.00
11	FMOR12-11	34.00	29.92	11.20	9.73	237.33	35.33	8.19	14.10
12	FMOR12-12	36.33	26.42	9.42	7.67	255.33	53.67	15.67	11.53
13	FMOR12-13	36.67	31.95	13.30	8.40	346.67	77.00	21.33	15.37
14	FMOR12-14	25.00	46.80	13.12	9.18	303.67	55.67	21.67	15.43
15	FMOR12-15	59.67	33.82	15.75	10.60	222.67	57.00	15.00	13.50
16	FMOR12-16	32.33	35.92	14.88	10.32	194.00	52.33	11.67	14.13
17	FMOR12-17	88.33	43.36	16.60	14.20	661.67	201.33	28.33	18.33
18	FMOR12-18	7.00	28.18	15.36	7.19	59.33	14.33	0.00	9.43
19	FMOR12-19	8.67	26.27	12.44	6.25	20.67	5.67	8.09	8.50
20	FMOR12-20	2.00	7.53	5.23	2.33	4.00	0.67	5.93	4.00
21	FMOR12-21	4.33	7.67	3.49	1.43	25.00	3.67	6.60	2.77
22	FMOR12-22	127.00	31.99	14.34	9.17	2129.33	464.33	20.69	15.97
23	FMOR 12-23	115.33	32.11	15.23	8.90	461.67	107.00	21.93	11.20
24	FMOR12-24	60.67	30.45	14.79	8.10	462.67	107.67	21.67	12.60
25	FMOR12-25	88.33	31.79	15.88	9.63	177.67	53.67	18.33	10.67
26	FMOR 12-26	61.00	29.42	14.06	7.93	374.00	77.33	21.67	13.73
27	FMOR12-27	21.00	9.27	4.09	2.03	97.33	19.00	7.00	3.40
28	FMOR12-28	72.33	18.46	10.59	6.33	356.67	83.33	7.67	8.47
29	FMOR12-29	58.67	30.67	14.52	8.63	241.33	56.33	23.00	13.97
30	FMOR12- 30	12.33	21.43	9.87	6.70	50.67	10.00	6.19	7.47
31	FMOR12-31	60.67	20.46	10.29	6.17	623.33	132.67	15.33	8.03
	Std Error	31.40	7.97	3.11	2.53	386.42	84.32	6.11	3.41
	Mean	44.60	33.79	14.55	9.50	407.84	92.41	18.28	14.15
	C.V %	121.94	52.18	47.05	58.98	212.09	204.77	74.75	53.40
	LSD	88.83	10.58	2.68	4.37	1068.00	233.67	11.81	4.41
	Significance	**Ns**	****	*****	**Ns**	*****	**ns**	**Ns**	*****

** = significant at 0.01 level of probability, *=significant at 0.05 level of probability, ns= non-significant difference, Tpod=number of pods per plot, PLt=pod length, PD= pod diameter, TSeed= number of seed per plot, Syield= seed yield per plot, HSW= 100 seed weight, seeds/p=number of seeds per pod

The Number of primary branches (Pbr) ranged from 2 to 15 branches, with a mean value of 7.01. Twelve accessions performed above the mean value. Accession FMOR12-9 and FMOR12-11 had the highest number of primary branches 15 and 11 branches, respectively. Accession FMOR 12-21 and FMOR12-19 had the least with 2 branches each.

Number of secondary branches (Sbr) ranged from 0.00 to 27 branches with a mean value of 10.97 branches. While accessions FMOR12-11 and FMOR12-12-17 were the best performing accessions with 27 and 22 branches, respectively. Accessions FMOR12-2 and FMOR12-10 had the least secondary branches, with 1 and 0, respectively.

Number of flowering branches (Flbr) ranged from 2 to 21 branches, with a mean of 10.44 branches. Accessions FMOR12-16 and FMOR12-9 had the highest Number of flowering branches with values 21 and 20, respectively. The least performing accessions are FMOR12- 2, FMOR12-18 and FMOR12-20 each having 2 flowering branches each.

Days to first flowering (DFF) ranged from 102 to 435 days, having an average of 162 days. FMOR12-1 and FMOR12-30 had early flowers with 102 and 105 days, respectively. FMOR12-21 and FMOR12-20 had delayed flowers of 358 and 435 days respectively.

Days to first fruiting (DFFR) ranged from 173 to 492 days, having an average of 242 days. Accession FMOR12-1 and FMOR12-29 had theirfirst fruiting earlier with 172 and 176 days, respectively. The accessions FMOR12-21 and FMOR12-20 had delayed fruiting with 419 and 492 days respectively.

Total number of pods harvested (Tpods) ranged from as low as 2.00 pods for accession FMOR12-20 to 127 pods for accession FMOR12-22 with a mean of 44.6 pods. Accessions FMOR12-22 and FMOR12-23 had the highest number of pods with 127 and 115 pods, respectively. The least producing accessions are FMOR12-21 and FMOR12-20 with 4 and 2 pods respectively.

The Pod length (PL) ranged from 7.53cm to 46.80cm with a mean of 33.79cm. Accessions FMOR12-14 and FMOR12-17 had the longest pods with values 46.80cm and 43.36cm,respectively,

while accessions FMOR12-21 and FMOR12-20 had the shortest pods with values 7.67cm and 7.53cm, respectively.

The pod diameter (PD) ranged from 3.49mm for accession FMOR12-21 to 16.60mm for accession FMOR12-17 with a mean of 14.55mm. The best performing accessions were FMOR12-17 and FMOR12-15 with diameter of 16.60mm and 15.88mm respectively. The least performing accessions are accessions FMOR12-10 and FMOR12-21 with 3.87mm and 3.49mm, respectively.

The pod weight (PW) ranged from 1.43g to14.20g with a mean of 9.50g. Accessions FMOR12-17 and FMOR12-5 had the heaviest pods weight with values 14.20g and 12.40g, respectively. Accessions FMOR12-27 and FMOR12-21 had the least pods weight with 2.03g and 1.43g.

The total number of seeds (Tseeds) produced per plot ranged from 4.00 seeds to 2129.33 seeds with a mean of 407.84seeds.Accessions FMOR12-22 and FMOR12-17 had the highest number of seeds, with 2129.33 seeds and 662 seeds respectively. Accessions FMOR12-19 and FMOR12-20 had the least number of seeds with 20.67 and 4.00 seeds, respectively.

The seed yield per plot (Syield) ranged from 0.67g to 464.33g with a mean of 92.41g.Accessions FMOR12-22 and FMOR12-17 had the highest yield with values 464.33g and 201.33g, respectively. Accessions FMOR12-21 and FMOR12-20 had the least yield with just 3.67g and 0.67g, respectively.

The 100 seed weight (HSW) for the accessions ranged from 0.00g to 28.33g, with a mean of 18.28g. Accessions FMOR12-17 and FMOR12-29 had the heaviest seeds density with 28.33g and 23g, respectively while accessions FMOR12-18 and FMOR12-2 had seed 0.00g 100 seeds.

The number of seeds per pod (S/pod) varied from 2.77 seeds to 18.33 seeds, with a mean of 11.06 seeds. The best performing accessions are FMOR12-17 closely followed by FMOR12- 5 with 18.33 and 17.10 seeds respectively. The least performing accessions were FMOR12-27 and FMOR12-21 with 3.40 and 2.77 seeds, respectively.

4.1.2 Analysis of variance

The results of the analysis of variance for the 17 traits evaluated are presented in Table 4.2. The mean squares for three of the characters surviving ability of the plant, pod diameter and seed /pod were significant at ninety five percent level of probability ($P< 0.05$). Five traits days to first flowering, days to first fruiting, pod length, plant height at sixmonths after establishment and number of secondary branches were highly significant at ninety nine percent level of probability ($P< 0.01$). The remaining nine charactersdid not reveal statistical difference among the accessions for the characters.

4.1.3 Variance components and heritability

The variance components generated from the analysis of variance table for all the traits are presented in Table 4.3. The genotypic variance ranged from 0.05 for pod diameter to 4604.09 for days to first flowering. Two of the traits total seed and seed yield recorded negative variances values. The genotypic coefficient of variation (GCV) ranged from 1.6 for pod diameter to 41.82 for days to first flowering. Pod diameter had the lowest GCV. Phenotypic variance ranged from 2.76 for pod diameter to 417471.30 for total number of seedswhile phenotypic coefficient of variation (PCV) ranged from 11.42 for plant pod diameter to 158.43 for total number of seeds. Four of the traits: pod diameter, pod weight, total seed and seed yield had GCV values less than 10%. Four of the traits: pod length, number of seed/pod, stem diameter at 6MAE and flowering branches had values between 10 and 20% while nine traits: survival ability, regrowth potential, days to fruiting, days to flowering, total number of pods, 100 seed weight, plant height at 6MAE, primary branches and secondary branches had values greater than 20%.All the traits measured had PCV values greater than 20% except pod diameter which recorded a PCV value between 10 and 20%.The heritability estimates are presented in Table 4.3. It ranged from 2.16% for pod diameter to

81.68%for days to flowering. All the traits had values between 0 and 30% only days to flowering and days to fruiting recorded values greater than 60%.

Table 4.2: Mean squares from analysis of variance for Drumstick (*Moringa oleifera)*

Source	DF	Sur	Rej	DFF	DFFr	Tpods	PL	PG	PW	Tseed	S/yield	100S	S/pods	SD6M	H6M	Pbr	Sbr	flbr
REP	**2**	0.68	0.85	2128.6	7523.63	2242.01	443.94	142.72	36.03	382034	15989.6	284.305	73.99	21.93	1305.31	62.43	122.57	440.32
PROV	**30**	7.52*	3.38	14844.63**	14238.83**	3339.50	347.46**	52.14**	29.48	455010	22741.4	162.786	60.88*	81.39	7487.99**	24.15	112.27**	77.80
ERROR	**60**	4.17	2.66	32.13	47.71	2957.89	190.54	28.98	19.22	447961	21330.7	112.158	34.87	55.10	3374.45	20.55	54.64	67.98

Sur…..Survival
Rej……Rejuvenation
DFF…..Days to first flower
DFFr …..Days to first fruiting
TPods…. Total pods
PL….Pod Length
PD…. Pod Diameter
PW….Pod weight
TSeed…. Total seed
Syied…. Seed yield
100 S…..100seed weight
S/pod…. Seeds per pod
SD6m….Stem diameter at 6 months
H6M…… Plant height at 6 months
Pbr……. Primary branches
Sbr….. Secondary branches
Flbr…..Flowering branches.

Table 4.3. Estimates of variance components, heritability phenotypic and genotypic coefficients of variation

S/N	Character	Mean	σ_g^2	σ_e^2	σ_{ph}^2	$H_b^2\%$	GCV	PCV
1	Survival	3.59	1.03	4.15	5.18	19.84	28.23	63.37
2	Regrowth	2.33	0.24	2.66	2.90	8.24	20.95	72.99
3	Stem Diameter	18.05	9.15	50.23	59.37	15.40	16.75	42.68
4	Plant height	133.09	1211.61	3242.25	4453.85	27.20	26.15	50.14
5	Number of primary branches	7.01	3.19	17.28	20.47	15.60	25.49	64.52
6	Number of sec. branches	10.97	18.43	52.71	71.14	25.90	39.14	76.90
7	Flowering branches	10.44	3.79	63.66	67.45	5.62	18.66	78.67
8	Days to first flowering	162.23	4604.09	1032.34	5636.43	81.68	41.83	46.28
9	Days to first fruiting	242.04	3987.54	2276.18	6263.72	63.66	26.09	32.70
10	Total pod	44.60	127.20	2957.88	3085.09	4.12	25.29	124.53
11	Pod length	33.79	12.15	41.98	54.14	22.45	10.32	21.77
12	Pod diameter	14.55	0.06	2.70	2.76	2.16	1.68	11.42
13	Pod weight	9.50	0.57	7.16	7.73	7.33	7.93	29.27
14	Total seeds	407.84	-10163.3	427634.6	417471.3	0.00	0.00	158.42
15	Seed yield	92.41	-346.77	20469.27	20122.5	0.00	0.00	153.50
16	100seed weight	18.28	17.74	52.31	70.05	25.32	23.04	45.79
17	Seed/ pod	14.15	3.03	7.28	10.32	29.39	12.30	22.69

σ_g^2 = genotypic variance, σ_e^2 = .error variance, σ_{ph}^2 = phenotypic variance, GCV= genotypic coefficient of variation, PCV = phenotypic coefficient of variation

4.1.4 Estimates of the correlation coefficients

The phenotypic correlations of the traits are presented in Table 4.4. Positive and highly significant correlations ($P \leq 0.01$) were obtained betweensurvival ability in the 3 years and the following vegetative characters namely: regrowth potential after abscission, stem diameter at six months after establishment and plant height six months after establishment. Positive and highly significant correlations ($P \leq 0.01$) were also obtained between total number of seeds and seed yield, pod weight and pod length, pod girth and pod length, secondary branches and flowering branches with values ranging from 0.76 and 0.99.Positive and significant correlations were also obtained between survival ability and the yield attributes namely: total pod, pod length, pod diameter, total number of seeds, seed yield, 100 seed weight and seed per pod having the correlation coefficient range from0.37 and 0.74.Negative significant correlations ($P \leq 0.05$) were obtained between days to first flowering, days to first fruiting and the Yield attributes: total pods, pod length, pod weight ranging from -0.23 to -0.4, also negative significant correlations were obtained between days to first flowering, days to first fruiting and Vegetative attributes: plant height at 6MAE and stem girth at 66MAE ranging from -0.18 and -0.45.

