CHARACTERISATION OF LOCAL AND EXOTIC ACCESSIONS OF MORINGA (*MORINGA OLEIFERA* LAMARCK)

This Thesis is submitted to;

Department of Crop Science
College of Agriculture and Consumer Sciences
University of Ghana

By

KENNETH OPARE-OBUOBI
(10329449)

In partial fulfillment of the Requirement for the Award of

MASTER OF PHILOSOPHY DEGREE

In

CROP SCIENCE

MARCH, 2012

DECLARATION

This is to certify that this thesis is the result of research undertaken by KENNETH OPARE-OBUOBI towards the award of Master Philosophy degree in the department of Crop Science of the of Agricultural and Consumer Sciences, University of Ghana.

.....

Kenneth Opare-Obuobi

(CANDIDATE)

.....

Prof. (Mrs.) Essie T. Blay

(SUPERVSIOR)

.....

Prof. Samuel K. Offei

(SUPERVSIOR)

ABSTRACT

Knowledge of existing diversity especially at the genotypic level in plant population is crucial for the effective breeding, conservation, management, and efficient utilization of plant genetic resources (PGR) (Mondini *et al.,* 2009). However, Moringa (*Moringa oleifera* Lamarck), a common and popular plant growing in almost every part of the country with several uses and benefits still remains underutilized tropical crop in Ghana. This is due to the fact that the crop has either received little or no attention with respect to research and breeding programmes. *Moringa oleifera* seeds were collected from ten provenances with eight from Ghana and one each from United State of America and India. A completely randomised complete block design and modification of AVRDC-GRSU record sheet was used to evaluate morphological characteristics of thirty seven accessions from these provenances. Eight out of twelve traits observed revealed variability among the accessions studied. For quantitative characters studied; tripinnatum leaf length ranged between 25.2cm and 61.6cm, tripinnatum leaf width ranged between 17.8cm and 48.5cm, tripinnatum leaflet length ranged from 10.8cm to 25.9cm and tripinnatum leaflet width was from 5.7cm and 17.6cm. There were also significant differences among the accessions of *Moringa oleifera* for the vegetative features. Dry leaf samples of Moringa from eight provenances with 40 accessions were used for both biochemical and molecular analysis. The dried leaf samples were found to contain 0.0014 - 0.0736% (Fe), 0.0006 – 0.0097% Mn, and 0.0005 – 0.111% Zn. The accessions also contained 40.3 – 79.3 mg/kg (Ca), 19.23 - 48.13 mg/kg (Mg), 87.5 -253.1 mg/kg (Na), 1549 – 2272 mg/kg (K) and 0.179 – 0.438 mg/kg (P). There were significant differences in the nutritional content of the dried Moringa leaf samples except for potassium (K). The heavy metal Pb ranged from 0.0005 -0.0058%. Percentage dry matter, crude protein and nitrogen

ranged from 14.63 – 25.37%, 12.9 – 26.3% and 2.064 – 4.208% respectively. Eleven distinct bands with a monomorphic locus at a distance of 1.2cm for the forty accessions were observed in gel electrophoresis of total protein from the accessions. The bands ranged from a length of 0.1cm to 3.9cm with two accessions, E40 and E41 producing all eleven bands. Forty genotypes from different ecological zones were assayed for polymorphism using twelve decamer RAPD markers. Ten primers amplified and showed distinct DNA fragments. A high polymorphic level was recorded with 95.1% of the fragments produced being polymorphic (4.9% monomorphic). Cluster analysis showed a separation of BNR 4 accession from the rest. Accessions E41 and UE49 could not be resolved by the RAPD marker. Cluster analysis of the combined data of total protein and RAPD produced two major clusters with accession BNR4 separating itself from the rest. In conclusion, enough variation existed among the accessions studied and can be used in the establishment of Moringa breeding programme to produce superior varieties.

DEDICATION

There are millions of excuses for not taking action, but to achieve, you have to live above all excuses. I dedicate this work to God, Almighty whose guidance and protection has brought me this far. I also dedicate this work to the fond memories of my beloved sister DIANA AWO AYIBEA OPARE-OBUOBI, (late).

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to my Supervisors Prof. (Mrs.) Essie T. Blay and Prof. Samuel K. Offei for their guidance throughout the period of the programme.

Special thanks to Dr. Harry M. Amoatey and Matilda Owusu-Ansah of Biotechnology and Nuclear Agriculture Research Institute (BNARI) for providing some Moringa accessions for the studies especially those from USA and India. My appreciation also goes to Mr. E. O. Ankrah, Mr. Patrick Mireku, Mr. Derick Djan, Mr. Richard Agyare (Tamale), Mr. Francis Kusi (SARI- Manga), Mr. A. Bajo and Mr. Samuel Acheampong (Baobab foundation) for their assistance in the collection of accessions.

I am also grateful to all members of laboratory division of Soil Research Centre, Accra (CSIR) especially Mr Felix O. Ababio (research scientist) and Regina Akusika Atsu for their support and assistance.

My sincere appreciation to all members of the Biotechnology Centre, University of Ghana, especially Messrs. Rodney Owusu Darko, Edward Addo, Benjamin Otu, Moses Adenusi, Martin Teye Ossom and Miss Mawulawoe Awitti for their assistance and guidance during my stay at the Laboratory. I also thank Messrs. William Ashirifie, Kofi Ajaganko and Richard Otoo of Crop Science, University of Ghana, Legon and Mr. Stephen Atsu of University of Ghana farms for their assistance with the field work.

I am grateful for the friendship of Misses Joyce Decutt and Princess Adjei-Frimpong; Messrs. Emmanuel O. Ghartey, Kingsley Abrokwah, Enoch Sapey, Rashid Tetteh,

Emmanuel Amoah, Eric Antwi Brobbey, Daniel Ntiamoah, Issac Agbeko course mates during the MPhil. Programme.

I am indebted to my siblings Daniella and Lawrence for their support and love. Finally, I remain indebted to my Father and Mother for all the sacrifices they made for me.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES/ MAPS

LIST OF PLATES

LIST OF ABBREVIATIONS, ACRONYMS AND UNITS

Abbreviations and Acronyms

Acc.	Accession
ANOVA	Analysis of Variance
ARS	Agricultural Research Station
AVRDC-GRSU	Asian Vegetable Research and Development Centre - Genetic Resource and Seed Unit
BNARI	Biotechnology and Nuclear Agriculture Research Institute
CSRPM	Centre for Scientific Research into Plant Medicine
FAO	Food and Agriculture Organisation
FDB	Food and Drugs Board
Fig	Figure
GSD	Ghana Standards Board
KNUST	Kwame Nkrumah University of Science and Technology
NTsys	Numerical Taxonomy System Programme
RDI	Recommended Daily Intake
SARI	Savanna Agriculture Research Institute
SAHN	Sequential, Agglomerative, Hierarchical and Nested
SRI	Soil Research Institute
UPGMA	Unweighted Pair Group Method with Arithmetic Averages
USDA	United States Department of Agriculture
WHO	World Health Organisation

Units

%	Percentage
°C	Degree celcius
cm	Centimetre
g	Gram
mg	Milligram
kg	Kilogram
ppm	Parts per million
TM	Trade mark
mm	Millimetre
ml	Millilitre
rpm	Revolutions per minute
*g	Times gravity
µl	Micro litre
µg	Microgram

CHAPTER ONE

INTRODUCTION

Moringa is the monogeneric name given to the species of trees and shrubs belonging to the family *Moringaceace* Martinov of the order *Capparales* Violes (Oslon, 2001; Hawthrone and Jongking, 2006). Of the thirteen species, *M. oleifera* is the most widely utilized and well-known species and is indigenous to Sub-Himalayan regions of northwest India. It is also the most researched species of the family and has been subjected to selection through breeding methods to produce new varieties in its country of origin. It is the most common species cultivated throughout the tropics, fast growing and not very demanding with regard to climate and soil quality (Muluvi *et al*., 1999; Tsaknis *et al*., 1999; Vlahov *et al*., 2002; Manzoor *et al*., 2007). *M. stenopetala*, an African species native to Ethiopia and Northern Kenya (Lalas *et al.*, 2003), is also widely grown, but to a much lesser extent than *M. oleifera*. *M. oleifera* is known by several common names, usually dependent on the country and tribe but in English it is commonly referred to as "Horseradish tree" (due to the flavour of its root), "Drumstick tree", "Never Die tree", "West Indian Ben tree", or "Radish tree" (Ramachandran *et al*., 1980). In Ghana, the species is called by names such as Yevu-ti, Kpokpoti, or Yevutsi by the Ewe community (Irwine, 1969), Zingeridende in Hausa, Obnukuo, Ornyyukuo, or Zangala in Dagari community, Kpokpotsor in Damgbe, Nasadua in Krusaal and Bimoba in Gambadua (Wormadey, 2007).

Moringa oleifera is a fast growing plant with a high fruit production rate per acre. The leading producer of Moringa fruits is India; with an annual production rate of 1.1 to 1.3 million tonnes from 38000ha of land (Rajangam *et al.*, 2001). Small scale farmers get a net income of about USD 1500 per hectare from the cultivation of

Moringa per annum (Rajangam *et al.,* 2001). In Ghana the plant is grown mainly for its leaves, which are usually consumed as beverage either fresh or dried. They are also grown and used as living fence posts to demarcate lands. There is little or no information on large scale production or income generated by producers, processors and retailers of Moringa products in Ghana, though significant amount of revenue is obtained from these operations.

The plant is an important food commodity which serves as a natural nourishment for the people of the tropics and sub-tropics. It is rich in both macronutrients and micronutrients which are essential for human nutrition for the daily development of the human body (Anwar *et al.,* 2007). The leaves and fruits of *Moringa oleifera* are commonly eaten as vegetables mostly in the eastern part of Africa. Nutritional composition of these parts compares satisfactorily with other crops such as beans, cowpea, turnip, cassava leaves, amaranthus leaves and pumpkin leaves and even essential nutrients from other non-plant sources (CSIR, 1962; Palmer and Pitman, 1972; Maroyi, 2006). The essential amino acids, including the sulfur-containing amino acids in the Moringa leaves are higher (Makkar and Beckar, 1997) than in foodstuff recommended by the Food and Agriculture Organization (FAO) (WHO, 1985), and with concentrations similar to those of soybean seeds.

The plant, for centuries has been used as medicine for many ailments for millions of people especially those of the tropics and subtropics (Caceres *et al*., 1991; Jahn, 1991). Virtually every part of the plant including the flowers, seed, roots and bark has some pharmacologically active compounds useful for the treatment of diseases such as nervous debility, catarrhal infections, rheumatism, intestinal worms, ascites,

dyspepsia, venomous bites, sore throat, diuretics, skin disease, and cures for scurvy and various bladder and prostate ailments (Irvine, 1961; von Maydell, 1986; Duke, 1987; Morton, 1991). The plant can also be used as an antiseptic, cardiac and circulatory stimulant (ECHO, 2008).