4.1.5 Rank summation index

Rank summation index on eight selected traits: total number of pods, total number of seeds, the seed yield, 100seed weight, pod weight, number of seeds per pod, secondary branches and flowering branches is presented in Table 4.5, FRINMOR12-17, FRINMOR12-22 and FRINMOR12-6 ranked first, second and third respectively, with RSI of 17, 35 and 53 respectively while FRINMOR12- 20ranked last with RSI of 239.

Table 4.4. Phenotypic correlations among the characters

Var	Sur	Rej	S6M	H6M	Pbr	Sbr	Fbr	DFF	DFR	TP	PL	PD	PW	TS	SY	HSW	S/P
Sur	**1.00**																
Rej	**0.88****	**1.00**															
SD6M	**0.55****	**0.52****	**1.00**														
H6M	**0.56****	**0.54****	**0.76****	**1.00**													
Pbr	0.13	0.31	**0.46***	0.24	**1.00**												
Sbr	0.05	0.05	**0.42***	0.33	**0.41***	**1.00**											
Flbr	0.13	0.17	**0.50***	**0.36***	**0.65****	**0.76****	**1.00**										
DFF	-0.23	-0.24	**-0.42**	**-0.42**	-0.20	-0.21	-0.34	**1.00**									
DFFR	-0.18	-0.25	-0.35	**-0.45***	-0.32	-0.20	-0.34	**0.88****	**1.00**								
Tpod	**0.58****	**0.53****	**0.67****	**0.74****	**0.42***	**0.51****	**0.56****	**-0.43**	**-0.40**	**1.00**							
PodL	**0.60****	**0.37***	**0.52****	**0.38***	0.21	**0.39***	**0.44***	-0.33	-0.27	**0.42***	**1.00**						
PodG	**0.70****	**0.47***	**0.60****	**0.55****	0.26	0.32	**0.38***	**-0.38**	-0.31	**0.52****	**0.88****	**1.00**					
PodW	**0.59****	**0.37***	**0.57****	**0.40***	0.34	**0.51****	**0.54****	-0.35	-0.28	**0.54****	**0.94****	**0.90****	**1.00**				
Tseeds	**0.41***	**0.36***	**0.55****	**0.65****	0.10	**0.48***	**0.37***	-0.29	-0.24	**0.72****	**0.38***	**0.37***	**0.41***	**1.00**			
S yield	**0.43***	**0.37***	**0.54****	**0.65****	0.12	**0.49***	**0.39***	-0.29	-0.23	**0.75****	**0.41***	**0.41***	**0.46***	**0.99****	**1.00**		
100s wgt	**0.52****	**0.47***	**0.54****	**0.56****	**0.44***	**0.38***	**0.56****	-0.22	-0.23	**0.66****	**0.66****	**0.58****	**0.65****	**0.50****	**0.54****	**1.00**	
seeds/p	**0.62****	**0.39***	**0.54****	**0.37***	0.27	**0.39***	**0.46***	-0.33	-0.26	**0.45***	**0.92****	**0.85****	**0.91****	**0.48***	**0.51****	**0.66****	**1.00**

Values in bold are significantly different from 0 with a significance level alpha=0.05

Surv=surviving ability, Reg=regrowth potential, SD6M= stem diameter, H6M= plant height, Pbr= number of primary branches, Sbr= number of secondary branches, flbr=number of flowering branches, DFF= days to first flowering ,DFFR= days to first fruiting, Tpod=number of pods per plot, PLt=pod length, PD= pod diameter, TSeed= number of seed per plot, Syield= seed yield per plot, HSW= 100 seed weight, seeds/p=number of seeds per pod.

Table 4.5: Rank Summation Indices on eight morphological traits

S/N	Accessions	Tpod	Tseeds	Syield	HSW	podwgt	seed/pod	Sec br	Flbr	Rank index
1	FRIN MOR12-17	4	2	2	1	1	1	2	4	17
2	FRIN MOR12-22	1	1	1	10	10	4	3	5	35
3	FRIN MOR12-6	3	5	5	13	3	8	7	9	53
4	FRIN MOR12-3	7	4	4	9	7	3	17	11	62
5	FRIN MOR12-24	9	6	6	6	15	15	12	7	76
6	FRIN MOR12-29	12	16	14	2	12	12	4	6	78
7	FRIN MOR12-5	23	11	8	3	2	2	18	19	86
8	FRIN MOR 12-23	2	7	7	4	11	17	23	15	86
9	FRIN MOR12-11	19	17	20	21	6	11	1	3	98
10	FRIN MOR12-14	21	13	16	5	9	6	10	18	98
11	FRIN MOR12-13	16	12	12	8	13	7	21	10	99
12	FRIN MOR12-16	20	19	19	18	5	10	8	1	100
13	FRIN MOR12-25	5	20	18	11	8	18	16	8	104
14	FRIN MOR12-31	10	3	3	15	24	23	11	17	106
15	FRIN MOR 12-26	8	9	11	7	16	13	25	20	109
16	FRIN MOR12-28	6	10	9	23	21	22	5	14	110
17	FRIN MOR12-4	15	8	10	16	14	5	26	22	116
18	FRIN MOR12-9	13	22	21	12	22	20	6	2	118
19	FRIN MOR12-12	17	15	17	14	17	16	13	12	121
20	FRIN MOR12-15	11	18	13	17	4	14	19	25	121
21	FRIN MOR12-7	14	14	15	25	25	26	9	13	141
22	FRIN MOR12-30	24	27	28	27	20	24	14	16	180
23	FRIN MOR12-8	25	28	25	19	27	28	15	21	188
24	FRIN MOR12-1	18	26	24	20	26	25	27	23	189
25	FRIN MOR12-18	28	23	23	30	19	19	20	30	192
26	FRIN MOR12-2	27	24	26	31	18	9	30	29	194
27	FRIN MOR12-27	22	21	22	24	30	30	28	24	201
28	FRIN MOR12-19	26	30	29	22	23	21	24	28	203
29	FRIN MOR12-10	29	25	27	28	29	27	31	26	222
30	FRIN MOR12-21	30	29	30	26	31	31	22	27	226
31	FRIN MOR12-20	31	31	31	29	28	29	29	31	239

Tpod –total pod,Tseeds-total number of seeds,Syield- seed yield, HSW- 100 seed weight, podwgt- pod weight, secbr-secondary branches, flbr- flowering branches

4.1.6 Principal component analysis

4.1.6.1 The eigenvalues

Eigenvalues from principal component analysis are presented in Table 4.6. The analysis computed 17 eigenvalues which is equivalent to the number of characters being considered.- However, five of the eigenvalues were selected for further analysis based on the criterion of retaining and interpreting only the components having eigenvalue that is greater than 1. The first Principal Component PC1 was responsible for a total of 50% variation out of the 100% expressed variability by all the components, which also accounted for the maximum amount of variation correlating with some of the characters analysed. PC2 was responsible for 11.3% of the variation expressed. The PC2accounted for the maximum amount of variance not expressed by PC1. PC2 only correlated with variables that were not displaying strong correlations with PC1. PC3 had a cumulative expression of 71.6% of the variation and PC 5 on which the truncating eigenvalue lied had a cumulative variability of 87.5% being expressed by the first five components.

4.1.6.2 Eigenvectors

The eigenvectors for the principal component analysis arepresented in Table 4.7.Eigenvectors with values greater than 0.20 contributed and had strong influence on each of the components.All the traits loaded in PC1 are having positive contribution to the component: pod weight, total pods, pod girth, number of seed/pod, stem diameter,pod length,100seed weight, plant height, seed yield, total seed, survival ability, regrowth potential and flowering branches respectively. Positive contributors to PC2 are survival ability, days to first fruiting, pod length, pod girth,number of seed/ pod, days to first flowering, regrowth potential and pod

weight respectively. Traits with negative effect areflowering branches, secondary branches and primary branches.Traits thatpositively contributed to PC3 are, plant height, regrowth potential,total number of seed, seed yield,survival ability, while those with negative effects areflowering branches,pod weight, secondary branches, pod length, primary branches and seed/pod.Traits loaded on PC4 are withpositive effect on the component they are,days to first fruiting,days to first flowering, seed yield, total number of seed andsecondary branches. The positive contributors to PC5 are primary branches, regrowth potential, days to first fruiting and survival ability while traits loaded with negative effects on PC5 are total seed and seed yield.

4.1.6.3 Factor loading of characters

The factor loadings of the different characters measured are presented in Table 4.8. Fifteen out of the seventeen characters are loaded on PC1. Howeverdays to first flowering and days to first fruiting were not loaded on PC1.Two characters survival ability and days to first fruiting are loaded on PC2. Regrowth potential and plant height are loaded on PC3. Four characters: days to first flowering, days to first fruiting, seed yield and total seed are loaded on PC4 while primary branches and regrowth potential are loaded on PC5.

Table 4.6: Eigenvalues of the Correlation matrix

S/N	Prin Comp	Eigenvalue	Proportion (%)	Cumulative(%)
1	**PC1**	**8.53**	**50.15**	**50.15**
2	**PC2**	**1.92**	**11.28**	**61.42**
3	**PC3**	**1.74**	**10.22**	**71.64**
4	**PC4**	**1.53**	**9.01**	**80.65**
5	**PC5**	**1.16**	**6.83**	**87.48**
6	PC6	0.51	3.03	90.51
7	PC7	0.45	2.67	93.17
8	PC8	0.34	1.98	95.16
9	PC9	0.25	1.44	96.60
10	PC10	0.21	1.22	97.81
11	PC11	0.15	0.88	98.69
12	PC12	0.08	0.49	99.18
13	PC13	0.06	0.35	99.53
14	PC14	0.04	0.211	99.74
15	PC15	0.02	0.144	99.89
16	PC16	0.02	0.097	99.98
17	PC17	0.00	0.017	100.00

Table 4.7: Eigenvectors of Principal Components for Characters

S/N	Traits	PC1	PC2	PC3	PC4	PC5
1	Survival	**0.24**	**0.38**	**0.24**	-0.07	**0.20**
2	Regrowth	**0.21**	**0.22**	**0.32**	-0.13	**0.43**
3	Stem diameter	**0.27**	-0.07	0.10	-0.03	0.16
4	Plant height	**0.26**	-0.10	**0.34**	-0.02	0.04
5	Pry branches	0.16	**-0.28**	**-0.26**	-0.10	**0.57**
6	Sec branches	0.19	**-0.34**	**-0.28**	**0.28**	-0.07
7	flowrg branches	**0.22**	**-0.34**	**-0.33**	0.09	0.15
8	DFF	-0.18	**0.24**	-0.08	**0.54**	**0.30**
9	DFFR	-0.16	**0.29**	-0.07	**0.57**	0.18
10	Total pod	**0.28**	-0.17	0.20	0.10	0.10
11	PodLt	**0.27**	**0.29**	**-0.28**	-0.04	-0.19
12	PodGt	**0.28**	**0.28**	-0.15	-0.11	-0.09
13	Podwt	**0.29**	**0.20**	**-0.30**	-0.01	-0.13
14	Totalseed	**0.24**	-0.14	**0.30**	**0.34**	**-0.28**
15	seed yield	**0.25**	-0.13	**0.27**	**0.34**	**-0.26**
16	HSW	**0.27**	0.05	-0.08	0.126	0.17
17	seeds/pod	**0.28**	**0.26**	**-0.24**	0.01	-0.18
	Eigenvalue	**8.5**	**1.9**	**1.7**	**1.5**	**1.2**
	Variability (%)	**50.1**	**11.3**	**10.2**	**9.0**	**6.8**
	Cumulative (%)	**50.1**	**61.4**	**71.6**	**80.7**	**87.5**

PC = Principal Component.

Only eigenvectors with values≥0.20 which largely influenced each PC axes are presented.

Surv=surviving ability, Reg=regrowth potential, SD6M= stem diameter, H6M= plant height, Pbr= number of primary branches, Sbr= number of secondary branches, flbr=number of flowering branches, DFF= days to first flowering ,DFFR= days to first fruiting, Tpod=number of pods per plot, PLt=pod length, PD= pod diameter, TSeed= number of seed per plot, Syield= seed yield per plot, HSW= 100 seed weight, seeds/p=number of seeds per pod

Table 4.8. Factor loadings of the characters on the Components

S/N	Traits	PC1	PC2	PC3	PC4	PC5
1	Survival	**0.71**	**0.53**	0.31	-0.09	0.22
2	Regrowth	**0.61**	0.30	**0.42**	-0.16	**0.46**
3	Stem diameter	**0.80**	-0.10	0.13	-0.03	0.17
4	Plant height	**0.75**	-0.14	**0.45**	-0.02	0.05
5	Pry branches	**0.46**	-0.39	-0.34	-0.12	**0.62**
6	Sec branches	**0.57**	-0.47	-0.37	0.35	-0.08
7	flowrg branches	**0.65**	-0.48	-0.44	0.11	0.17
8	DFF	-0.52	0.33	-0.10	**0.67**	0.321
9	DFFR	-0.48	**0.40**	-0.10	**0.70**	0.20
10	Total pod	**0.83**	-0.24	0.26	0.12	0.10
11	PodLt	**0.80**	0.40	-0.37	-0.05	-0.20
12	PodGt	**0.82**	0.39	-0.20	-0.14	-0.10
13	Podwt	**0.84**	0.28	-0.40	-0.01	-0.14
14	Totalseed	**0.71**	-0.19	0.39	**0.41**	-0.29
15	seed yield	**0.73**	-0.18	0.36	**0.42**	-0.28
16	HSW	**0.78**	0.07	-0.10	0.16	0.18
17	seeds/pod	**0.81**	0.34	-0.32	0.01	-0.19

PC = Principal Component.
Only factor loadings with values≥0.40 are the loads on the components
Surv=surviving ability, Reg=regrowth potential, SD6M= stem diameter, H6M= plant height, Pbr= number of primary branches, Sbr= number of secondary branches, flbr=number of flowering branches, DFF= days to first flowering ,DFFR= days to first fruiting, Tpod=number of pods per plot, PLt=pod length, PD= pod diameter, TSeed= number of seed per plot, Syield= seed yield per plot, HSW= 100 seed weight, seeds/p=number of seeds per pod.

4.1.6.4 Contribution of accessions to the components

The contributions of each accession to the components are presented in Table 4.9. The best five performing accessions are considered as having a strong impact on the characters of each of the components. The PC1 had accessions FRIN MOR12-17, FRIN MOR12-10, FRIN MOR12-21, FRIN MOR12-22 and FRIN MOR12-20 with the values 7.16%, 9.70%, 12.04%, 14.55% and 14.61% respectively. On the other hand, PC2 had accessions FRIN MOR12-14, FRIN MOR12-11, FRIN MOR12-22, FRIN MOR12-5 and FRIN MOR12-2 with values 6.13%, 7.14%, 8.03%, 9.61% and 18.16% respectively. PC3 had accessions FRIN MOR12-17, FRIN MOR12-16, FRIN MOR12-31, FRIN MOR12-22 and FRIN MOR12-11 with values 6.69%, 12.23%, 13.83%, 17.19% and 20.29% respectively. PC4 has high contributions from accessions FRIN MOR12-1, FRIN MOR12- 2, FRIN MOR12- 21, FRIN MOR12-22 and FRIN MOR12-20 with values 5.95%, 6.98%, 13.94%, 17.62% and 26.20% respectively. PC5 has high contributions from accessions FRIN MOR12-25, FRIN MOR12-31, FRIN MOR12-19, FRIN MOR12-22 and FRIN MOR12-9 with values 4.78%, 8.19%, 9.06%, 12.95% and 34.24% respectively were having highest contributions. The summary of the characters and accessions load on the components is shown in Table 4.10.

Table 4.9: Contribution of the *Moringa oleifera* accessions to the Components (%)

S/N	Accessions	PC1	PC2	PC3	PC4	PC5
1	FRIN MOR12-1	2.53	4.44	0.03	**5.95**	1.05
2	FRIN MOR12-2	0.55	**18.16**	2.90	**6.98**	0.22
3	FRIN MOR12-3	2.07	2.45	0.06	1.08	0.59
4	FRIN MOR12-4	0.27	3.40	0.13	0.11	0.70
5	FRIN MOR12-5	0.90	**9.61**	2.27	0.36	0.98
6	FRIN MOR12-6	2.64	0.41	0.05	0.98	0.57
7	FRIN MOR12-7	1.42	6.72	0.13	0.14	3.83
8	FRIN MOR12-8	6.92	1.94	0.03	0.71	0.00
9	FRIN MOR12-9	0.37	4.02	1.52	0.18	**34.24**
10	FRIN MOR12-10	**9.70**	2.23	0.23	0.73	3.11
11	FRIN MOR12-11	0.07	**7.14**	**20.29**	2.66	0.00
12	FRIN MOR12-12	0.04	0.96	1.38	0.26	0.04
13	FRIN MOR12-13	0.25	0.20	1.97	0.34	0.01
14	FRIN MOR12-14	0.42	**6.13**	2.32	0.13	0.26
15	FRIN MOR12-15	0.32	4.94	0.01	0.87	0.24
16	FRIN MOR12-16	0.33	0.33	**12.23**	0.02	0.44
17	FRIN MOR12-17	**7.16**	0.20	**6.69**	5.65	0.22
18	FRIN MOR12-18	1.34	2.01	0.17	1.15	1.75
19	FRIN MOR12-19	1.90	0.75	0.01	2.70	**9.06**
20	FRIN MOR12-20	**14.61**	3.50	0.20	**26.20**	4.02
21	FRIN MOR12-21	**12.04**	1.65	1.79	**13.94**	2.26
22	FRIN MOR12-22	**14.55**	**8.03**	**17.19**	**17.62**	**12.95**
23	FRIN MOR 12-23	3.83	0.62	5.62	1.45	3.13
24	FRIN MOR12-24	2.01	0.00	1.28	0.08	0.22
25	FRIN MOR12-25	1.76	0.03	0.14	0.55	**4.75**
26	FRIN MOR 12-26	1.74	0.26	0.98	2.58	4.10
27	FRIN MOR12-27	5.28	3.00	3.55	0.78	0.09
28	FRIN MOR12-28	0.00	2.51	1.19	0.53	0.65
29	FRIN MOR12-29	1.63	2.02	1.51	0.77	0.02
30	FRIN MOR12- 30	1.51	1.41	0.32	4.17	2.30
31	FRIN MOR12-31	1.86	0.97	**13.83**	0.34	**8.19**

Table 4.10: Component loads of Characters and Accessions

Components	Traits	Accessions
PC1	Pod/ seed yield attributes	FRINMOR12- 22, FRINMOR12-21, FRINMOR12-20, FRINMOR12-10, FRINMOR12-17.
PC2	Survival and adaptable	FRIN MOR12-2, FRINMOR12-5, FRINMOR12-22, FRINMOR12-11 and FRINMOR12-14.
PC3	Vegetative growth for leaves	FRIN MOR12-11, FRINMOR12-22, FRINMOR12-31, FRINMOR12-16 and FRINMOR12-17.
PC4	Early flowering	FRIN MOR12-20, FRINMOR12-22, FRINMOR12-21, FRINMOR12-2 and FRINMOR12-1.
PC5	Bushy vegetative pattern	FRIN MOR12-9.FRINMOR12-22, FRINMOR12-19, FRINMOR12-31 and FRINMOR25.

4.1.6.5 The scatter plot

The principal component analysis gave a scatter plot of PC 1 versus PC 2as shown in Figure 4.1 and PC2 versus PC 3 as shown in Figure 4.2. The Plot revealed the relationship existing among the accessions which were scattered and found in the four quadrants. Interestingly it was observed from Figure 4.1 which was responsible for 61.42% of the variation that the accessions grouped in the upper region of the dendrogram were found located at the left part of the quadrant either upper part or the lower part. While the accessions located mid-way down the dendrogram were found located at the right side of the quadrant either upper or lower quadrant. From Figure 4.2 some of the accessions relatedness not clearly expressed in Figure 4.1 were seen in same quadrant. Most accessions in the lower part of the dendrogram were found in the upper part of the quadrant either at the positive or negative side of the axis.The plot of PC1 versus PC2 showed FRIN MOR12-22 located at the extreme lower positive side of the axis farthest to FRIN MOR12-2. While FRIN MOR12-5 located at upper positive side of the axis was farthest to FRIN MOR12-7 located at lower negative side of the axis. The plot of PC2 against PC3 showed FRIN MOR12-22 and FRIN MOR12-31 located at the positive left side of the axis farthest to FRIN MOR12-3 located at the negative right side of the axis. While FRIN MOR12-2 and FRIN MOR12-23 located at the positive right side of the axis are farthest to FRIN MOR12-11 located at the lower negative side of the axis.