For years, the seeds of *Moringa oleifera* have been shown to be one of the most effective primary coagulants for water treatment especially in rural communities (Folkard *et al.,* 1993; Doer, 2005; Onwuliri and Dawang, 2006). This is better because plant extracts possessing both coagulating and antimicrobial properties are safer for human health when used in water treatment, rather than the commonly used inorganic coagulant (alum) (Muyibi and Okuofu, 1995; Okuda *et al.,* 2001; Ali *et al.,* 2004; Akinnibosun *et al.,* 2008 and 2009) which has the tendency to induce Alzheimer's disease and has strong carcinogenic properties because of the presence of aluminium in the compound (Crapper *et al.,* 1973; Malleavialle *et al.,* 1984; Najm *et al.,*1998). The plant's usefulness as a non-food product has also been extensively described, including being used as lumber, fencing, charcoal and as lubricating oil (Oliveiera *et al.,* 1999; Ghasi *et al.,* 2000; Kalogo and Verstraete, 2000; Saleem and Meinwald, 2000; Jahn 2001). The biomass of the plant can also be used as soil amendment material to improve poor soils as well as plant growth enhancer (Foidl *et al.,* 2001).

The late Major Courage Quashigah (former health minister of Ghana) knowing the numerous benefits of the plant called for appropriate modalities to be fashioned out into the research, processing and packaging of the Moringa plant for use as an alternative to imported vitamin and nutrient supplements administered in health

facilities (Ghanaweb, 2007). The Moringa Association of Ghana with backing from the Centre for Development of Enterprises and GSB has now completed a food certification process, inspection manual as well as code of practice documents for Moringa leaf and products (GSB, 2009a, b and c). Professor Agyeman Badu Akosa, former Director General of Ghana Health Service, also believed that if Ghanaians had good drinking water; immunized their children under five years, with Moringa taking care of the nutrition problems, the country's health burden could be lessened (Ghanaweb, 2007). The FDB of Ghana, at present recognises and accepts the use of Moringa products as food just like the Royale Cocoa Powder beverage because of the nutritional and medicinal benefits (Sumalia, 2008, personal communication).

In spite of the numerous benefits and acceptance of *M. Oleifera* by both the Ghanaian public and state institutions like GSA and the FDB, the plant has received little attention with regard to breeding programmes to produce varieties with superior agro-morphological traits. For an efficient breeding programme, there is the need to characterise and estimate the genetic relationship that exist among the local accessions via the use of biochemical and molecular markers so that elite accessions that show genetic divergence may be crossed to generate the necessary variability from which improved varieties with better morpho-agronomic characters can be developed.

Biochemical and molecular markers have been recognised as useful markers for characterisation of germplasm with higher efficiency of distinguishing among accessions than using morpho-agronomic traits (Krapovickas, 1973; Smith and Smith, 1989). These markers have proven to be reliable method for fingerprinting accessions

in germplasm collection (Liu and Furniers, 1993; Bachmann, 1994). Biochemical markers such as total protein which refers to the protein accumulated in the vegetative parts of a crop or plant has helped differentiate between accessions (Powell, 1992). These biochemical markers are among the quickest and cheapest marker systems in use. They have been used to study large numbers of samples and remain an excellent choice for projects that only need to identify low levels of genetic variation. Molecular markers further extend and compliment characterisation based on morphological or biochemical description, providing more accurate and detailed information than classical phenotypic data (Karp *et al.*, 1997).

Despite its wide distribution and fast growing the plant still, to a large extent, remains an underexploited crop or under-utilised species (NUS) in the world and specifically in Ghana (PROTA Foundation, 2008; Oliver-Bever, 2009). For maximum utilisation of the crop, proper characterisation especially at the biochemical and molecular level is very important for the identification of accessions with superior agro-morphological traits. The gene pool of local accessions of the crop has been shaped and maintained mainly through natural selection. This is because accessions in the country have not been subjected to any form of selection at the genotypic level for use in breeding programmes especially when wide variation is observed in morphological characters like fruit shape, bark stem colour, petiole pigmentation of the leaves and growth habit of the plant (Owusu-Ansah, 2010). Also, in Ghana, not much research on characterisation of Moringa accessions via the use of either biochemical or molecular markers has been documented. Following the potential increase in the use of Moringa products resulting from the recent publicising of the nutritional and medicinal benefits of the plant, there is therefore the need to identify

and characterise the various accessions existing locally as well as investigate the presence or absence of harmful chemicals in the crop using the technologies that are available such as AAS.

The objectives of the study were therefore to;

1. Characterise the accessions of Moringa in Ghana based on their morphological features and biochemical characteristics.
2. Determine the level of variability and the relationship between the accessions.
3. Analyse the biochemical characteristic of the Moringa leaves and determine the lead (Pb) levels in the leaves.

CHAPTER TWO

LITERATURE REVIEW

2.1 General Botany

Moringa (Adans) belongs to the order *Capparales* and is the sole genus of family of shrubs and trees called *Moringaceae* (Ramachandran *et al.*, 1980; Jahn *et al.*, 1986; Olson, 2002). *M. oleifera* the most researched of all the species is a diploid (2n = 28) and the mating pattern is estimated to be 26% selfing and 74% outcrossing (Muluvi *et al.*, 2004). Pollination of the plant is usually by bees and other insects, as well as by birds (World Agroforestry Centre database; Morton, 1991; Jyothi *et al.*, 2004).

Moringa grows well in the humid tropics or hot dry lands, survives destitute soils, and has the ability to tolerate drought (Morton, 1991; Mahmood *et al*., 2010). It also grows under wide range of rainfall with minimum annual rainfall requirements estimated at 250 mm and maximum at over 3000 mm and a pH range of 5.0–9.0 (Palada and Changl, 2003). The plant is a fast growing perennial tree which can reach a maximum height of 7-12 m and a diameter of 0.2-0.4 m at chest height with the stem usually straight but occasionally poorly formed (Fahey, 2005; Mahmood *et al*., 2010). It reaches a height of 1.5-2 m before it begins branching but can reach up to 3.0 m (Foidl *et al.*, 2001). Its tripinnate compound leaves are feathery with green to dark green elliptical leaflets. Conspicuous, lightly fragrant flowers are borne on inflorescences 10–25 cm long, and are generally white to cream coloured, although they can be tinged with pink in some varieties. The fully grown dehiscent capsules, mature about 3 months after flowering, and remain on the tree for several months, releasing the seeds that are dispersed mainly by wind and water, but probably also by

seed-eating animals. Seeds do not retain their viability in storage at ambient temperatures for longer than 2 months (Sharma, 1982) germination percentages of 60, 48 and 7.5 % were reported for seeds after 1, 2, and 3 months respectively in India (Morton, 1991). The fruits are tri-lobed capsules, and are frequently referred to as "pods." Immature pods are green and in some varieties have some reddish colour. The ripe fruit turns brown and on the average contains 10–12 seeds (Anwar and Bhanger 2003). Seeds are large with three papery wings. Seed hulls are generally brown to black, but can be white if kernels are of low viability. Virtually every part of the plant is useful with the leaves, flowers, immature pods (which are called long green pods), and roots being edible for both man and farm animals respectively (Foidl *et al.,* 2001; Reyes, 2006).

Propagation of *Moringa oleifera* is either by seeds or cuttings with fruit production beginning as early as 6 to 8 months when planting is done from stem and branch cuttings (Ramachandran *et al*, 1980). Plants raised from seeds, are reportedly slower to flower with inferior quality of fruit produced (Ramachandran *et al*, 1980). Fruit yields in the first 2 years are generally low, but from the third year onward, a single tree can yield between 600 and 1,600 fruits each year (Ramachandran *et al*, 1980; Booth *et al.,* 1988; Morton, 1991).

2.2 Origin, Domestication and Distribution

Moringa oleifera is a deciduous tree which is truly wild only in the western Himalayas and the eastern Punjab (Dastur, 1951). It was cultivated in India and distributed throughout the tropical Asia and Oceannia in prehistoric times (Irvine, 1961). The tree was then introduced to other parts of the world mostly by

missionaries and voyagers. In the case of Netherlands New Guinea (West Papua), Indonesians took the plant there (Massal and Barrau, 1956) whilst the British brought it to Africa as an ornamental plant from India (Berger *et al.*, 1984). The British as well sent the plant to Jamaica in 1784 (Fawcett and Rendle, 1914). It is currently cultivated in several parts of the world including most countries in the Carribean islands West Indies, Bahamas (Brilton and Millspaugh, 1920) and Bermuda (Britton, 1918). In Latin America, the plant is also grown particularly in Cuba, Haiti, Jamaica, Puerto Rico, all of the Virgin Islands, Barbados, Trinidad, the Netherlands Antilles, Panama, E1 Salvador, Honduras, Costa Rica, Belize, Mexico, Brazil, and Venezuela (Arnoldo- Broeders, 1967, 1971; Adams, 1972; Correll and Correll, 1982; Proctor, 1984). Moringa was introduced to the United State of America by the Office of Foreign Seed and Plant Introduction of the United States Department of Agriculture from Cuba in 1915 *(Bur. PI. Indu,* 1915*)*

M. oleifera is now cultivated and has become naturalized in other parts of the world especially in countries located in the tropical and subtropical region. These include countries like Pakistan, India, and Nepal, as well as in Afghanistan, Bangladesh, Sri Lanka, Southeast Asia, West Asia, the Arabian peninsula, East and West Africa, throughout the West Indies and southern Florida, in Central and South America from Mexico to Peru, as well as in Brazil and Paraguay (Ramachandran *et al*, 1980; Jahn *et al.*, 1986; Lahjie and Siebert, 1987; Vivien, 1990; Francis and Liogier, 1991).

2.3 Species of Moringa and their distribution

According to Olson (2002), the thirteen species of Moringa fall into three groups that reflect life form and geography, namely the bottle trees, slender tree and trees, shrubs and herbs of north-east Africa.

2.3.1 Bottle trees

These species of Moringa are huge trees with bloated water-storing trunks and small radially symmetrical flowers. Three out of the thirteen species belong to this group and occur in the southern hemisphere. Namibia and Angola in south-western Africa are home to *M. ovalifolia*, while *M. drouhardii* and *M. hildebrandtii* are endemic to Madagascar (Olson, 2002).

2.3.2 Slender trees

This group of Moringa trees have a tuberous juvenile stage and cream to pink slightly bilaterally symmetrical flowers. The group has three species that occur in the red sea area, Arabia, and the Indian subcontinent. The group also includes the family's best-known and most economically valuable species, *M. oleifera* which is now cultivated in all the countries of the tropics. *M. oleifera* and *M. concanensis* are mainly from India and Pakistan, barely reaching Bangladesh. *M. peregrina* the third species in the group has the widest range of all, growing from the Dead Sea area sporadically along the Red Sea coasts to northern Somalia and around the Arabian Peninsula to the mouth of the Persian Gulf which seems to be native to sub-Himalayan India.

2.3.3 Trees, shrubs and herbs of north-east Africa

The group consists of eight *Moringa* species found in northeast Africa. All but *M. peregrina* are endemic to northeast Africa. These species are tuberous adults or tuberous juveniles maturing to fleshy-rooted adults; colourful, bilaterally symmetrical flowers. The eight species in addition to *M. ovalifolia* occur in a geographical location termed as the Horn of Africa. The Horn of Africa is the centre of *Moringa* diversity and includes a variety of life forms. *M. peregrina* is found in northern Somalia, Arabia, and the Red Sea coasts north to the Dead Sea. The remaining seven species occur in North-Eastern Africa mainly in Kenya, Somalia and Ethiopia and include *M. arborea , M. borziana, M. longituba, M. pygmaea, M. rivae, M. ruspoliana and M. stenopetala*. The densest concentration of *Moringa* species is in Mandera District, in the extreme northeast of Kenya, where *M. arborea, M. longituba, M. rivae*, and *M. ruspoliana* can be found, though the species are spatially isolated from one another.