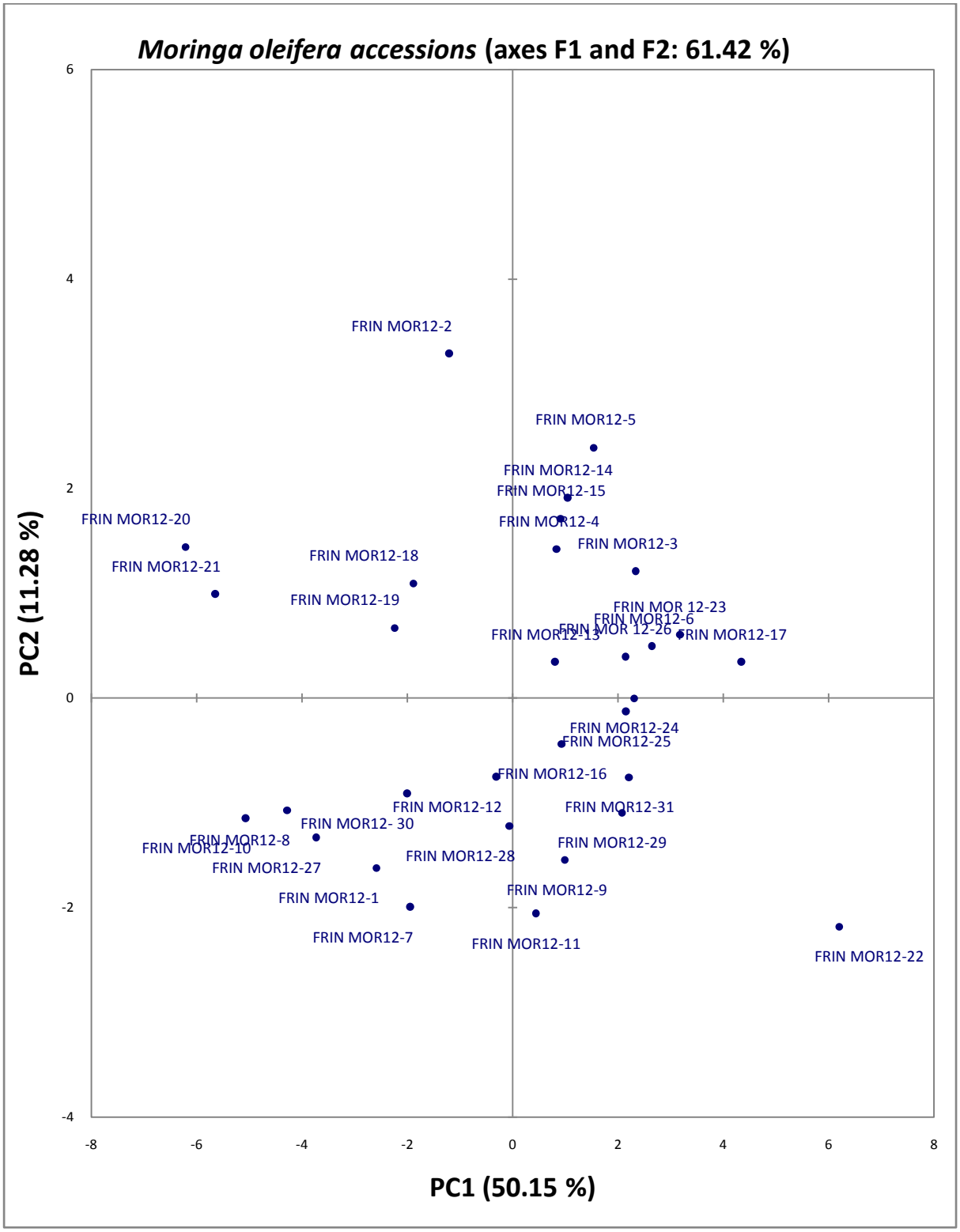


Figure 4.1 The two dimensional scattered plot of PC1 and PC2

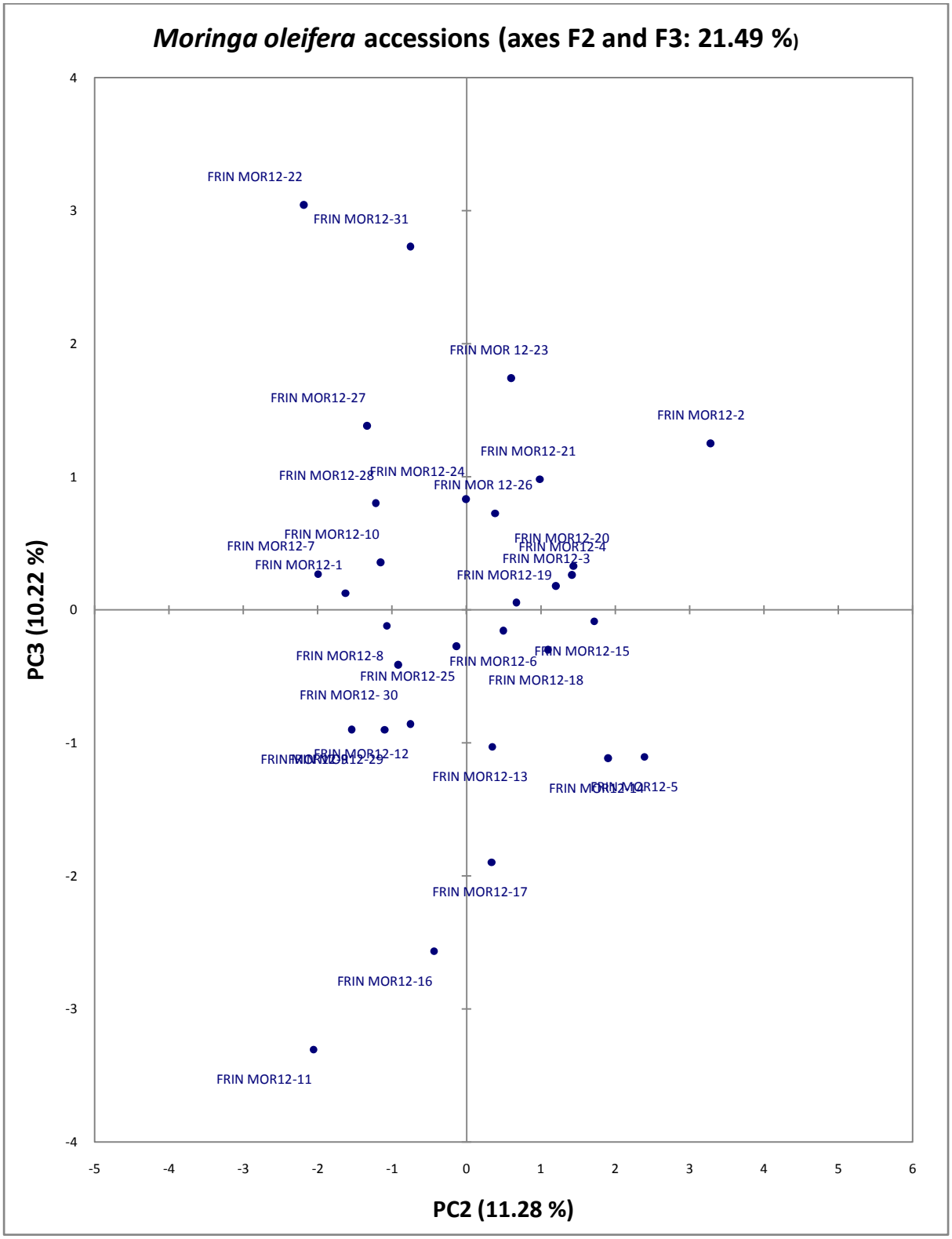


Figure 4.2 The two dimensional scattered plot of PC2 and PC3

4.1.6.6 Cluster analysis based on morphological characters

The dendrogram generated from the cluster analysis based on the seventeen morphological characters ispresented in Figure 4.3. The cluster analysisgrouped the 31 accessions into three main clusters (I, 2 and 3) when the constructed dendrogram was truncated at 85% similarity coefficient level.Cluster 1 was made up of two accessions FRIN MOR12-19 and FRIN MOR12-20 at 85% similarity coefficient.These two accessions in cluster 1 were found to be the least performing with regards to most of the measured characters especially the yield related characters. Cluster 2 was made up of twenty one accessions at 93% coefficient of similarity having only FRIN MOR12-25 linked at different level of coefficients to the remaining twenty accessions at 98% similarity coefficient;twenty of the accessions are sub clustered into 2a and 2b. Cluster 2a was made up of twelveaccessions FRIN MOR12-23,FRIN MOR12-28, FRIN MOR12-5, FRIN MOR12-4, FRIN MOR12-13, FRIN MOR12-22, FRIN MOR12-17, FRIN MOR12-31, FRIN MOR12-3, FRIN MOR12-4, FRIN MOR12-6 and FRIN MOR12-26.Cluster 2b was made up of eightaccessions FRIN MOR12-27, FRIN MOR12-15, FRIN MOR 12-29, FRIN MOR12-16, FRIN MOR12-11, FRIN MOR12-14, FRIN MOR12-7, FRIN MOR12-12.These twenty one accessions were among the best performing for either one or more of the evaluated characters. Cluster 3 was made up of two sub clusters 3a and 3b. 3a was made up of two accessions FRIN MOR12-1 and FRIN MOR12-9 clustered at 98% similarity coefficient. While Cluster 3b had six accessions FRIN MOR12-10, FRIN MOR12-8, FRIN MOR12-21, FRIN MOR12-30, FRIN MOR12-2 and FRIN MOR 12-18 clustered at 92% similarity coefficients.The eight accessions in cluster 3 were falling within the average ranked for performance as relating to almost all the characters.

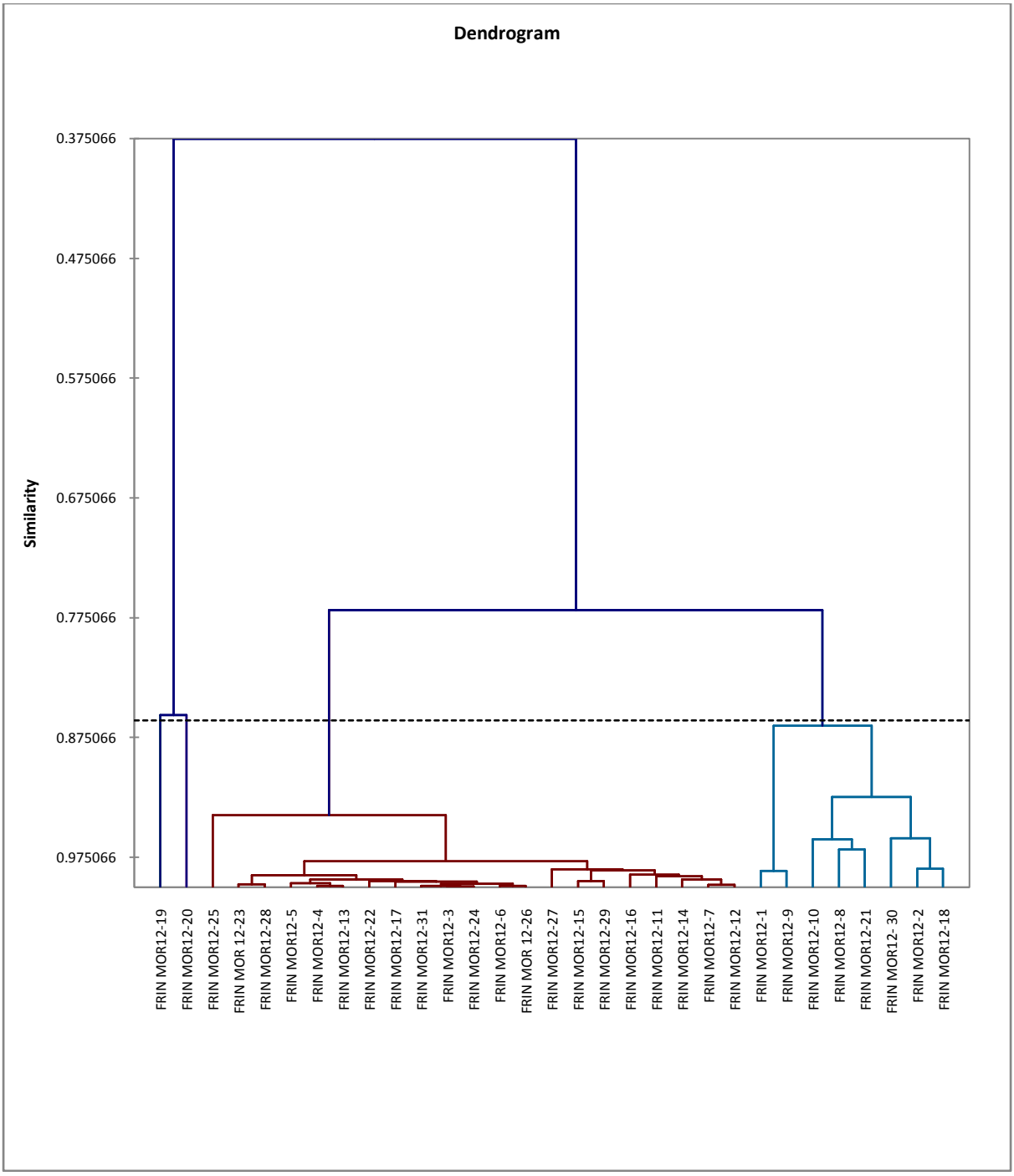


Fig 4.3: Dendrogram from PCA showing the clusters formed using the morphological characters

4.1.6.7 Euclidean similarity/dissimilarity index

The Euclidean distance index between the accessions is presented in Table 4.11. The Euclidean genetic similarity generated using the morphological character showed that only 5.4% (26 out of 480 pairs) of the accessions were having 0.0 genetic distances in between them. Only 11% (57 out of 480 pairs) of the accessions were closely related with distances between 0.1 and 0.3. While 20% (96 out of 480 pairs) had distances between 0.7 and 1.0 and 62.7% (301 out of 480 pairs) of the accessions had large dissimilarity distances with genetic distances greater than 1.00. Cumulatively, 16.4%of the 480 pairs were closely related or share some similarities approximately 82.7% of the 480 pairs are dissimilar to each other.

Table 4.11: EUCLIDEAN Similarity or Distance Index between the 31 accessions of *Moringa oleifera*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1	0.0																														
2	4.7	0.0																													
3	3.0	1.7	0.0																												
4	2.3	2.3	0.7	0.0																											
5	2.3	2.3	0.7	0.0	0.0																										
6	3.0	1.7	0.0	0.7	0.7	0.0																									
7	0.3	5.0	3.3	2.7	2.7	3.3	0.0																								
8	0.7	5.3	3.7	3.0	3.0	3.7	0.3	0.0																							
9	2.0	2.7	1.0	0.3	0.3	1.0	2.3	2.7	0.0																						
10	1.7	6.3	4.7	4.0	4.0	4.7	1.3	1.0	3.7	0.0																					
11	0.7	5.3	3.7	3.0	3.0	3.7	0.3	0.0	2.7	1.0	0.0																				
12	1.0	3.7	2.0	1.3	1.3	2.0	1.3	1.7	1.0	2.7	1.7	0.0																			
13	1.3	3.3	1.7	1.0	1.0	1.7	1.7	2.0	0.7	3.0	2.0	0.3	0.0																		
14	2.7	2.0	0.3	0.3	0.3	0.3	3.0	3.3	0.7	4.3	3.3	1.7	1.3	0.0																	
15	3.0	1.7	0.0	0.7	0.7	0.0	3.3	3.7	1.0	4.7	3.7	2.0	1.7	0.3	0.0																
16	1.0	3.7	2.0	1.3	1.3	2.0	1.3	1.7	1.0	2.7	1.7	0.0	0.3	1.7	2.0	0.0															
17	2.7	2.0	0.3	0.3	0.3	0.3	3.0	3.3	0.7	4.3	3.3	1.7	1.3	0.0	0.3	1.7	0.0														
18	0.7	4.0	2.3	1.7	1.7	2.3	1.0	1.3	1.3	2.3	1.3	0.3	0.7	2.0	2.3	0.3	2.0	0.0													
19	0.7	4.0	2.3	1.7	1.7	2.3	1.0	1.3	1.3	2.3	1.3	0.3	0.7	2.0	2.3	0.3	2.0	0.0	0.0												
20	0.3	5.0	3.3	2.7	2.7	3.3	0.0	0.3	2.3	1.3	0.3	1.3	1.7	3.0	3.3	1.3	3.0	1.0	1.0	0.0											
21	1.0	3.7	2.0	1.3	1.3	2.0	1.3	1.7	1.0	2.7	1.7	0.0	0.3	1.7	2.0	0.0	1.7	0.3	0.3	1.3	0.0										
22	2.7	2.0	0.3	0.3	0.3	0.3	3.0	3.3	0.7	4.3	3.3	1.7	1.3	0.0	0.3	1.7	0.0	2.0	2.0	3.0	1.7	0.0									
23	4.3	0.3	1.3	2.0	2.0	1.3	4.7	5.0	2.3	6.0	5.0	3.3	3.0	1.7	1.3	3.3	1.7	3.7	3.7	4.7	3.3	1.7	0.0								
24	2.3	2.3	0.7	0.0	0.0	0.7	2.7	3.0	0.3	4.0	3.0	1.3	1.0	0.3	0.7	1.3	0.3	1.7	1.7	2.7	1.3	0.3	2.0	0.0							
25	2.3	2.3	0.7	0.0	0.0	0.7	2.7	3.0	0.3	4.0	3.0	1.3	1.0	0.3	0.7	1.3	0.3	1.7	1.7	2.7	1.3	0.3	2.0	0.0	0.0						
26	3.0	1.7	0.0	0.7	0.7	0.0	3.3	3.7	1.0	4.7	3.7	2.0	1.7	0.3	0.0	2.0	0.3	2.3	2.3	3.3	2.0	0.3	1.3	0.7	0.7	0.0					
27	0.3	5.0	3.3	2.7	2.7	3.3	0.0	0.3	2.3	1.3	0.3	1.3	1.7	3.0	3.3	1.3	3.0	1.0	1.0	0.0	1.3	3.0	4.7	2.7	2.7	3.3	0.0				
28	2.0	2.7	1.0	0.3	0.3	1.0	2.3	2.7	0.0	3.7	2.7	1.0	0.7	0.7	1.0	1.0	0.7	1.3	1.3	2.3	1.0	0.7	2.3	0.3	0.3	1.0	2.3	0.0			
29	1.0	3.7	2.0	1.3	1.3	2.0	1.3	1.7	1.0	2.7	1.7	0.0	0.3	1.7	2.0	0.0	1.7	0.3	0.3	1.3	0.0	1.7	3.3	1.3	1.3	2.0	1.3	1.0	0.0		
30	0.3	5.0	3.3	2.7	2.7	3.3	0.0	0.3	2.3	1.3	0.3	1.3	1.7	3.0	3.3	1.3	3.0	1.0	1.0	0.0	1.3	3.0	4.7	2.7	2.7	3.3	0.0	2.3	1.3	0.0	
31	3.3	1.3	0.3	1.0	1.0	0.3	3.7	4.0	1.3	5.0	4.0	2.3	2.0	0.7	0.3	2.3	0.7	2.7	2.7	3.7	2.3	0.7	1.0	1.0	1.0	0.3	3.7	1.3	2.3	3.7	0.0

4.2 Molecular Characterization Results

4.2.1 Primers characteristics

Ten RAPD primers and ten Microsatellite SSR markers were used for the study, however none of the RAPD primers amplified at the electrophoresis stage. From the 10 Microsatellites SSR markers that were used five produced amplified bands which were scored and used in the assessment of the genetic diversity. Four out of the markers MO1, MO10, MO15 and MO41 as shown in Figures (4.4a,4.4c, 4.4d and 4.4e) respectively were polymorphic in nature while the marker MO6 Fig 4.4b produced only a monomorphic band.Polymorphism Information Content (PIC) value was calculated for four polymorphic primers out of the five primers used in the analysis as given in the Table 4.12. Polymorphic information content valuewhich estimates the quantity of information that can be obtained from a particular primer was highest for the primer MO41 with 0.75 followed by primer MO1 with 0.68 while, the lowest PIC value was recorded from the primer MO15 with 0.11.Polymorphic information content reveals the quantity of information that can be obtained from a particular primer. The polymorphic information content (PIC) ranged from 0.1134 for MO15 to 0.7519 for MO41 with an average of 0.4813.The gene diversity ranged from 0.1207 for MO15 to 0.7825 for MO41 with average value of 0.5224. The major allele frequency calculated ranged from 0.3226 for MO41 to 0.9355 for MO15 with average of 0.5726. The four SSR markers produced 19 alleles and the number of alleles ranged from 2 to 9 with an average of 4.75 alleles per locus in the 31 accessions. The maximum number of amplified products was generated by primer MO41 with nine alleles followed by MO1 with 5 alleles. Temperature of amplification for the SSR markers ranged from 55^0C for MO 41 to 61^0 C for MO6. These were used in generating amplification profiles for the 31individual accessions of Drumstick.

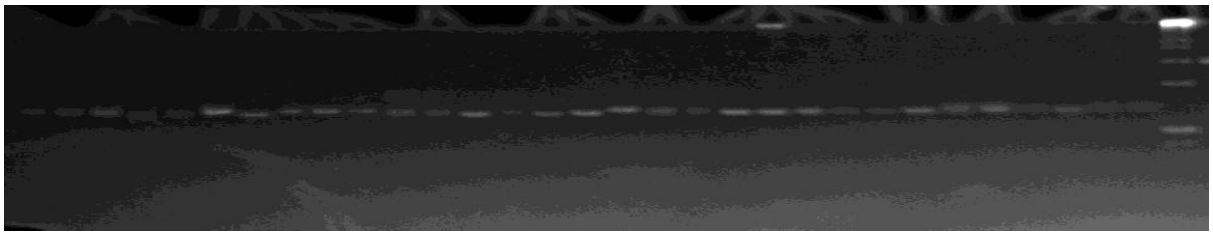


Fig 4.4a RIMER 1(MO1) F TTGTCTGCCTCCTTTTGTCA
R AACTGTCACCCTCCTATCCA

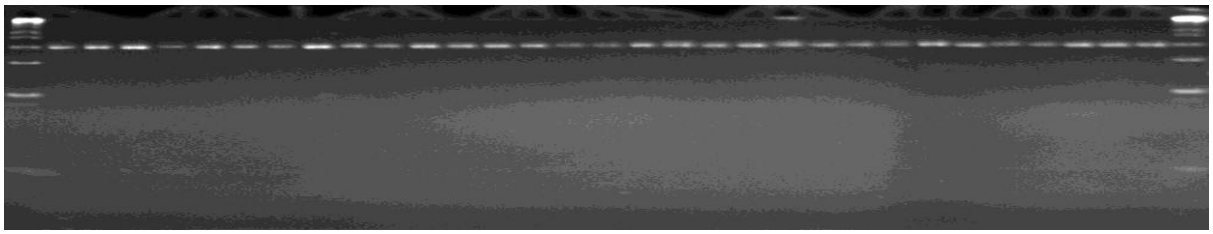


Fig 4.4b PRIMER2 (MO6) F GCATAGCCACCTTTACTCCT
R GACTTTTGAACTCCACCACC

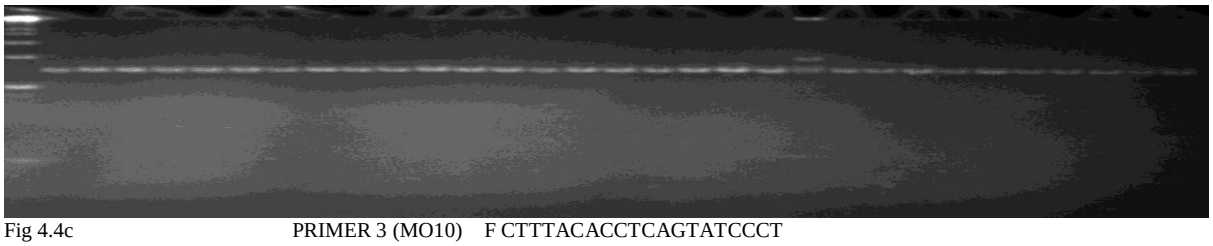


Fig 4.4c PRIMER 3 (MO10) F CTTTACACCTCAGTATCCCT
R GTTCGGCTTATGTTCTCGTT

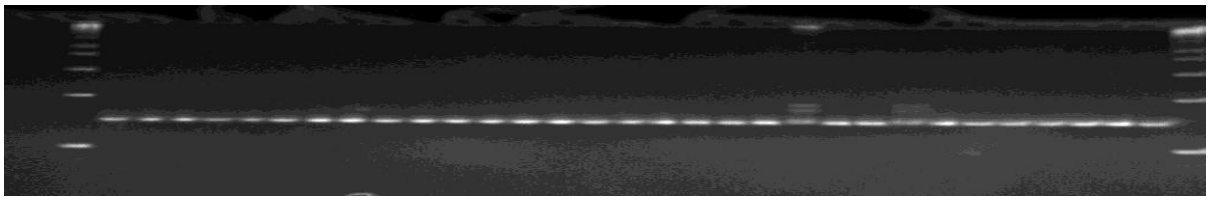


Fig 4.4d PRIMER4 (MO15) F CCCCTCTATTTCCATTTTCC
R GCTCCATAAACCCTCTTGCT

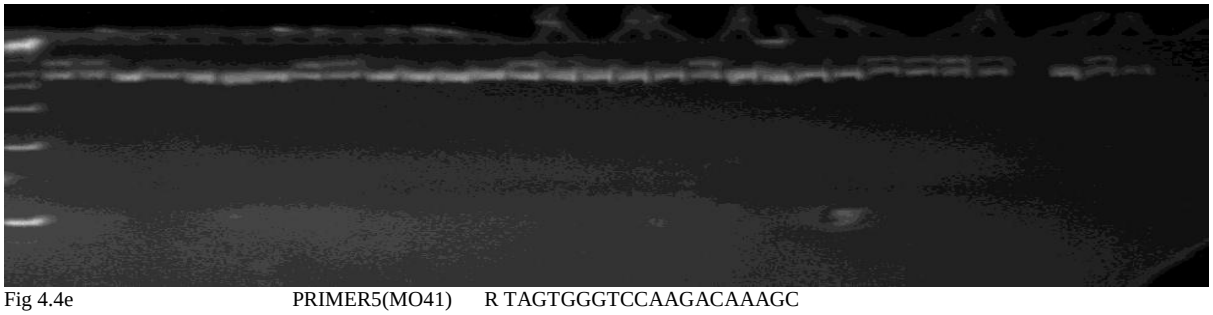


Fig 4.4e PRIMER5(MO41) R TAGTGGGTCCAAGACAAAGC
F TGGGATTAGGGCATTAGAAA

Table 4.12: Status of SSR markers used with respect to allele frequency, allele number, gene diversity and polymorphic information content (PIC)

Marker		Allele Frquency	SampleSize	No. of obs.	AlleleNo	Availability	GeneDiversity	PIC
MO1	F TTGTCTGCCTCCTTTTGTCA	0.3548	31	31	5.0000	1.0000	0.7305	0.6827
	R AACTGTCACCCTCCTATCCA							
MO6	F GCATAGCCACCTTTACTCCT	-	31	31	-	1.0000	-	-
	R GACTTTTGAACTCCACCACC							
MO10	F CTTTACACCTCAGTATCCCT	0.6774	31	31	3.0000	1.0000	0.4558	0.3773
	R GTTCGGCTTATGTTCTCGTT							
MO15	F CCCCTCTATTTCCATTTTCC	0.9355	31	31	2.0000	1.0000	0.1207	0.1134
	R GCTCCATAAACCCTCTTGCT							
MO41	R TAGTGGGTCCAAGACAAAGC	0.3226	31	31	9.0000	1.0000	0.7825	0.7519
	F TGGGATTAGGGCATTAGAAA							
Mean		**0.5726**	**31**	**31**	**4.7500**	**1.0000**	**0.5224**	**0.4813**

4.2.2 Cluster analysis from the SSR markers

The cluster analysis from the molecular characterization using five microsatellite markers generated a dendrogram which ispresented in Fig 4.5 below. Two main clusters (1 and 2) were generated from the cluster analysis separating at 0.04 and 0.13 coefficients. There are two distinct accessions FRIN MOR12-27 and FRIN MOR12-31 at coefficients 0.04. The remaining twenty nine accessions are clustered at 0.13 coefficients. At 0.13 coefficients, there are twodistinct sub clusters 2a and 2b. At 0.08 coefficients (Cluster 2a),six accessions clustered together and the remaining twenty three accessions were clustered at 0.06 coefficients (Cluster 2b).