2.4 Uses of Moringa

2.4.1 Moringa as food

Moringa oleifera can be found in the wild but domestically it is cultivated throughout the plains, especially as hedges and in house yards, thrives best under the tropical insular climate, and is plentiful near the sandy beds of rivers and streams (The Wealth of India, 1962; Qaiser, 1973). The plant is an essential food commodity which has had myriad attention as the 'natural nutrition of the tropics'. Parts of the plant such as the leaves, fruits, flowers and immatured pods, also called long green pods, are used in many countries especially in India, Pakistan, Philippines, Hawaii and many parts of

Africa as a highly nutritive vegetable and nutritional supplementation (D'souza and Klukarni, 1993; Anwar and Bhanger, 2003; Anwar *et al.*, 2005). The tender leaves taste like watercress and, along with the flowers, is eaten cooked or raw. The green pods with seeds have a similar flavour to that of asparagus when cooked. The seeds from ripe fruits have a peanut flavour when fried. In certain parts of the world, these parts are eaten as fresh vegetables and can be frozen or canned. Combined with curries, the parts of the plant are commonly prepared with chicken or sea food as a soup (Anwar and Bhanger 2003).

2.4.2 Moringa as animal feed and plant growth enhancer

Moringa leaves are also fed to livestock as well as pigs, chickens and rabbits and can be fed to fishes as food. A study in Fiji reports significant weight gain over traditional fodder when 50% of the fodder contained Moringa (Aregheore, 2002). Olugbemi (2010) reported that the inclusion of *Moringa oleifera* in cassava based broiler diets up to 5% is possible without negatively affecting productivity or haematological indices. Foidl and Reyes reported that adding Moringa leaves to cattle feed increased their daily weight gain by 32% (Trees for Life, 2005). The milk production of cattle increased by 43% after being supplemented with 15-17 kg of fresh Moringa leaves and milk production increased further by 58% and 65% when cows were fed with 2kg and 3kg of dry matter of Moringa as supplement per day respectively (Trees for Life, 2005). The adoption of Moringa as animal feed especially in third world nations will greatly improve the weight of animals as well as the production of animal milk which can boost the dietary protein supply in the population. Juice extract from green matter of the plant has been tested and is effective as a plant growth enhancer in the production of crops like soybeans, corn, turnips, black beans, red beans, white beans,

cowpeas, bell peppers, chia, sunflowers, mung beans, onions, coffee, tea, chilli peppers, melons and sorghum (Fahey 2005). Crops sprayed with green matter juice extract of the plant comparatively resulted in increased crop size. The juice extract was discovered to stimulate the growth of the plant's roots enhancing the uptake of soil nutrient as demonstrated in sugarcane production (Trees for Life, 2005). Leaf extracts have been found to increase *Rhizobium* root nodulation, nodule weight, and nitrogenase activity in mung bean (*Vigna mungo* (L.) HEPPER) when applied to seeds or as a root dressing (Bandana *et al.*, 1987).

2.4.3 Medicinal uses of Moringa

Myriad of medicinal properties have been attributed to parts of the plant. The high quality protein of the leaves has led to its widespread use by doctors, healers, nutritionists and community leaders, to treat malnutrition and a variety of illnesses. The seeds of *Moringa* are considered to be antipyretic, acrid, bitter (Oliveira *et al.*, 1999) and reported to show antimicrobial activity (The Wealth of India, 1962). The plant also contains numerous phytochemicals, some of which are of high interest due to their medicinal properties. Current studies demonstrate that isothiocyanates have antitumor activity in cancers of the lung, breast, skin, oesphagus, and pancreas (Kalkunte *et al.*, 2006; Satyan *et al.*, 2006) and this particular plant family is rich in a fairly unique group of glycoside compounds called glucosinolates and isothiocyanates. Small proteins/ peptides were isolated from the leaves of *Moringa oleifera* possessing antifungal and antibacterial activity (Dahot, 1998). Methanolic extracted from the roots has moringine and moringinine which have been reported to possess analgesic and anticonvulsive properties (Grupt *et al.*, 1999). Below are some medicinal uses of the various parts of the Moringa plant.

Table 2.1: Tissues of Moringa and their medicinal uses

Plant Part	Medicinal Uses
Root	Antilithic, rubefacient, vesicant, carminative, antifertility, anti-inflammatory, stimulant in paralytic afflictions; act as a cardiac/circulatory tonic, used as a laxative, abortifacient, treating rheumatism, inflammations, articular pains, lower back or kidney pain and constipation (The Wealth of India, 1962; Dahot, 1988; Padmarao *et al.*, 1996; Ruckmani *et al.*, 1998; Anwar *et al.*, 2007)
Leaves	Purgative, applied as poultice to sores, rubbed on the temples for headaches, used for piles, fevers, sore throat, bronchitis, eye and ear infections, scurvy and catarrh; leaf juice is believed to control glucose levels, applied to reduce glandular swelling. It also acts as an antiulcer and antihyperlipidemic agent (The Wealth of India, 1962; Dahot, 1988; Morton, 1991; Pal *et al.*, 1995; Makonnen *et al.*, 1997; Ghasi *et al.*, 2000; Fuglie, 2001; Patel *et al.*, 2010)
Stem Bark	Rubefacient, vesicant and used to cure eye diseases and for the treatment of delirious patients, prevents enlargement of the spleen and formation of tuberculous glands of the neck, to destroy tumors and to heal ulcers. The juice from the root bark is put into ears to relieve earaches and also placed in a tooth cavity as a pain killer, and has anti-tubercular activity (Bhatnagar *et al.*, 1961; Pal *et al.*, 1995; Siddhuraju and Becker, 2003; Anwar *et al.*, 2007)
Gum	Used for dental caries, and is astringent and rubefacient; Gum, mixed with sesame oil, is used to relieve headaches, fevers, intestinal complaints, dysentery, asthma and sometimes used as an abortifacient, and to treat syphilis and rheumatism (Fuglie, 2001).
Flowers	High medicinal value as a stimulant, aphrodisiac, abortifacient, cholagogue; used to cure inflammations, muscle diseases, hysteria, tumors, and enlargement of the spleen; lower the serum cholesterol, phospholipid, triglyceride, VLDL, LDL cholesterol to phospholipid ratio and atherogenic index; decrease lipid profile of liver, heart and aorta in hypercholesterolaemic rabbits and increased the excretion of faecal cholesterol (Bhattacharya *et al.*, 1982; Dahot, 1998; Mehta *et al.*, 2003; Siddhuraju and Becker, 2003)
Seed	Seed extract exerts its protective effect by decreasing liver lipid peroxides, antihypertensive compounds thiocarbamate and isothiocyanate glycosids have been isolated from the acetate phase of the ethanolic extract of Moringa pods. The seeds also possesses anti-inflammatory, antioxidant, antmicrobial and anticancer properties (Caceres *et al.*, 1992; Faizi *et al.*, 1998; Lalas and Tsaknis, 2002; Patel *et al.*, 2010)

2.4.4 Moringa as oil

The seeds contain 35-40% non-drying oil, and the remaining seed cake after extraction is reported to be very high in crude protein (nearly 60%), making it a desirable source of animal fodder (Duke, 1983). Known commercially as "ben oil", its oil qualities are similar to that of olive oil and are rich in palmetic, stearic, behmic, and oleic acids (Verma *et al.*, 1976; Nautiyal and Venhataraman, 1987) and is used for human consumption, and in cosmetics and soaps (Delaveau and Boiteau, 1980; Ramachandran *et al*, 1980; Szolnokim, 1985). The degummed oil has favourable characteristics for use as a replacement for petroleum diesel (Tsaknis, *et al.,* 1999). The oil is highly valued by perfumers for its power of absorbing and retaining odours, and by watchmakers as a lubricant (Ramachandran *et al*, 1980)

2.4.5 Moringa seeds as water purifying agent

Moringa seeds are one of the best natural coagulants discovered so far (Ndabigengesere and Narasiah, 1998) and crushed seeds are a viable replacement of synthetic coagulants (Kalogo *et al.*, 2000). The seeds are effective for high turbidity water and show similar coagulation effects to alum (Muyibi and Evison, 1995b). Muyibi and Evison, (1995b) also reported that coagulation effectiveness of Moringa seeds could reduce turbidity by between 92% and 99% though its effectiveness depended on the initial turbidity of the water. Rural women in Sudan, use Moringa seed extract to treat the high turbid Nile water instead of alum due to fear of alum causing gastrointestinal disturbances and Alzheimer's disease (Crapper *et al.*, 1973; Miller *et al.*, 1984; Martyn *et al.*, 1989; Muyibi, 1994). Moringa seeds also possess antimicrobial properties (Madsen *et al.*, 1987; Olsen, 1987) making it possible for it to be used as an antiseptic in the treatment of drinking water (Obioma and Adikwu,

1997). Broin *et al.*, (2002) reported that a recombinant protein in the seed is able to flocculate Gram-positive and Gram-negative bacteria cells, giving the seed of the plant an anti-microbial activity. *Moringa* seeds could be used as a less expensive biosorbent for the removal of cadmium (Cd) from aqueous media (Sharma *et al.*, 2006).

2.4.6 Other uses

Moringa plants are used in alley cropping because it has a large tap root and few lateral roots, it does not compete for nutrients in the subsoil with other crops (ECHO, 2008). It also adds to the nutrients available as it produces many protein rich leaves. They grow very quickly but do not provide too much shade due to the structure of their leaves (ECHO, 2008). They are also very good for reclaiming marginal land. The plant is used in the textile industry for shuttles and picking-sticks and is also suitable for pulp production for newsprint (Singh *et al.*, 1983; Guha *et al.*, 1968), cellophane, and textiles (Nautiyal and Venhataraman, 1987; Mahajan and Sharma, 1984). The corky bark yields a coarse fibre, which is utilized in making mats, paper, and cordage. The stem exudes a mucilaginous gum that is used in leather tanning and calico printing (Ramachandran *et al*, 1980; Nautiyal and Venhataraman, 1987). The plant is also used as a natural herbicide. This is usually done by digging Moringa leaves into the soil before planting; damping off disease (*Pythium debaryanum*) can be prevented among seedlings. In some parts of Nigeria, crushed leaves of the plant are used as domestic cleaning agent for cleaning cooking utensils or even walls (Fuglie, 2001).

2.5 Nutritional content and benefits

The leaves of Moringa have been reported to contain high amount of β-carotene, protein, vitamin C, calcium and potassium and serve as a good source of natural antioxidant and thus enhance the shelf-life of fat containing foods due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids (Dillard and German, 2000; Siddhuraju and Becker, 2003). "Gram for gram, fresh leaves of *M. oleifera* have four times the vitamin A of carrots, seven times the vitamin C of oranges, four times the calcium of milk, three times the potassium of bananas, three quarters the iron of spinach, and two times the protein of yogurt" (Trees for Life, 2005). The plant is referred to as "mother's best friend" in the Philippines simply because of its ability to increase a nursing mother's milk production when consumed (Estrella *et al.*, 2000) and is at times prescribed for anaemia (Siddhuraju and Becker, 2003). Fuglie (2005) reported that serving 8g of dried Moringa leaf powder will satisfy a child within ages 1-3 with 14% of the protein, 40% of the calcium, 23% of the iron, and nearly all the vitamin A that the child needs in a day.

The numerous nutritional benefits of the plant complemented by the ability of the plant to thrive in most areas in the tropics and subtropics gives it the potential of curbing the malnutrition and hunger problems of the world. The areas with high malnutrition of the world have the plant thriving there as shown in figure 2.1. Health workers in countries like Senegal and Haiti have adopted the use of Moringa powder to combat malnutrition especially in small children, pregnant and nursing mothers (Price, 1985a). Zija™, a commercially-promoted *Moringa oleifera* containing product sold in the United States of America is an important nutritional supplement

with several medicinal properties and contains low calories, quality protein dietary supplementation (containing 19 of the 20 most common amino acids), low carbohydrate, lower-fat alternative to common liquid dietary supplements, and may serve to reverse the effects of many nutrient-depleting prescription and non-prescription drugs (Johnson, 2005).

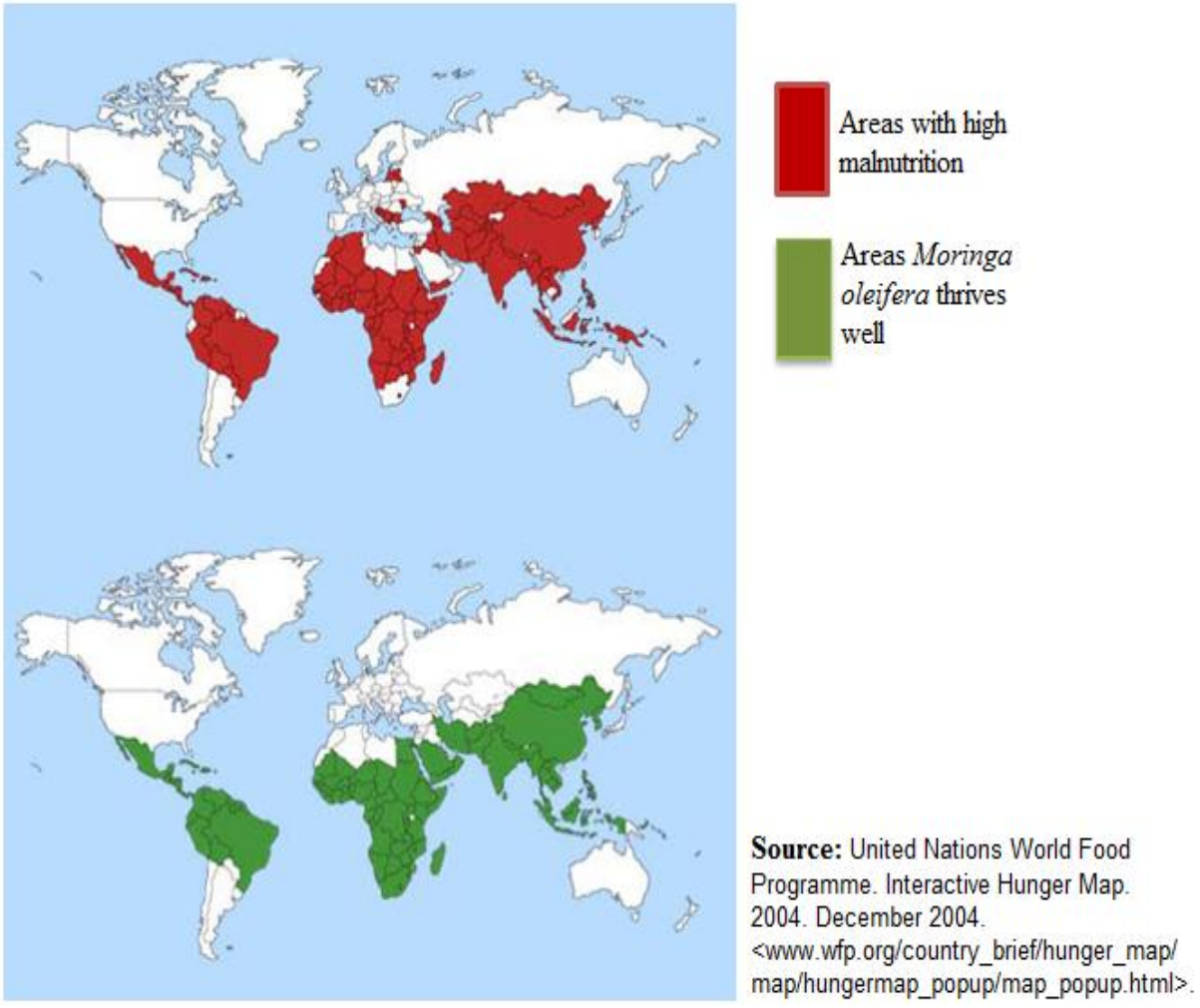

Fig 2.1: Map showing areas of malnutrition and areas where the *Moringa oleifera* plant grows

2.6 Characterisation and Diversity

Characterisation of biological organisms deals with identifying or accessing the variations that exist between the organisms either at the phenotypic or genotypic level (Mondini *et al.*, 2009). Efficiently managed and effectively utilized germplasm collection must first and foremost be well-characterized. Historically, characterization of germplasm collections has been carried out at several levels, from descriptions of taxonomy (Hilu, 1989), biogeography (Lyman, 1984; Nabhan, 1985; Brush, 1989; Zimmerer *et al.*, 1991), and morphology and agronomic characters (Chapman, 1989) to biochemical analysis (Doebley, 1989; Gepts, 1990) and the study of molecular traits and markers (Clegg, 1990; Gepts, 1995). Diversity originally was assessed by measuring variations that occur at the phenotypic traits (i.e. morphological variation) of crop plants such as growth habit, flower colour, or quantitative agronomic traits like yield potential but soon became inadequate because they are subjected to environmental variations. Due to these limitations, molecular tools were then developed to measure the variations that occur at the genetic level between species, accessions or varieties. The assessment of genetic diversity within and between populations is routinely performed at the molecular level using various laboratory-based techniques such as allozyme, total protein or DNA based analysis, which measure levels of variation directly. Genetic diversity may also be estimated using morphological, and biochemical characterization and evaluation.

2.6.1 Importance of Characterisation

The assessment of genetic variability and relatedness in any germplasm collection is very vital for effective utilisation of the germplasm for breeding programmes, identification of conservation priorities (Bekele, 1983; Demissie and Bojørnstand,

1996) and/or elimination of duplicates in the gene stock and establishment of core collections (Nisar *et al.*, 2007). A practical application of the knowledge of genetic diversity is its use in sorting of populations for genome mapping experiments (Kaga *et al*., 1996). Diversity studies especially at the molecular level of cultivated plants and their wild relatives bring important information for establishment of appropriate breeding strategies, especially when interspecific crosses are necessary for mapping purposes or for the incorporation of new features (Benko-Iseppon, 2001).

2.6.2 Morphological characterisation

This form of characterisation deals with variations that exist at the phenotypic level, in other words visually observed characters. It often does not require expensive technology but large tracts of land are frequently required for these experiments, making it sometimes more expensive than molecular assessment. The traits measured often show phenotypic plasticity; this allows assessment of diversity in the presence of environmental variation. Oslon (2002) in a study on both morphological features such as leaf form, leaf glands life form, woody anatomy, gum duct (articulated laticifers), flowers and fruit and testa anatomy; molecular data on chloroplast genome (cpDNA), gene sequence of ribulose -1-5-2 bisphosphate carboxylase / oxygenase (rbcL) revealed that Caricaceae and Moringaceae are sister taxa. Resmi *et al.,* (2005) observed variability among drumstick (*Moringa oleifera* Lam.) accessions from central and southern Kerala based on the observation that accessions from Thiruvananthapuram (MO 13, 24 and 26) had three distinct flowering peaks in a year, while others showed one or two peaks. Moringa also exhibits considerable variability in weight of cotyledons, seed size or yield, and phenology, and especially in the length, appearance, and quality of pods (Duke 1987; Jahn 1989; Morton 1991).

2.6.3 Biochemical characterisation

Biochemical analysis is based on the separation of proteins into specific banding patterns through the use of biochemical markers (Mondini *et al.,* 2009). The use of biochemical markers involves the analysis of total protein, seed storage proteins and isozymes. The isozymes technique uses enzymatic functions and is comparatively inexpensive yet powerful method of measuring allele frequencies for specific genes. It is a fast method which requires only small amounts of biological material. However, only a limited number of enzymes are available and thus, the resolution of diversity is limited.

Characterization of germplasm using biochemical fingerprinting has got special attention due to its increased used in crop improvement and the selection of desirable genotypes for breeding crops (Mondini *et al.,* 2009). Genetic markers and protein profiling are also successfully used to resolve the taxonomic and evolutionary problems of several crop plants (Khan, 1990; Murphy *et al*., 1990; Nakajima, 1994; Das and Mukarjee, 1995; Ghafoor *et al*., 2002) It is also known that variation in protein bands elaborate the relationship among the collection from various geographical regions (Satija, 2002; Asghar *et al*., 2003; Ghafoor *et al*., 2003).

2.6.4 Molecular characterisation

Molecular form of diversity is based on a large variety of DNA molecular markers, which can be employed for analysis of variation. Different markers have different genetic qualities. They may be dominant or co-dominant can amplify anonymous or well characterized loci, can contain expressed or non-expressed sequences, etc. Molecular markers work by highlighting differences (polymorphisms) within a

nucleic sequence between different individuals. These differences include insertions, deletions, translocations, duplications and point mutations. However, they cannot determine the activity of specific genes in the genome of the organism. Currently, molecular markers complement the use of morphological and biochemical markers and also introduce many new possibilities to increase our understanding of the genetic constitution of plants (Tanksley, 1993; Karp *et al.,* 1997).

The development of new PCR-based molecular markers, such as Randomly Amplified Polymorphic DNA (RAPDs), Simple Sequence Repeats (SSRs), Amplified Fragment Length Polymorphisms (AFLPs) and Single Nucleotide Polymorphism (SNP) has created the opportunity for fine-scale genetic characterization of germplasm collections that were previously impossible. The PCR-based markers generate relatively large amounts of data per unit time (Powell *et al.,* 1996) due to the high polymorphic nature and the ease with which they are processed. The high levels of variation detected with these molecular markers has allowed germplasm managers, plant breeders, and geneticists to pursue with new vigour, important questions relating to crops and their genetic resources (Bretting and Widrlechner, 1995).

2.6.4.1 RAPDs

Random Amplified Polymorphic DNAs were the first PCR-based molecular markers to be employed in genetic variation analyses (Welsh *et al.,* 1990; Williams *et al.,* 1991). This class of molecular markers are generated through the random amplification of genomic DNA using short primers (decamers or hexamers), 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. DNA polymorphisms are then produced by "rearrangements or deletions at or between oligonucleotide primer binding sites in the genome" (Williams *et al.,* 1991). The main advantages of the RAPD technology are suitability for the work on anonymous genome, application to the problem where only limited amount of DNA is available and its efficiency and cost effectiveness (Hadrys *et al*., 1992; Tingey and Tufo, 1993; Rabouam *et al*., 1999). A study of genetic variability using RAPDs in evaluating 75 accessions of *Moringa oleifera* from the Sudan and Guinea savannah zone in Nigeria produced high degree of polymorphism (74% polymorphic) (Abubakar *et al.,* 2011). Beyene, (2005) studied the genetic variation in 19 accessions with 71 individuals of *Moringa stenopetala* germplasm from Ethiopia by using RAPD as genetic marker. He reported a mean diversity range estimate of Shannon's index from 0.00 for Lasho and Berber to 0.3623 for Konso Gamolle within populations. The entire population was found to have an index of 0.3124. He concluded that there was a low genetic variability among the accessions studied. Muluvi *et al.,*(1999) analysed the genetic variation in *M. oleifera Lam* using four pairs of amplified fragment length polymorphism (AFLP) primers which revealed polymorphism between and within populations and reported significant differences between populations from different regions, even though outcrossing perennial plants are expected to maintain most variation within populations. Muluvi *et al.,* (1999) further suggested that provenance source is an important factor in the conservation and exploitation of *M. oleifera* genetic resources.