At 0.57 similarity index cluster 2 hadtwo separate distinct clusters that grouped the remaining twenty nine accessions. At 0.43 the remaining twenty nine accessions were grouped into two sub clusters having 0.08 and 0.06 coefficients. At 0.08coefficients six accessionswere grouped with their genetic difference having FRIN MOR12-2 at 0.35, FRIN MOR12-10 at 0.31. At 0.17 genetic distances FRIN MOR12-15, FRIN MOR12-4,FRIN MOR12-26 and FRIN MOR12-13 are sharing same genetic composition.The other sub cluster (2bi) of 0.06 was sub clustered with the rest accessions at 0.39, at this coefficient, nine accessions are sub clustered. 29% (nine out of thirty-one) of the accessions under study clustered at this distributing them at different genetic distance. The cluster comprised of FRIN MOR12-1, FRIN MOR12-23, FRIN MOR12-18, FRIN MOR12-14, FRIN MOR12-19, FRIN MOR12-5, FRIN MOR12-8, FRIN MOR12-3 and FRIN MOR12-7 respectively.Under sub cluster 2b (ii),45.2% (fourteen out of thirty one) were grouped together at 0.39. Cluster 2bi comprised of: FRIN MOR12-29, FRIN MOR12-24, FRIN MOR12-25, FRIN MOR12-30, FRIN MOR12-21, FRIN MOR12-28, FRIN MOR12-20, FRIN MOR12-9,

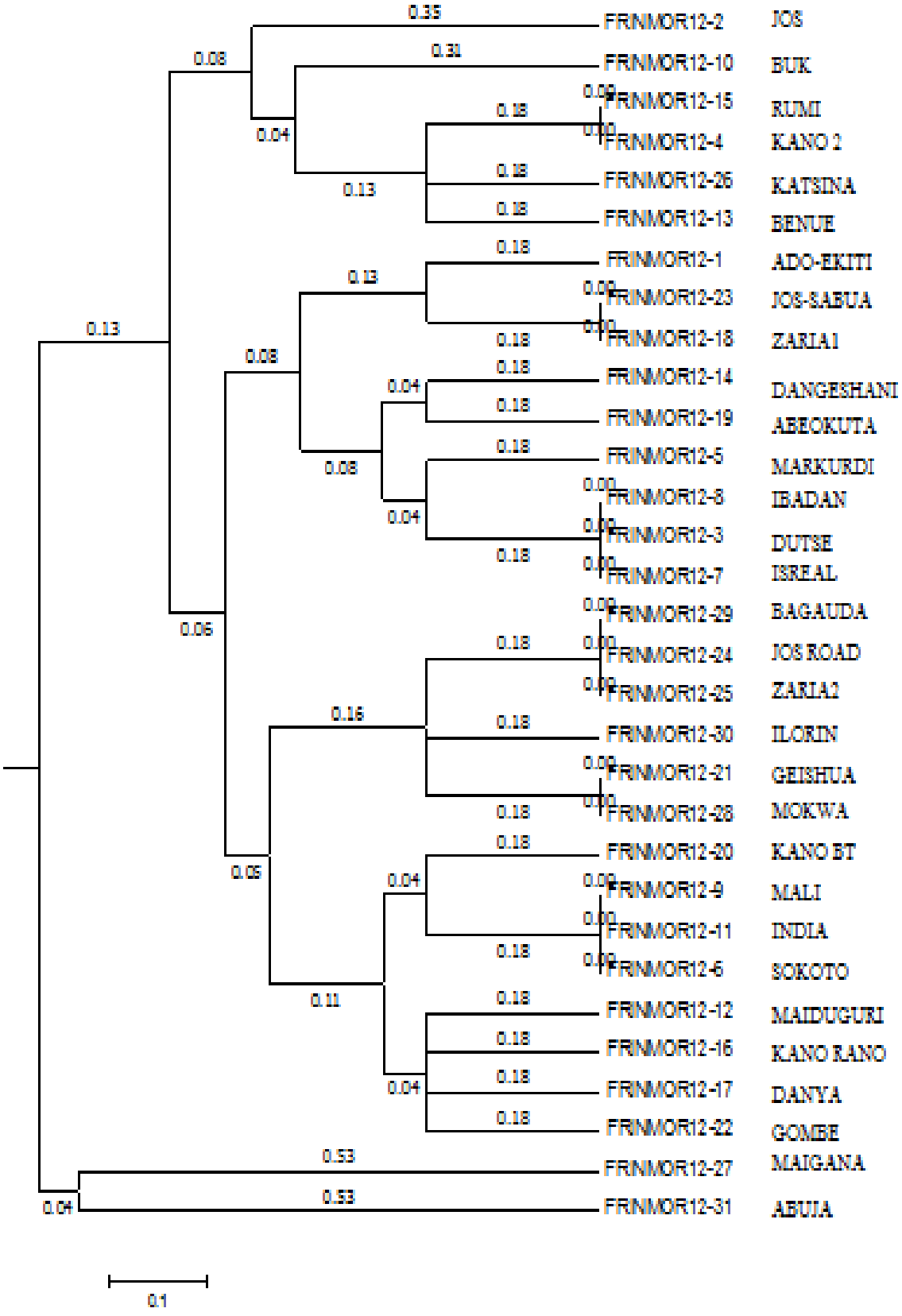


Figure 4.5: The dendrogram from the cluster analysis with the SSR marker

FRIN MOR12-11, FRIN MOR12-6, FRIN MOR12-12, FRIN MOR12-16, FRIN MOR12-17 and FRIN MOR12-22. They were grouped at various genetic distances having 0.16 and 0.11 coefficient of genetic distance. At 0.16 six accessions: FRIN MOR12-29, FRIN MOR12-24, FRIN MOR12-25, FRIN MOR12-30, FRIN MOR12-21 and FRIN MOR12-28 were clustered together. The remaining eight accessions were clustered at coefficient 0.11 having 0.04 genetic distance level in-between them. The dendrogram at 0.00 genetic distance showed some duplications or very close relations among the accessions in FRIN MOR12- 15 and FRIN MOR12- 4; FRIN MOR12-23 and FRIN MOR12-18; FRIN MOR12-8, FRIN MOR12-3 and FRIN MOR12-7; FRIN MOR12-29,FRIN MOR12-24 and FRIN MOR12-25; FRIN MOR12-21 and FRIN MOR12-28 also FRIN MOR12-9, FRIN MOR12-11 and FRIN MOR12-6.

CHAPTER FIVE

5.1 DISCUSSIONS

The initial analysis of the accessions on the field brought out wide variation for all the seventeen traits measured. This wide variation among the accessions can be ascribed to the different locations and sources of collection of the planting materials. Variation among the accessions had also been reported to result from genetic differences caused by the adaptation to diverse environmental conditions (Ginwal *et al*., 2005). The observed variation in 100 seed weight in this result may partly be due to the different position of seed on mother plants or due to different environmental conditions towhich the mother plants were subjected to during the growing season (Singh *et al.,* 2010).Earlier studies has reported variation amongseed sources with respect to seed traits (length,width, thickness and weight) in many forest species including *F. albida* (Dangasuk *et al*.,1997; Fredrick *et al*., 2015), *Acacia karroo* (Abdelkhair *et al*., 2003), *Pinusroxbrughii* (Ghildiyal *et al*., 2009), *Dalbergia melanoxylon*(Amri *et al*., 2008), and *Celtis australis* (Singh *et al*.,2006). A lower range of coefficients of variation usually implies low variability and it is an indication of uniformity among accessions. In this study, coefficients of variation (CV) for all the characters evaluated had high values reflecting the existence of a high level of variability among the accessionssuggestingtheir sensitivity to environmental factors as reported by Fredrick *et al*., (2015).

A high proportion of genotypic coefficient of variation to the phenotypic coefficient of variation reflects a greater influence of the genetic composition on expression of characters which is desirable in breeding programmes. However, results from this study revealed that phenotypic variances ($\sigma^2 p$) and PCVs were slightly higher than genetic variances ($\sigma^2 g$) and

GCVs for all the characters measured, this suggests the influence of environment on the expression of these characters.These patterns of high level differences or variability have been recorded by Popoola *et al.* (2014) in their study on thirteen accession collected from Nigeria realised significant levels of intraspecific variations.Number of total seeds in this study is the most varied of the measured traits closely followed by seed yield and they gave negative variances and were regarded as zero following the suggestion by Chandra (2015). The negative variance had been attributed to the large differences among the data obtained from different replications of the trial. The estimates of heritability were low for all the characters except days to first fruiting and days to first flowering which recorded high heritability values. The low estimates of heritability indicate the influence of the environment on their expression and direct selection for these traits will be ineffective. According to Andualem, *et al*., (2013) selection may be practically impossible or considerably difficult resulting from the masking effect of the environment on the trait. Traits with moderate to high heritability values are more likely to be transmitted to offspring since a greater percentage of the expressed variance is being controlled by the genes rather than the environment. According to Singh (2001) andAndualem, *et al*., (2013), if heritability value of a character ishigh, selection for such traits could be fairly easy and such traits could be more easily improved. This is because there would be a close correspondence between the genotype and the phenotype due to the relative small contribution of the environment to the phenotype.

The high positive significant correlation existing between pairs of characters is indicative of their ability to be used as an aid to selection. The pod yield had high positive significant correlation with characters like regrowth potential, plant height and stem diameter that can be measured at early stage of plant establishment they can also be used as aids to selection for

pod yield in *Moringa oleifera*. This same trend of accessions with longer fruits pod producing higher number of seeds per pod and consequently higher seed set percentage had been reported by Ganesan*et al.*,(2014) and Popoola *et al*., (2016). Early flowering and fruiting are not reliable aids to selection characters for pod/ seed yield. This is suggesting that the early flowering accessions might not necessarily produce viable seeds by the harvesting period due to pseudo fertilization or even flower abortion in the course of flower maturity. Early fruiting and flowering charactersare actually important to the breeder this negative correlation recorded with yield related characters in this study needs to be a major improvement focus in the already established repository of drumstick. Correlated quantitative characters have been used in improvement program, as theimprovement of one character may cause simultaneous changes in the other character (Loha *et al*., 2006; Shu*et al.*, 2012).

Result from the rank summation index indicated that accessions FRINMOR12-17, FRINMOR12-22 and FRINMOR 12-6 from Kano, Sokoto and Gombe states respectively all belonging to cluster 2 are the best materials in terms of total number of pods, total number of seeds, the seed yield, 100seed weight, pod weight, number of seeds per pod, secondary branches and flowering branches. These accessions are therefore considered the best potential materials for the improvement of *Moringa*in Nigeria.

The Principal Component Analysis brought out the variations existing between accessions tor the various characters. PC1 recorded 50.1% and is mostly correlated with all the traits except with the number of days to first flowering and number of days to firstfruiting. A total variation of 50.1% isbeing accounted for by the yield related traits suggesting their stability and consistency. This trend has been reported earlier on Moringa diversity study by Ganessan *et al*., (2014) and Popoola *et al*., (2016). PC1 is being heavily loaded with the pod yield

related characters. PC2 is highly loaded with adaptability and survival attributes, PC3 is having high load for vegetative growth, this has to do more with the leaves production potential, PC4 is heavily loaded with earliness to flowering and fruiting attributes while PC5 is also having high load with primary branches and regrowth potential which are the major vegetative characters. The more the primary branches the more the ability of the tree to tend towards leaves production. This gives it a bushy growth pattern which can be good as an ornamental tree. More primary branches is an indicative of high leaves production, this will aid selection by a breeder depending on production goals. The variation shown by the scatter plot of the first 2 PC scores were almost similar to the ones observed in the dendrogram fromcluster analysis. Despite the fact that no one accession was found to be performing best overall for all the characters measured, cluster analysis has successfully given a broad category where each of the accessions belongs. The Scatter plot also confirms the grouping from the cluster by plotting the accessions on the quadrant with respect to their genetic relatedness. The further placed accessions on the quadrants are well dispersed and can be considered for further detailed study in the breeding works. The cluster successfully brought out the diversities and similarities existing among the accessions showing the pattern of variation and genetic relatedness. Accessions with similar morphological performance on the field were brought together in same cluster whileaccessions in different clusters are dissimilar to each other. The cluster did not express influence of geographical distribution or environment showing that they are genetically distinct from one another. This pattern was also recorded by Popoola *et al*. (2014).