2.7 Biochemical (Physico-chemical) Analysis

The food we consume is basically needed to provide the body with the minimum requirements of the six groups of nutrients namely carbohydrates, fats, proteins, mineral elements, vitamins and water (Alfred and Patrick, 1985). One food group that contains nutrients which can be absorbed by the body to be used as energy sources, body building, regulatory and protective material is vegetables. Leafy vegetables are a category of vegetables that is widely eaten throughout the world and are comparatively rich in fibre while cereals, root vegetables and other foodstuff are relatively poor sources (Brain and Allan, 1986). The low calories and negligible quantities of utilizable energy of vegetables make them ideal for obese people who can satisfy their appetite without consuming too much carbohydrate (Oke and Ojofeitmi, 1988). Generally vegetables have low amounts of protein content but Moringa leaves are exceptional and there is increasing awareness of the importance of it in maintaining health, particularly in areas where animal protein is scarce (Baker and Griffin, 1967). Pallavi and Dipika, (2010) after assessing the effect of different methods of drying (sun, shade and oven drying) on the nutritive value of the *Moringa oleifera* leaf with its fresh counterparts reported that there was significant increase ($p < 0.01$) in all the nutrients in the dried samples of the leaves making them a concentrated source of nutrients. Shade dried samples had the highest nutrient retention followed by sun drying and oven dried samples but the differences were not statistically significant ($p > 0.05$).

Oduro *et al*., (2008) evaluated nutritional potential of *M. oleifera* and seven varieties of sweet potato (*Ipomea batatas*) leaves. They reported that the *M. oleifera* leaves contain higher levels of crude protein, crude fibre, iron and calcium making it a very

rich source of dietary nutrient compared to *Ipomea batatas*. Kwenin *et al.*, (2011) also evaluated the nutritional value of some African indigenous green leafy vegetables in Ghana. They concluded that *Moringa oleifera* was high in protein content with value of 6.60% compared to other locally consumed vegetables such as *Xanthosoma sagittifolia* (Kontomire) and *Talinum triangulare* (Bokoboko) with protein content of 4.65% and 5.10% respectively. Fuglie, 2005 also reported that 8g serving of dried leaf powder will satisfy a child within the ages 1-3 with 14% of the protein, 40% of the calcium, 23% of the iron, and nearly all the vitamin A that the child needs in a day. Also a 100g portion of leaves could provide a woman with over a third of her daily need of calcium and give her important quantities of iron, protein, copper, sulphur, and B-vitamins. Reddy *et al*., (2005) after utilizing extracts of three plant foods, namely amla (gooseberry), drumstick leaves (*M. oleifera*) and raisins as sources of natural antioxidants in the preparation of biscuits, reported that even though all the above three extracts exhibited a high percentage of antioxidant activity compared with the effect of butylatedhydroxyanisole, extracts from drumstick leaves and amla were more effective than raisins in controlling lipid oxidation during storage of biscuits. The leaves of the plant had the richest amount of protein source with an adequate profile of amino acids and ash, while the immature pods show a high content of dietary fibre and low lipid content (Sánchez-Machado *et al.*, 2009). Abdulkarim *et al.*, 2005 also reported high levels of total proteins (383.0 SD=13.0g/kg dry matter) in *Moringa oleifera*, which were greater than the protein level (18-25%) for important leguminous seeds used for human nutrition and nearly double the contents of cereals (Singh and Singh, 1992). The seed lipid content (412.0 SD= 22.2g/kg dry matter) reported by Oliveira *et al.*, (1999) is greater than that of some soybean varieties (149-220g/ kg meal) (Vasconcelos *et al.*, 2001).

2.8 Presence of Lead (Pb) in Plants

The toxicity of heavy metals like lead is a problem for ecological, evolutionary and environmental reasons (Nagajyoti *et al.,* 2008). The metal is a highly toxic pollutant as it is introduced into the atmosphere through automobile exhausts (Lagerwerff and Specht, 1970). Dense traffic releases detrimental exhaust gases and toxic pollutants like unburnt and partially burnt hydrocarbons, lead compounds and other elements that are contained in petrol polluting the city environment (Iqbal *et al.,* 2001). In the soil, it may be caused by broken-down lead paint, residues from lead-containing gasoline or pesticides used in the past, contaminated landfills, or from nearby industries such as foundries or smelters (Woolf *et al.,* 2007). Much of the global lead contamination has occurred as a result of mining and iron smelting activities (Huang *et al.,* 1996). According to FAO/WHO standard (Codex Alimentarius Commission, 2001) the safe level of lead in vegetables is 2 mg/kg above which it becomes toxic for human consumption. The soil being a major source of heavy metals, consciousness of the critical contents or limit on safe crop production needs be observed. The following critical levels of heavy metals for arable land have been established; Zn – 300 ppm; Cu – 100 ppm; Cr – 100 ppm; Pb – 100 ppm; Ni – 50 ppm; Cd – 3 ppm and Hg – 2 ppm (Mengel and Kirby 1987). In plants, the critical levels established for heavy metals are Zn (150-200 ppm); Cu (15-20 ppm); Cr (1-2 ppm); Cd (5-10 ppm); Pb (10-20 ppm); Hg (2-5 ppm); Ni (20-30 ppm) and Co (20-30 ppm). Higher contents than these are very likely to be toxic (Sauerbeck 1982).

Exposure of the human body to lead may cause a decrease in lifespan and have health effects in the long term (Needleman, 2004). Death rates from a variety of causes have been found to be higher in people with elevated blood lead levels; these include

cancer, stroke, and heart disease, and general death rates from all causes (Rossi, 2008). The metal is considered a possible human carcinogen based on evidence from animal studies (Merrill *et al.,* 2007). The metal also has an adverse effect on plants as it inhibit germination and retard plant growth as commonly observed at toxicity level (Lerda, 1992; Wierzbicka and Obidzinsca, 1998; Antosiewicz and Wierzbicka, 1999; Shaukat *et al.,* 1999; Iqbal and Shazia, 2004). The toxicity of lead negatively impacts on seed germination and seedling growth of some tree species (Shafiq and Iqbal, 2005). Studies on concentrations of lead in some leafy vegetables of Makarfi, Nigeria revealed 321 mg/kg of lead in the dry weight of Moringa leaves studied (Garba *et al.,* 2010). This far exceeds FAO/WHO standard (Codex Alimentarius Commission, 2001) the safe level of lead in vegetables. Magat *et al.,* (2009) reported lead level of 90 mg/kg in some Moringa accessions studied in the Philippines giving enough reason that the plant can pick and accumulate lead concentrations like it has been reported in Chinese cabbage (Wong *et al.,* 1996; Chove *et al.,* 2006). Foliar application of lead affected growth and yield of wheat (Rashid and Mukhirji, 1993). High significant effects of lead on shoot, root lengths and seedling was observed in dry biomass of *Lythrum salicaria* (Joseph *et al.,* 2002).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of Moringa Accessions

Moringa seeds and cuttings were collected from seven regions of Ghana, namely Greater Accra, Central, Western, Eastern, Ashanti, Volta, Northern, Upper East and Upper West representing the different ecological zones of the country. Fourteen additional collections from the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of Ghana Atomic Energy Commission (GAEC) were included of which two accessions were collected from United States of America (USA) and India as shown in figure 3.1.

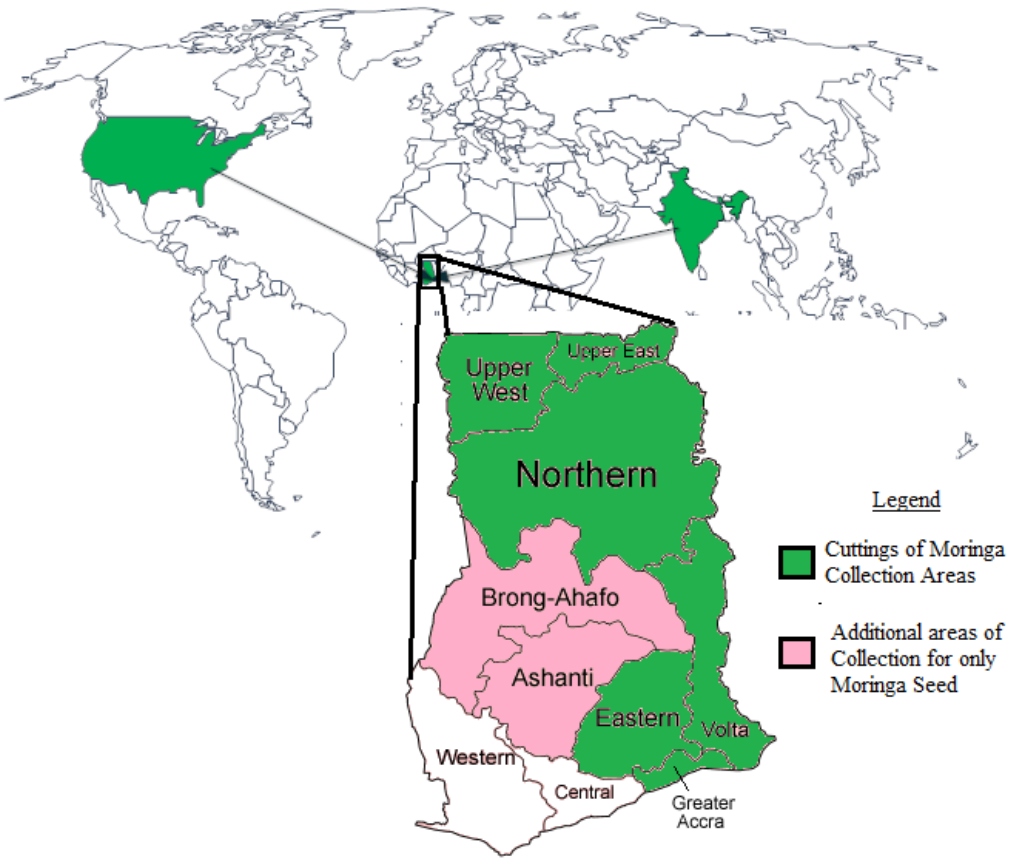

Fig 3.1: Origins of *Moringa oleifera* accessions used in the study

3.2 Morphological Characterisation

The accessions were planted at the University of Ghana farm, Legon on the soil belonging to the Nyigbenya-Hatso series characterised by well drained, red, sandy clay loam to clay with abundant rough stone concretions and quartz gravels classified by the soil research institute (FAO/UNESCO, 1990). A randomised complete block design was used for the experiment with three replicates. Seeds were nursed, pricked out and transplanted onto the permanent plot after four weeks. Planting was done at a distance of 2.5m within rows and 2.5m between rows. A total land area of 656.25m^2 was used for the study with seven plants per row. A Moringa record sheet with modification from AVRDC-GRSU was used for the morphological characterisation. The passport data of the various accessions used for the morphological studies are shown in appendix 1.

3.2.1. Data Collection

Quantitative and qualitative data were taken on the vegetative and morphology of the plant. Mean values were computed by dividing the sum of the parameters measured by the number of samples selected from the observation unit. Data was taken six months after planting. Data collected were as follows;

1. *Tripinnatum leaf length:* This is the average length of the leaf measured from the petiole attached to the stem to the tip or apex of the leaf.

2. *Tripinnatum leaf width*: This measured the average width recorded from the tip of the longest opposite leaflets in the leaf.

3. *Tripinnatum leaflet length*: This parameter measured the average length of a leaflet from the tip of the leaflet to the point of attachment to the midrib of the leaf.

4. *Tripinnatum leaflet width*: This was measured from the furthest points of the leaflet across the midrib.

5. *Growth habit*: Plants were classified as exhibiting compact, intermediate or tall (erect)

6. *Leaf type:* leaf was scored either as simple or compound for the various accessions in study.

7. *Leaf arrangement*: Plant data for the accessions on this parameter was recorded as opposite, alternate, pinnate or palmate.

8. *Leaflet arrangement*: Data for the accessions with regard to leaflet arrangement was recorded as opposite, alternate, pinnate or palmate.

9. *Leaflet shape*: The leaflet shape of the plants was recorded as simple or compound.

10. *Leaf colour*: For each accession the colour of the leaf was recorded as *light* green, green or dark green.

11. *Petiole pigmentation*: the petiole pigmentation was observed as green, dark green, purple or dark purple.

12. *Colour of the stem bark*: The colour of the woody stem observed and recorded as whitish, green, brown or dark brown.

3.3 Biochemical Studies

3.3.1 Experimental material

Dried leaves of plants established from stem cuttings of *Moringa oleifera* from eight different locations were used for both biochemical analysis and molecular

characterisation. The passport data of the various accessions used for both the biochemical and molecular studies are shown in appendix 2.

3.3.2 Dry matter determination

The hot empty crucible was weighed (W_2) and 1.0 g (W_1) of air dried sample transferred into it. The crucible with the sample in it was placed in an oven at 105 °C overnight. The hot crucible with the dried sample was weighed (W_3) and the percentage dry matter content computed.

$$\text{Dry matter \%} = \frac{(W_3 + C_2) - (W_2 + C_1) * 100}{W_1}$$

Where W_1 = weight of air dried sample

W_2 = weight of empty crucible

W_3 = weight of crucible + oven dried sample

C_1 = correction for W_2 read from the balance (due to hot weighing)

C_2 = correction for W_3 read from the balance (due to hot weighing)

3.3.3 Crude protein determination

0.3 g ground dried leaf sample was weighed into a digestion tube. 1 g catalyst mixture of K_2SO_4 and anhydrous $CuSO_4$ was added in the ratio 10:1.5 ml conc. H_2SO_4 was then added and the tube placed in the digester with the temperature raised to 350 °C. The sample was digested for about an hour and a half or until the colour changed to light blue. The tube was removed from the block and allowed to cool. 30 ml of distilled water was carefully added to the digestion tube. The steam distillation procedure was then used to determine the ammonium in the digestion mixture. This

was done by first placing the digestion tube onto the Tecator steam distillation apparatus. The distiller was then set according to manufacturer's instruction. The digestion tube was inserted into the system and 150 ml of distillate collected into the receiver flask containing 25 ml 4% boric acid. The distillate was then titrated against a standard acid (0.1N HCl). The colour change was from green to steel-blue then pink with the end-point occurring when a drop of acid resulted in a colour change from steel-blue to light pink. The percentage nitrogen content computed and multiplied by 6.25 to give the percentage crude protein.

$$\% \text{ N} = \frac{14.01 * (V - B) * n}{W * 10 * DM\%}$$

$$\% \text{ Crude Protein} = N * 6.25$$

Where:

N = Nitrogen
V = volume of HCl consumed
B = blank titration
n = normality of HCl
W = weight of sample taken
DM% = Dry matter of sample

3.3.4 Determination of macronutrients

1.0 g of the plant sample was weighed into a silica crucible and heated to ash in a muffle furnace for 5 hours at 500–600 °C. The ash residue was dissolved in dilute HNO_3 and filtered through acid-washed filter paper in a 100 ml volumetric flask and the volume made up to the mark with distilled water. A blank digest were prepared of the same amounts of reagents to account for any contamination through the acids used in the digestion. The estimation of calcium (Ca) magnesium (Mg) and potassium

(K) was done using the atomic absorbance spectrometer (AAS). For sodium (Na) the flame photometer was used.

3.3.4.1 Determination of Ca, Mg and K using AAS

5 ml aliquot of the above extract was transferred into a 100 ml volumetric flask and made up to the volume. The sample solution was then atomised and the absorbance observed. The corresponding concentration for the absorbance recorded represented the content of Ca and Mg in the sample solution. The percentage calcium, magnesium or potassium was computed.

$$\text{Ca/Mg/ K (g) in 100g (\%Ca/ Mg/ K)} = \frac{C*df*100}{1000000} = \frac{C*2000*100}{1000000} = \frac{C}{5}$$

Where C = concentration of Ca (µg/ml) as read from the standard curve;
df = dilution factor, which is 100 × 20 = 2 000, as calculated below:
1 g of sample made to 100 ml (100 times);
5 ml of sample solution made to 100 ml (20 times).
1 000 000 = factor for converting µg to g.

3.3.4.2 Determination of phosphorus using spectophotometric vanadium phosphomolybdate method

5 ml of the digest was transferred into a 50-ml volumetric flask, and 10 ml of vanadomolybdate reagent added. The volume was made up to the mark with distilled water, and thoroughly mixed. It was then kept for 30 minutes for a yellow colour to develop and read on spectrophotometer at 420 nm. The percentage phosphorus was then computed as follows:

$$\text{P content (g) in 100g (\% P)} = \frac{C * df * 100}{1000000} = \frac{C * 1000 * 100}{1000000} = \frac{C}{10}$$

Where C = concentration of P (µg/ml) as read from the standard curve;

df = dilution factor, which is 100 × 10 = 1 000, as calculated below:

1 g of sample made to 100 ml (100 times);

5 ml of sample solution made to 50 ml (10 times).

1 000 000 = factor for converting µg to g.

3.3.5 Determination of micronutrients and lead (Pb)

0.3 g of dry Moringa leaf sample was weighed into a clean 125 ml 'pyrex' conical flask. 4 ml of concentrated H_2SO_4 was then added and the flask swirled carefully to ensure that the entire sample is wetted. The flask and its content were heated in a fume hood chamber on an electric sand bath plate set at medium heating. The flask was removed, cooled and about 10 drops of H_2O_2 added to the solution. 3-4 drops of H_2O_2 were added at a time again slowly to avoid vigorous reaction of the contents. The flask was swirled with the contents kept at the bottom of the flask and reheated avoiding excessive heating that causes spattering. The flask and its content were cooled and 6 drops of H_2O_2 added before reheating. Cooling and adding drops of H_2O_2 were done until there was a change in colour from black to dark brown. The temperature of the heater was then increased on the hot electronic sand-bath plate and cooling continued while adding 6 drops of H_2O_2 and heating. When the solution stayed colourless upon cooling, peroxide was added and left for the last time on the burner at high temperature for 10-15 minutes. The solution was cooled and contents transferred into a 100 ml volumetric flask and brought to the mark with distilled water, cooled and mixed thoroughly. The solution was then used to determine iron, manganese, zinc and lead levels. Two blank digests were prepared of the same

amounts of the reagents (i.e. H_2SO_4 and H_2O_2). All data were corrected against the mean blank value and the percentage metal content computed.

$$\% \text{ Metal} = \frac{(A-B) * V * 100}{1000 * 1000 * W}$$

Where; A = AAS reading
B = Blank reading
V = Volume of extract
W = Weight of sample

Table 3.1: The wavelength, slit and current at which the various metals read.

Metal	Wavelength (nm)	Slit	Current (mA)
Fe	248.33	1.8/1.5	30
Zn	213.86	2.7/1.8	15
Mn	279.48	1.8/0.6	20
Pb	283.31	2.7/1.05	10

3.4 Molecular Characterisation

3.4.1 Total protein studies

3.4.1.1 Total protein extraction

A modified protocol of Bio-Rad Laboratories (Philadelphia, USA) was used for electrophoretic studies of total protein variation at 10% sodium dodectyl sulphate (SDS) polyacrylamide gel. The collected accessions were screened for variations in the total protein fingerprints using fresh leaf samples. About 0.2 g of Moringa leaves

were washed with distilled water. The washed leaves were then macerated using ceramic mortar and pestle with 500 µl of Tris-citric buffer of pH 7.0 (extraction buffer). The macerated leaves were transferred into eppendorf tubes and centrifuged at 15000 rpm for six minutes. The supernatant were then collected and transferred into new eppendolf tubes.

3.4.1.2 Preparation of gel for electrophoresis

Polyacrylamide gel electrophoresis was used to separate the DNA samples studied. Two glass plates, a comb and Perspex spacers were prewashed in 6M HCl and rinsed several times in distilled water. The plate sets were blocked with plasticine to prevent the gel from leaking away until it solidified. The comb was inserted and 1cm below the comb marked with a marker. The plate set was then filled with a prepared separating gel (appendix 4g) to about 2 cm to the brim, topped with a few drops of n-butanol and allowed to polymerise in about 40 minutes. The n-butanol was then washed off with distilled water and the space left filled with the prepared stacking gel (figure 4d) almost to the brim. The comb was immediately inserted in the stacking gel and the gel allowed to polymerise for about 30 minutes.

3.4.1.3 Loading and running the electrophoresis

After the gel had set, the plate sets were removed from the plasticine and the gel assembly set placed in the electrophoresis tank. The electrode buffer pH 8.3 was poured into the bottom and the top compartments to make contact with the ends of the gel and form a circuit. The comb was removed and each well loaded with 15 µl of total protein extracts of an accession. The power leads were then connected to make an anionic contact (black lead) to the top and cathode contact (red lead) to the bottom.

The system was then run at constant voltage of 50V until the front/tracking dye moved into the separating gel. The voltage was adjusted to 80V and allowed to run until the front/tracking dye moved within 2mm to the bottom of the separating gel.

3.4.1.4 Staining and destaining the gel

The gel was removed from the plate and placed in a plastic tray and stained with Coomassie brilliant blue (appendix 3b) for about 2 hours. The gel was then destained by washing in destaining solution as (appendix 3c) until the background became clear with only the protein bands taking up the stain. The gel was then stored in 20% glycerol solution, photographed and the bands scored. The buffer systems, running conditions and staining procedures for total protein studies are shown in appendix 3 and 4.

3.4.2 Molecular genotyping using Random Amplified Polymorphic DNA (RAPD)

3.4.2.1 DNA extraction (D'neasy plant mini-kit)

About 0.2 g of plant material was ground under liquid nitrogen to a fine powder using mortar and pestle. The tissue powder and the liquid nitrogen were transferred into an eppendorf tube and the liquid nitrogen allowed to evaporate before the sample thawed. 400 µl of buffer AP1 and 4 µl of RNase A stock solution (100 mg/ml) was added to a maximum of 100 mg of ground plant tissue and vigorously vortexed. The mixture was incubated for 10 minutes at 65°C and was mixed 2-3 times during incubation by inverting tube. 130 µl of buffer AP2 was added to the lysate, mixed and incubated for 5 minutes on ice. The lysate in a 2 ml collection tube was centrifuged

for 2 minutes at a speed of 8000 rpm. The supernatant was carefully collected to a new tube without disturbing the cell-debris pellet. 255 µl buffer AP3 and 450 µl of ethanol (96-100%) was added to the 450 µl of clear lysate and mixed by pipetting. 650 µl of the mixture was then transferred to a new tube and centrifuged for a minute at ≥ 6000 g (i.e. ≥ 8000 rpm) and the supernatant collected and discarded. The process was repeated and the supernatant discarded. 500 µl of buffer AW was added to the precipitate and centrifuged for 1 minute at a speed ≥ 6000 x g (i.e. ≥ 8000 rpm) and the supernatant collected and discarded. 100 µl of preheated (65°C) buffer AE was pipetted and added to the precipitate. The mixture was incubated for 5 minutes at room temperature and then centrifuged for 1 minute at ≥ 6000 g (i.e. ≥ 8000 rpm) to elute. The elution process was repeated.