The microsatellite SSR markers analysis gavethe polymorphic Information Content average value of 0.5 in this study. This agrees with the results of Salvakumari and Ponnuswami (2015) in their genetic study on 34 ecotypes of *Moringa oleifera* using 20 SSR markers. A

contrary result was obtained by Ganessan *et al.* (2014) who reported a lower average PIC value of 0.15. Saini *et al*. (2013) used RAPD, ISSR, Cytochrome P_{450} markers also for *Moringa oleifera* and reported high PIC values of 0.72, 0.81 and 0.68 respectively which are all higher compared to what is obtained with SSR marker in this study. This indicates sufficient polymorphism exists within the present collection and can be exploited for genetic linkage maps. The selected markers reflected a level of efficiency despite the insufficient number used. The average gene diversity value is 0.52 in this study also indicates a wide variability among the accessions. A contrary result was observed by Ganesan *et al*. (2014) study on *Moringa oleifera* using SSR markers who reported gene diversity range between 0.01 and 0.49 with an average of 0.18.The amplified alleles with an average value of 4.75 alleles per locus in the 31 accessions is also contrary to the report ofGanesan *et al.* (2014) of 35 alleles with an average of 1.84 per locus in diversity assessed in their study with 300 individuals of *Moringa oleifera* using SSR markers. Kuo (2002) reported 75 RAPD markers with an average of 6.98 bands per primer. This is much higher than the ones reported for SSR markers. Muluvi *et al.* (1999) also reported 59 bands per primer with AFLP. It is clearly seen that SSR markers produce the least number of bands. This is because they are locus specific and normally only two allelles are expected from each locus. The molecular analysis in this study revealed a relatively high polymorphism and gene diversity which reveals the high level of variability among the accessions. Primers MO 1 and MO 41 should be considered in future study for the genetic resource management in *Moringa oleifera.*

The cluster analysis of 31 M*oringa oleifera* accessions based on UPGMA suggested the formation of two main clusters with four sub clusters formed at different genetic distances.The clustered groups comprised of accessions from different ecological locations being grouped together, there is no clear geographical isolation of the accessions

studied.Thismight be attributed to genetic component, breeding system and phenotypic similarities. Also according to Fredrick *et al*.(2015) the accessions were raised in a common environment and subjected to similar treatments which invariably might have reduced the effects of the environment on their phenotypic expression. The absence of clustering based on geographical location indicates that individuals from different locations are not significantly different genetically. This is similar to previous report on ninety seven accessions using SSR markers in India by Rajalakshmi *et al*. (2017);Ganesan *et al*. (2014) and Rufai *et al*. (2013) using RAPD markers.Clustering of individuals from the same population in different clusters indicates high genetic variation within population which may be attributed to the use of seed sources, mutation or breeding system which is in agreement with the fact that it is predominantly an out-crossed plantMgendi *et al*. (2010). However, such results are contrary to the study ofMuluvi *et al.*(1999) where clustering of accession was based on their geographic origin.The cluster analysis clearly showed the genetic diversities among the accessions and went further to show 15 of the accessions were duplicated andwere now grouped into just 6 accessions. This duplicate could have resulted from low level of genetic distances between the concerned accessions or resulting from low frequency of the allele obtained with the five primers used in this study.The similarities may also be as a result of gene flow between adjacent populations or spread from cuttings and seeds used in planting although Muluvi*et al*. (1999); Zenglu and Randal (2002) had reported grouping of genotypes based on geographic origin. It is inferred from the cluster analysis that Cluster 1 and cluster 2a are most differed at the molecular level and the accessions in the clusters can be employed in improvement programme of *Moringa oleifera.* The groupings of the accessions within the main clusters and sub- cluster were different for both molecular markers and morphological characters. Molecular data, analyzed separately for genomic and functional loci, resulted in slightly different dendrograms, indicating that each approach of characterization differently

accesses the variability present among the accessions. These observations indicate great diversity exists among the accessions and also demonstrate that the selected primers are highly informative and useful for further studies on *Moringa oleifera* genetic diversity study and improvement programmes.

CHAPTER SIX

6.1 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1.1 Summary

*Moringa oleifera*has attracted several scholars resulting from diverse usesthedifferent parts of the plant has been subjected towhichstretch from food andmedicinal uses to water purification, biopesticide and production of biodiesel. In this study, morphological traits and molecular markershave been used for characterisation and studying genetic distancesamong thirty one accessions of drumstick.

Data analysis results has shown the presence of wide genetic diversity among the accessions, The significant and positive correlationsobserved among the yield related traits is an indication that selection for one trait results in progress for all traits with positive correlation and retrogression for the negatively correlated. The higher estimates observed for phenotypic coefficients of variation than the genotypic coefficient of variation for all traits indicates the presence of environmental influence to some extenton the phenotypic expressions of traits. Variability was discovered in the collection.

The principal component analysis brings out the variability with seventeen components out of which the first five components having eigenvalues greater than one were able to detect 87.5% of the variation that exists in the collection and were further used in the results interpretations. Principal component 1 actually brought out 50% of the total variabilitywith maximum positive loading factor of thirteen out of the seventeen traits on it. The overall variation of PC2 was 11.3%while PC3 was responsible for 10.2% of theexpressed variation,

PC4 shared 9.0% of the total diversity among the accessions and PC5 accounts for 6.8% of the total variation. The principal component analysis therefore, discriminated and separated the 31 accessions in terms of pod/seed yield traits, survival and adaptable traits, vegetative traits, early flowering traits and bushy tree form traits. Based on the cluster analysis it was evident that there was no correlation between grouping of the genotypes and geographical origins of the materials.

Micro satellite markers have proven powerful tool for analyzing and determining the amount of genetic differentiation among the *Moringa* accessions. Only four SSR markers produced polymorphic bands with polymorphic information content of 0.48, gene diversity of 0.52 and allele frequency of 0.57 and these results indicated there was actually gene diversity existing among the collections. High polymorphism and gene diversity were observed from the primers MO41 and MO1indicating the deduced information from the primers can be used with high level of certainty in subsequent analysis. The cluster analysis grouped the 31 accessions into two main clusters with three sub clusters.This characterization was able to detect duplications of fifteen accessions which were merged to six sub clusters. Simultaneous use of both methodshas provided better and more comprehensive description that assists to a great extent the interpretation of biological diversities.

6.1.2 Conclusions

The evaluation of the accessions using morphologicaltraits depicted that the accessions possess sufficient genetic diversity and significant positive associations among most of the yield related traits. The correlation studyhas shown high level of correlation between some important traits: seed yield with plant height at 6MAE, rejuvenation with survival ability,

total seed with total pods, seeds per pod with pod length, seed per pod with pod diameter and seed per pod with pod weight at 0.65, 0.88, 0.72, 0.92, 0.85 and0.91 respectively.The heritability estimates were high for days to first flowering and days to first fruiting thus these traits could be improved more easily compared to others.The molecular analysis showed a relatively high polymorphism and gene diversity which indicates high level of variability exists among the accessions. PrimersMO41 and MO1 exhibited high polymorphism and gene diversity thus recommended for subsequent analysis. Cluster analysis was able to detect duplications in the characterization of the *moringa* collection.

The computed rank summation index has ranked accessions FRINMOR12-17, FRINMOR12-22 and FRINMOR12-6 as superior materials with respect to total number of pods, total number of seeds, the seed yield, hundred seed weight, pod weight, number of seeds per pod, secondary branches and flowering branchesand these can be used for further improvement on *Moringa* in Nigeria.

The principal component analysis shows five out of the seventeen principal components extracted with eigenvalues greater than one and cumulatively explained 87.5% of the variation.Accessions can be selected from any of PC1, PC2, PC3, PC4 or PC5 depending on breeding interest. However, accessions FRINMOR12-22 and FRINMOR12-17 are exhibiting most of the principal components traits.

6.1.3 Recommendations

i. Accessions FRINMOR12-14 and FRINMOR12-5 are recommended for superior performance relating to pod length and number of seeds per pod, accession FRINMOR12-1 and FRINMOR12-9 had their first fruit earlier while accessions FRINMOR12-22 and FRINMOR12-17 performed best in yield related traits.

ii. Days to first flowering and days to first fruiting are the most varied traits and could be regarded as an important trait for evaluation in breeding programme.

iii. There is need to broaden the accession collection and carry out the biochemical composition of the accessions. This present study has basically considered yield from the pods, seeds and the vegetative growth. However there is need to undertake further work to elucidate the diversity existing among the collections for biochemical composition of the primary products of *Moringa oleifera* which are the plant seeds, leaves, the root and the bark of the plant. The determination of the biochemical composition of the seeds, leaves, roots, plant bark will assist in knowing the best accessions that can be recommended for consumption giving more nutritional benefits and having higher production yield for leaves producers

iv. Gene diversity of *Moringa oleifera* is effectively investigated using microsatellite markers. These observations indicate that the selected primers are highly informative and useful for further studies on *Moringa oleifera* genetic diversity. Primers MO 1 and MO 41 should be considered in future study.

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APPENDICES

Appendix 1:*Moringa oleifera* plantation (Location SFRS, Zaria)



Appendix 2: Fruiting *Moringa oleifera* tree (Location Orogun, Ibadan)