3.4.2.2 DNA quantification

DNA concentration and its purity were determined by measuring the optical density using the UV absorbance spectrophotometer. The quality of DNA was assessed on a 0.8 % (w/v) agarose gel electrophoresis that contains 0.5X tris borate EDTA (TBE) buffer. The DNA concentration was estimated with a Hoefer TKO 100 mini fluorometer. The intensity of orange florescence was also used for rough estimation of the concentration of DNA together with UV absorbance. The purity was measured by spectrophotometry.

3.4.2.3 Polymerase chain reaction and gel electrophoresis

PCR amplification conditions were: pre-denaturation at 94 ºC for 4 mins, 45 cycles of denaturation at 94 for 1 min, annealing at 34 ºC for 1 min, elongation step at 72 ºC for

2 mins. Final cycle at 72 ºC for 5 mins and final product was then cooled at 4 ºC. RAPD-PCR products were then analysed by electrophoresis on a 1.2% agarose gel with 1XTAE buffer and stained with 5 µl ethidium bromide. Power was supplied at 70V for 2 hours. DNA banding patterns were then visualized using Benchtop 3UV™ transilluminator (Cambridge, UK).

Table 3.2: List of the RAPD primers and their nucleotide sequences used in the study

Primer No.	Primer Code	Nucleotide Sequence (5' - 3')
1	OPC-08	TGGACCGGTA
2	OPC-04	CCGCATCTAC
3	OPC-07	CTCCCGACGA
4	OPC-16	CACACTCCAG
5	OPC-06	GAACGGACTC
6	OPC-13	AAGCCTCGTC
7	OPC-18	TGAGTGGGTG
8	OPC-15	GACGGATCAG
9	OPC-01	TTCGAGCCAG
10	OPC-19	GTTGCCAGCC

3.5 Data Analysis

3.5.1 Morphological data

Quantitative data obtained were analysed using the analysis of variance (ANOVA). All statistical comparisons were done at 5% significance level. Where the analysis of

variance indicated significant differences among treatments, least significance difference (L.S.D) was used to compare the treatment means. The standard errors and ranges were calculated from the mean values for each accession. Qualitative data obtained for the accessions were grouped based on their descriptor state. Qualitative data were scored on presence (1) and absence (0) basis. The data obtained were entered onto an excel sheet where characters were given codes using numbers and used to compute the similarity coefficient and to generate a dendrogram to reflect their relatedness using the NTSYS-pc version 2.2i, (Applied Biostatistics Inc, USA) and to generate a matrix determining similarity among samples according to Dice's coefficients. The similarity matrix was then used to draw a dendrogram with the clustering method UPGMA via the SAHN module in the software.

3.5.2 Biochemical Data

Biochemical data obtained were analysed using the analysis of variance (ANOVA) with all statistical comparisons done at 5% level of significance. Where the analysis of variance indicated significant differences among treatments, least significance difference (L.S.D) was used to compare the treatment means. The standard errors and ranges were calculated from the mean values for each accession. Graphs were also used to represent analysed data.

3.5.3 Molecular data

3.5.3.1 Total protein

For analysis of total protein data, the samples were genotyped for the presence and absence of bands as 1 and 0, respectively. The data were then used to draw a

dendrogram. NTSYS-pc version 2.2i, (Applied Biostatistics Inc, USA) was used to generate a matrix determining similarity among samples according to Dice's method. The similarity matrix was then used to draw a dendrogram with the clustering method UPGMA via the SAHN module in the software. Both total protein and RAPD data were combined and used to draw a dendrogram.

3.5.3.2 RAPD

Amplification products were scored for presence (1) or absence (0) of bands in a binary matrix at the same molecular weights. Where a PCR product was not obtained, data for specific locus and genotype were treated as missing data. Only polymorphic bands were included in the binary data set, and similarities were calculated using Dice coefficient (Dice 1945; Nei and Li, 1979)

$$\text{Dice's coefficient } (D_{ij}) = \frac{2a}{(2a+b+c)}$$

Where D_{ij} = similarity between two individuals i and j

a = number of bands present in both i and j,

b = present in i but not in j,

c = present in j but absent in i.

Cluster analyses using the UPGMA (unweighted pair-group method with arithmethic averages; Sokal and Michener, 1958) were carried out on the similarity matrices phenograms (dendrogram) constructed via the SAHN module using the software NTSYS-pc version 2.2i, (Applied Biostatistics Inc, USA).

CHAPTER FOUR

RESULTS

4.1 Morphological analysis

4.1.1 Variation in vegetative characteristics of the Moringa accessions

At six months after planting (MAP), mean tripinnatum leaf length ranged between 25.2 cm and 61.6 cm whilst the tripinnatum leaf width ranged between 17.8 cm and 48.5 cm (Table 4.1). For both parameters the minimum values were recorded on accessions collected from Ashanti region of Ghana while the maximum was recorded on accessions from the Upper East region. Tripinnatum leaflet length and tripinnatum leaflet width ranged from 10.8 cm to 25.9 cm and 5.7 cm and 17.6 cm respectively (Table 4.1). Accessions from Ashanti region had the lowest tripinnatum leaflet length and tripinnatum leaflet width with values of 10.8 cm and 5.7 cm respectively (Table 4.1). Accessions collected from Upper East had the highest tripinnatum leaflet length of 25.9 cm whilst those assembled from Greater Accra with a value of 17.6 cm had the maximum tripinnatum leaflet width. There were significant differences among the accessions of *Moringa oleifera* for the vegetative features determined in this study ($P \leq 0.05$, Appendices 6a, 6b, 6c and 6d).

Table 4.1: Vegetative characteristics of *Moringa oleifera* accessions.

Origin of accession	Mean vegetative features at 6 MAP ± SE (cm)			
	Tripinnatum leaf length	**Tripinnatum leaf width**	**Tripinnatum leaflet length**	**Tripinnatum leaflet width**
USA	54.4 ± 6.55	43.1 ± 5.37	23.9 ± 3.10	13.9 ± 1.55
AR	25.2 ± 2.70	17.8 ± 0.99	10.8 ± 0.58	5.7 ± 0.27
BA	37.8 ± 3.16	23.5 ± 2.40	14.4 ± 2.23	8.9 ± 1.14
ER	41.6 ± 6.91	29.9 ± 5.65	16.8 ± 2.99	9.2 ± 1.43
GA	50.3 ± 1.69	38.2 ± 3.97	20.1 ± 1.30	**17.6 ± 5.88**
INDIA	43.8 ± 1.79	33.0 ± 2.35	17.9 ± 1.29	9.6 ± 0.77
NR	34.7 ± 1.67	23.5 ± 2.58	14.9 ± 1.13	8.8 ± 0.46
UER	**61.6 ± 3.11**	**48.5 ± 5.90**	**25.9 ± 1.78**	13.3 ± 1.25
UWR	44.5 ± 2.83	34.4 ± 3.70	20.6 ± 1.74	12.6 ± 1.81
VR	37.3 ± 5.35	27.5 ± 4.88	14.9 ± 2.64	9.0 ± 1.16
L.s.d p< 0.05	**11.59**	**11.93**	**5.99**	**6.28**
%CV	**21.0**	**29.1**	**25.9**	**45.1**

Note; SE = standard error of the mean, minimum values are underlined whilst maximum values are bolded. AR= Ashanti region, BA = Brong Ahafo region, ER = Eastern region, GA = Greater Accra region, NR = Northern region, UER = Upper East region, UWR = Upper West region, VR = Volta region and USA = United States of America.

4.1.2. Qualitative attributes of the *Moringa oleifera* accessions

There was variation in the morphological traits in the *Moringa oleifera* accessions studied (Table 4.2). Some of the variations observed were in traits such as colour of leaf, petiole pigmentation and stem bark colour of the accessions. Leaf colour varied from light green to dark green. Most of the accessions had purple petioles, others petioles were dark purple and a few were green in colour. Four accessions showed a growth habit of intermediate between erect and compact with two accessions exhibiting compactness. The remaining accessions exhibited erect growth form. Variations in the stem bark colour of the accessions comprised whitish/ silvery, green and dark brown. Three of the accessions had a whitish bark colour with an accession exhibiting a brown colour. In the case of leaf type, leaf arrangement, leaflet shape and leaflet arrangement the accessions appeared to be monomorphic for the traits.

Table 4.2: Qualitative attribute of the *Moringa oleifera* accessions

ID	Leaf colour	Leaf type	Leaf arrangement	Leaflet shape	Leaflet arrangement	Petiole pigmentation	Growth habit	Bark Colour
AS01	Light green	Compound	Alternate	Compound	Opposite	Purple	Erect	Green
AS02	Light green	Compound	Alternate	Compound	Opposite	Purple	Erect	Dark brown
BA01	Dark green	Compound	Alternate	Compound	Opposite	Dark Purple	Erect	Whitish
BA02	Dark green	Compound	Alternate	Compound	Opposite	Purple	Intermediate	Green
BNR04	Dark green	Compound	Alternate	Compound	Opposite	Purple	Erect	Green
BNR05	Dark green	Compound	Alternate	Compound	Opposite	Purple	Erect	Green
BNR06	Light green	Compound	Alternate	Compound	Opposite	Purple	Intermediate	Green
BNR07	Light green	Compound	Alternate	Compound	Opposite	Dark Purple	Erect	Green
BNR08	Light green	Compound	Alternate	Compound	Opposite	Purple	Erect	Green
BNR09	Dark green	Compound	Alternate	Compound	Opposite	Purple	Erect	Dark brown
BNR10	Dark green	Compound	Alternate	Compound	Opposite	Purple	Intermediate	Dark brown
BNR11	Dark green	Compound	Alternate	Compound	Opposite	Dark Purple	Erect	Green
BNR12	Dark green	Compound	Alternate	Compound	Opposite	Purple	Erect	Green
BNR13	Light green	Compound	Alternate	Compound	Opposite	Dark Purple	Erect	Green
BNR14	Light green	Compound	Alternate	Compound	Opposite	Purple	Erect	Green
BNR15	Dark green	Compound	Alternate	Compound	Opposite	Dark Purple	Erect	Green
E02	Light green	Compound	Alternate	Compound	Opposite	Green	Erect	Dark brown
E04	Light green	Compound	Alternate	Compound	Opposite	Purple	Erect	Brown
E05	Dark green	Compound	Alternate	Compound	Opposite	Dark Purple	Erect	Dark brown
E10	Dark green	Compound	Alternate	Compound	Opposite	Purple	Compact	Green
E11	Dark green	Compound	Alternate	Compound	Opposite	Purple	Compact	Whitish