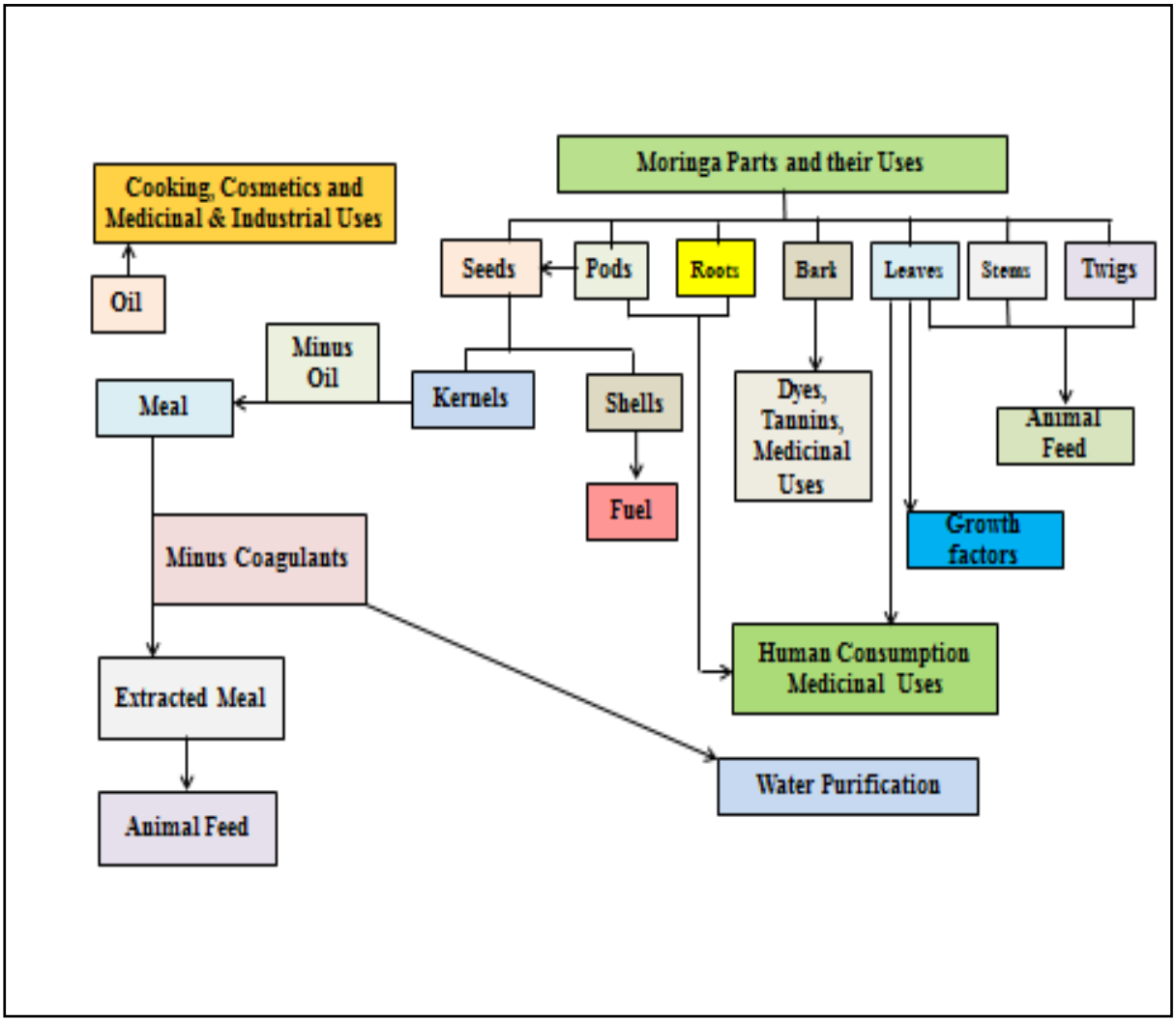


Appendix 3: *Moringa oleifera* seeds



Appendix 4 Moringa oleifera flower



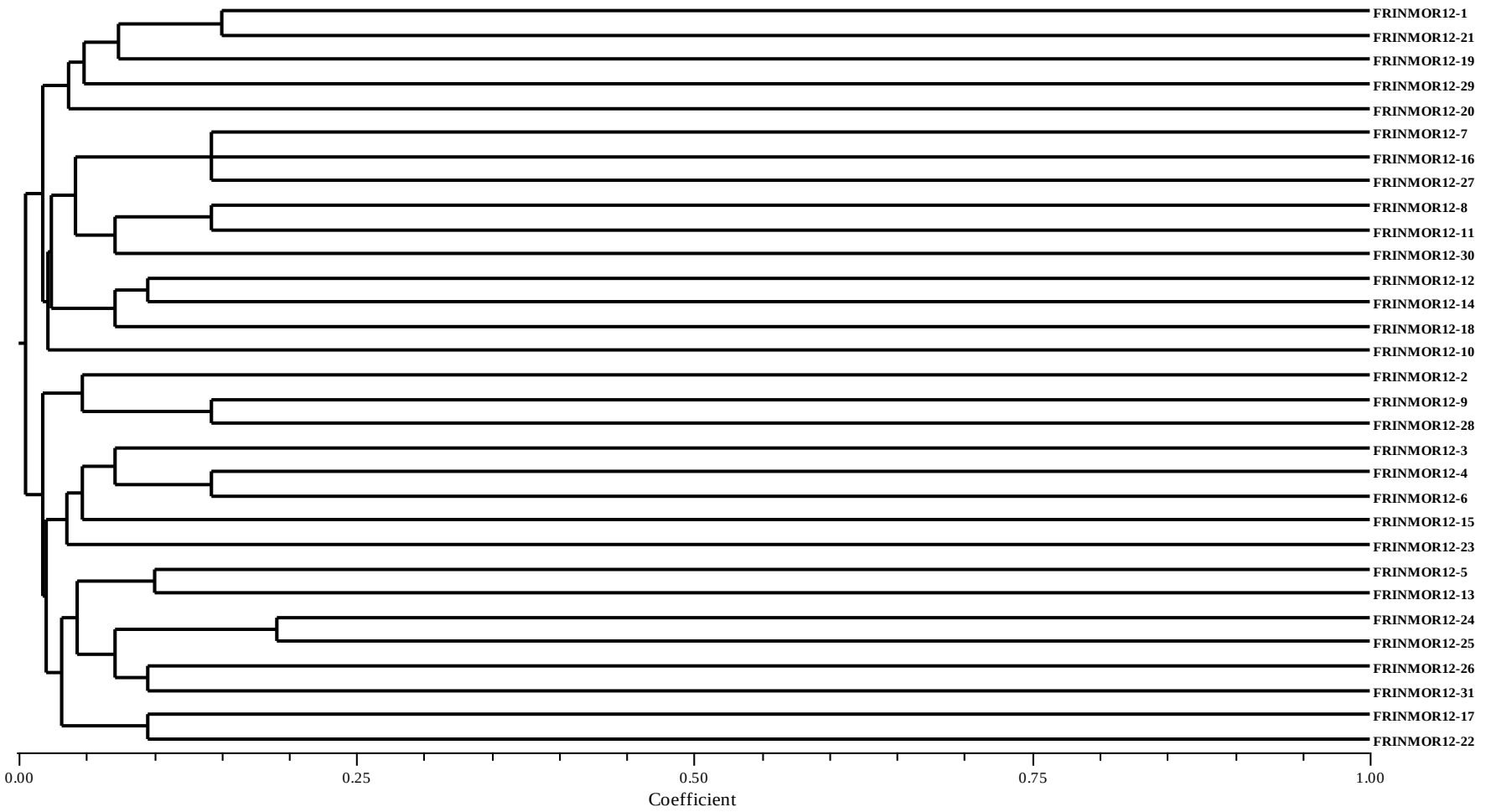


Appendix 5: Pictorial presentation of the potentials of *Moringa oleifera*plant parts

APPENDIX 6

DNA Count and Concentration of the *Moringa oleifera* accessions

#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	1	NACGRAB	01-Aug-14 11:36:34 AM	1802.9	ng/µl	36.059	18.345	1.97	1.62	DNA	50
2	2	NACGRAB	01-Aug-14 11:37:29 AM	1113	ng/µl	22.261	11.601	1.92	1.37	DNA	50
3	3	NACGRAB	01-Aug-14 11:38:28 AM	2253.9	ng/µl	45.078	22.966	1.96	1.75	DNA	50
4	4	NACGRAB	01-Aug-14 11:39:25 AM	1614	ng/µl	32.28	16.462	1.96	1.59	DNA	50
5	5	NACGRAB	01-Aug-14 11:40:10 AM	1231.2	ng/µl	24.625	12.507	1.97	1.68	DNA	50
6	6	NACGRAB	01-Aug-14 11:40:58 AM	1476.7	ng/µl	29.533	15.523	1.9	1.38	DNA	50
7	7	NACGRAB	01-Aug-14 11:41:36 AM	2137.1	ng/µl	42.743	21.839	1.96	1.74	DNA	50
8	8	NACGRAB	01-Aug-14 11:42:17 AM	1832.2	ng/µl	36.644	18.523	1.98	1.7	DNA	50
9	9	NACGRAB	01-Aug-14 11:43:16 AM	2223.2	ng/µl	44.464	22.512	1.98	1.76	DNA	50
10	10	NACGRAB	01-Aug-14 11:43:57 AM	1674.8	ng/µl	33.497	17.006	1.97	1.79	DNA	50
11	11	NACGRAB	01-Aug-14 11:44:35 AM	2439.6	ng/µl	48.791	25.42	1.92	1.66	DNA	50
12	12	NACGRAB	01-Aug-14 11:45:14 AM	1938.3	ng/µl	38.766	19.758	1.96	1.72	DNA	50
13	13	NACGRAB	01-Aug-14 11:45:40 AM	1429.6	ng/µl	28.591	14.534	1.97	1.78	DNA	50
14	14	NACGRAB	01-Aug-14 11:46:10 AM	2800.6	ng/µl	56.011	28.016	2	1.86	DNA	50
15	15	NACGRAB	01-Aug-14 11:46:34 AM	1893.5	ng/µl	37.87	19.209	1.97	1.85	DNA	50
16	16	NACGRAB	01-Aug-14 11:47:08 AM	2339.7	ng/µl	46.793	24.376	1.92	1.45	DNA	50
17	17	NACGRAB	01-Aug-14 11:48:10 AM	2676.9	ng/µl	53.537	26.971	1.98	1.91	DNA	50
18	18	NACGRAB	01-Aug-14 11:48:41 AM	2942.1	ng/µl	58.842	29.806	1.97	1.7	DNA	50
19	19	NACGRAB	01-Aug-14 11:49:07 AM	1264.6	ng/µl	25.292	12.81	1.97	1.72	DNA	50
20	20	NACGRAB	01-Aug-14 11:49:37 AM	2514.6	ng/µl	50.292	26.051	1.93	1.66	DNA	50
21	21	NACGRAB	01-Aug-14 11:50:02 AM	2420.4	ng/µl	48.408	25.413	1.9	1.62	DNA	50
22	22	NACGRAB	01-Aug-14 11:50:27 AM	1983.9	ng/µl	39.679	20.678	1.92	1.67	DNA	50
23	23	NACGRAB	01-Aug-14 11:51:09 AM	2174.8	ng/µl	43.496	21.742	2	1.91	DNA	50
24	24	NACGRAB	01-Aug-14 11:51:31 AM	2212.9	ng/µl	44.258	22.692	1.95	1.73	DNA	50
25	25	NACGRAB	01-Aug-14 11:52:28 AM	2398.9	ng/µl	47.978	24.498	1.96	1.73	DNA	50
26	26	NACGRAB	01-Aug-14 11:53:00 AM	2333.8	ng/µl	46.676	24.168	1.93	1.52	DNA	50
27	27	NACGRAB	01-Aug-14 11:53:29 AM	1995.2	ng/µl	39.905	20.805	1.92	1.51	DNA	50
28	28	NACGRAB	01-Aug-14 11:54:01 AM	2187.9	ng/µl	43.758	22.206	1.97	1.84	DNA	50
29	29	NACGRAB	01-Aug-14 11:54:30 AM	1665.1	ng/µl	33.302	16.586	2.01	1.97	DNA	50
30	30	NACGRAB	01-Aug-14 11:54:55 AM	1919.4	ng/µl	38.389	19.763	1.94	1.65	DNA	50
31	31	NACGRAB	01-Aug-14 11:55:27 AM	2313.3	ng/µl	46.266	23.771	1.95	1.71	DNA	50



Appendix 7: Dendrogram generated by NTSYS

	MO1			MO6		MO10		MO15				MO41			
FRIN MOR12-1	0	0	0	0	1	1	0	1	0	0	0	0	0	1	0
FRIN MOR12-2	0	1	0	0	1	1	0	1	0	0	0	1	0	1	1
FRIN MOR12-3	0	0	0	1	0	1	0	1	0	0	0	0	0	1	1
FRIN MOR12-4	1	0	0	0	1	1	0	1	0	0	0	0	1	0	0
FRIN MOR12-5	1	0	1	1	0	1	0	1	0	0	0	0	0	1	1
FRIN MOR12-6	1	0	0	1	0	1	0	1	0	0	0	1	0	0	0
FRIN MOR12-7	0	0	0	1	0	1	0	1	0	0	0	0	0	1	1
FRIN MOR12-8	0	0	0	1	0	1	0	1	0	0	0	0	0	1	1
FRIN MOR12-9	1	0	0	1	0	1	0	1	0	0	0	1	0	0	0
FRIN MOR12-10	1	0	1	0	1	1	0	1	0	0	0	1	0	0	0
FRIN MOR12-11	1	0	0	1	0	1	0	1	0	0	0	1	0	0	0
FRIN MOR12-12	1	0	0	1	0	1	0	1	0	0	0	0	1	0	0
FRIN MOR12-13	1	0	0	0	1	1	0	1	0	0	0	1	0	0	0
FRIN MOR12-14	0	0	0	1	0	1	0	1	0	0	0	1	0	0	0
FRIN MOR12-15	1	0	0	0	1	1	0	1	0	0	0	0	1	0	0
FRIN MOR12-16	1	0	0	1	0	1	0	1	0	0	0	0	1	1	1
FRIN MOR12-17	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0
FRIN MOR12-18	0	0	0	0	1	1	0	1	0	0	0	0	0	1	1
FRIN MOR12-19	0	0	0	1	0	1	0	1	0	0	0	1	1	1	0
FRIN MOR12-20	0	0	1	1	0	1	0	1	0	0	0	1	0	0	0
FRIN MOR12-21	0	1	0	1	0	1	0	1	0	0	0	0	0	1	1
FRIN MOR12-22	1	0	0	1	0	1	0	1	0	0	0	0	0	1	1
FRIN MOR 12-23	0	0	0	0	1	1	0	1	0	0	0	0	0	1	1
FRIN MOR12-24	0	1	0	1	0	1	0	1	0	0	0	0	1	0	0
FRIN MOR12-25	0	1	0	1	0	1	0	1	0	0	0	0	1	0	0
FRIN MOR 12-26	1	0	0	0	1	1	0	1	0	0	0	0	0	0	0
FRIN MOR12-27	0	1	0	0	0	1	0	0	1	1	1	0	0	1	1
FRIN MOR12-28	0	1	0	1	0	1	0	1	0	0	0	0	0	1	1
FRIN MOR12-29	0	1	0	1	0	1	0	1	0	0	0	0	1	0	0
FRIN MOR12- 30	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0
FRIN MOR12-31	1	0	1	1	0	1	1	0	1	1	1	1	0	0	0

Appendix 8: Scored Pattern of the SSR Markers