Table 4.2: Continued qualitative attribute of the *Moringa oleifera* accessions

ID	Leaf colour	Leaf type	Leaf arrangement	Leaflet shape	Leaflet arrangement	Petiole pigmentation	Growth habit	Bark Colour
GA02	Light green	Compound	Alternate	Compound	Opposite	Green	Erect	Green
GA03	Light green	Compound	Alternate	Compound	Opposite	Green	Erect	Dark brown
GA04	Light green	Compound	Alternate	Compound	Opposite	Purple	Erect	Whitish
GA07	Dark green	Compound	Alternate	Compound	Opposite	Dark Purple	Erect	Dark brown
GA10	Dark green	Compound	Alternate	Compound	Opposite	Dark Purple	Erect	Dark brown
GA15	Light green	Compound	Alternate	Compound	Opposite	Purple	Erect	Dark brown
N03	Light green	Compound	Alternate	Compound	Opposite	Purple	Erect	Green
N05	Light green	Compound	Alternate	Compound	Opposite	Purple	Erect	Dark brown
N08	Light green	Compound	Alternate	Compound	Opposite	Purple	Erect	Dark brown
UE02	Dark green	Compound	Alternate	Compound	Opposite	Purple	Erect	Green
UE05	Dark green	Compound	Alternate	Compound	Opposite	Purple	Intermediate	Dark brown
UW01	Dark green	Compound	Alternate	Compound	Opposite	Purple	Erect	Dark brown
UW02	Dark green	Compound	Alternate	Compound	Opposite	Purple	Erect	Dark brown
V01	Dark green	Compound	Alternate	Compound	Opposite	Dark Purple	Erect	Dark brown
V02	Dark green	Compound	Alternate	Compound	Opposite	Dark Purple	Erect	Green

4.1.3. Cluster analysis for Qualitative attributes of the *Moringa oleifera* accessions

Cluster analysis resolved the qualitative data for the *Moringa oleifera* accessions into two major clusters A and B at a similarity level of 70% (figure 4.1). Major cluster A contained sixteen accessions; AS01, BNR 08, BNR 14, N03, AS02 GA15, N05, N08, E04, GA04, BNR06, BNR07, BNR13, GA02, E02 and GA03. Cluster A further separated into two sub-clusters at 78% similarity level with accession E02 and GA03 separating themselves from the rest of the accessions in major cluster A. Major cluster B contained; BA01, E05, GA07, GA10, V01, BNR11, BNR15, C05, V02, BA02, BNR04, BNR05, BNR12, UE02, E10, BNR09, UW01, UW02, BNR10, UE05 and E11. It further separated at 75% similarity level into two sub-clusters containing BA01, E05, GA07, GA10, V01, BNR11, BNR15 and V02 in one group and BA02, BNR04, BNR05, BNR12, UE02, E10, BNR09, UW01, UW02, BNR10, UE05 and E11 in the other group. Accessions AS01, BNR 08, BNR 14 and N03; AS02 GA15, N05 and N08; BNR07 and BNR13; E02 and GA03 in major cluster A and E05, GA07, GA10 and V01; BNR11, BNR15 and V02; BNR04, BNR05, BNR12 and UE02; BNR09, UW01 and UW02; BNR10 and UE05 in major cluster B could not separate at 100% similarity level suggesting that they are duplicates or they are the same based on the traits studied.

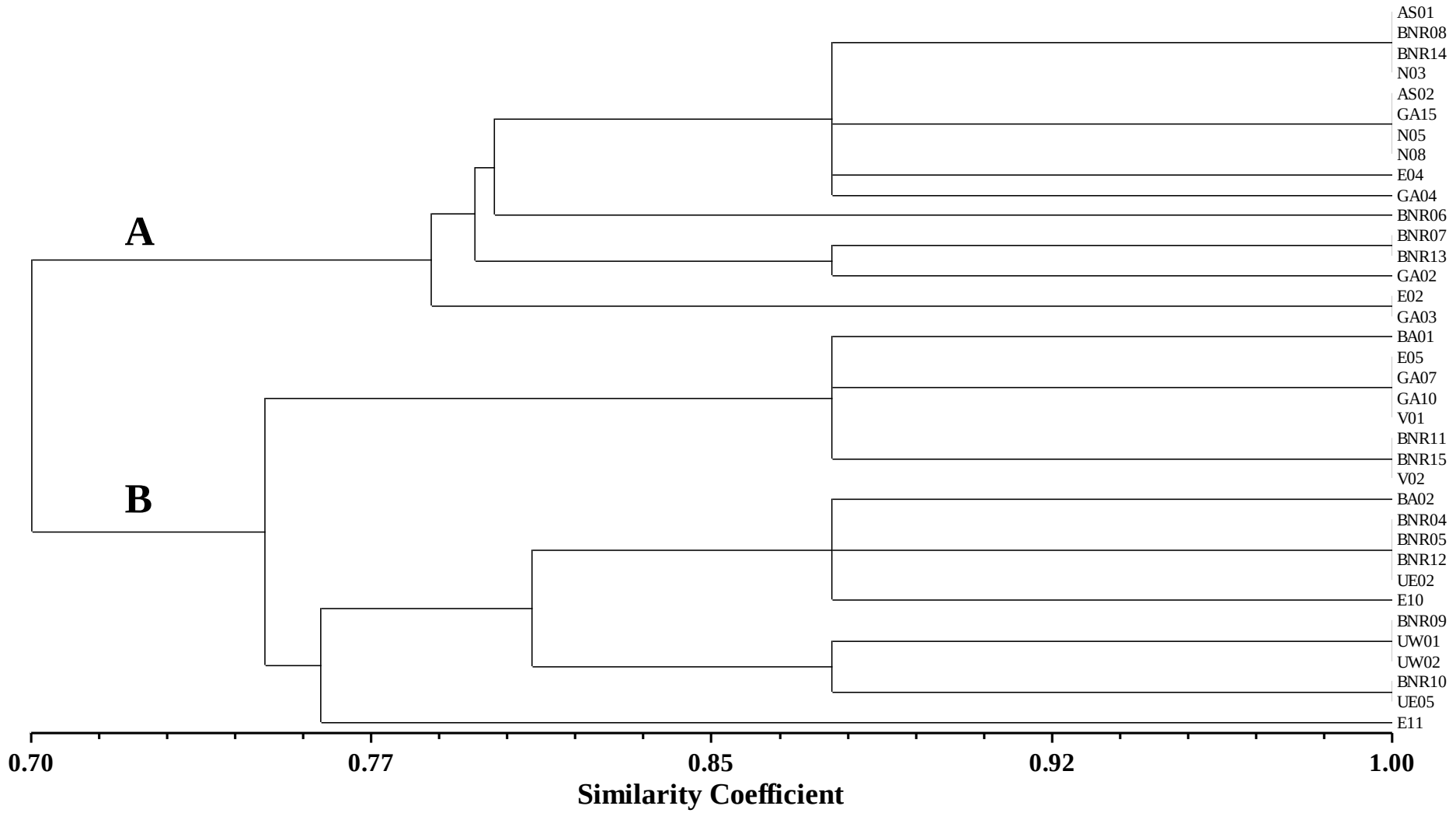

Fig 4.1: Dendrogram generated by UPGMA cluster analysis showing the relationship among Moringa accessions based on Dice similarity from qualitative data.

4.2. Biochemical analysis

4.2.1 Concentration of micro minerals in the dried leaf powder of *Moringa oleifera* accessions

Table 4.3 shows the levels of micro-minerals such as iron (Fe), manganese (Mn) and zinc (Zn) in the dried leaf samples of *Moringa oleifera* accessions collected from different locations. Fe content in the dried leaf samples ranged from 0.0014% to 0.0736%. The accessions from Upper West region had the lowest Fe content and those from India had the highest. The concentration of Mn detected in the samples varied from 0.0006% to 0.0097%. Accessions from Upper East region had the minimum amount of Mn whereas those from India gave the highest concentration of the mineral element. The accessions collected from Northern region had the lowest concentration of Zn with a value of 0.0005% and the highest quantity (0.0111%) obtained from accessions assembled from India. There were significant differences among the accessions of *Moringa oleifera* studied for concentration of micro-minerals ($P \leq 0.05$, Appendices 7a, 7b and 7c). The quantity of heavy metal lead (Pb) detected in the dried leaf samples ranged from 0.0005% to 0.0056%. Accessions from USA had the lowest Pb concentration whilst those from India had the highest concentration of the metal. There was a significant difference among the accessions examined ($P \leq 0.05$, Appendix 7d).

Table 4.3: Micro mineral composition in the dried leaf of *Moringa oleifera* accessions

Origin of accession	**Mean Micro Mineral Composition (%) ± SE**			
	Fe (%)±SE	**Mn (%)±SE**	**Zn (%)±SE**	**Pb (%)±SE**
USA	0.0033 ± 0.00017	0.0011 ± 0.00031	0.0006 ± 0.00006	0.0005 ± 0.00006
ER	0.0039 ± 0.00054	0.0007 ± 0.00006	0.0031 ± 0.00245	0.0019 ± 0.00020
GR	0.0044 ± 0.00012	0.0008 ± 0.00025	0.0006 ± 0.00009	0.0016 ± 0.00009
India	**0.0736 ± 0.00202**	**0.0097 ± 0.00088**	**0.0111 ± 0.00065**	**0.0056 ± 0.0007**
NR	0.0027 ± 0.00043	0.0007 ± 0.00003	0.0005 ± 0.00010	0.0013 ± 0.00015
UER	0.0044 ± 0.00123	0.0006 ± 0.00006	0.0007 ± 0.00003	0.0010 ± 0.00007
UWR	0.0014 ± 0.00003	0.0007 ± 0.00036	0.0005 ± 0.00003	0.0008 ± 0.00006
VR	0.0026 ± 0.00026	0.0010 ± 0.00019	0.0008 ± 0.00006	0.0027 ± 0.00031
L.s.d (p< 0.05)	**0.0025**	**0.0010**	**0.0027**	**0.0008**
%CV	**12**	**31**	**68.9**	**23.7**

Note: SE = standard error of the mean, minimum values are underlined whilst maximum values are bolded. ER = Eastern region, GA = Greater Accra region, NR = Northern region, UER = Upper East region, UWR = Upper West region, VR = Volta region and USA = United States of America. Conversion rate: 1% = 10000 ppm; 1ppm =1 mg/kg

4.2.2. Concentration of macro minerals in the dried leaf powder of *Moringa oleifera* accessions

Table 4.4 shows the mean macro mineral composition of dried leaf of *Moringa oleifera* accessions collected from the various provenances. Samples from USA had the lowest concentration of Ca (40 mg/kg) whilst those accessions from Northern region of Ghana had the highest Ca concentration of 79.3 mg/kg. The lowest quantity of Mg detected was from Volta region (19.23 mg/kg) accessions whilst accessions from Northern region had the highest quantity of Mg of 48.13 mg/kg. The Na content of the *Moringa oleifera* samples ranged from 87.5 mg/kg to 253.1 mg/kg (Table 4.4). Accessions from Volta region had the lowest Na concentration whilst those from Greater Accra region had the highest level of Na in the dried leaves of Moringa. The accessions from the Upper East had the lowest concentration of 1549 mg/kg of potassium (K) in the dried leaves sample of Moringa whilst Upper West region had the highest K concentration of 2272 mg/kg. The phosphorus concentration in the accessions ranged from 0.179 mg/kg recorded in the accessions from India to 0.438 mg/kg in accessions from Greater Accra region as shown in Table 4.4. There were significant differences between the accessions for the macro minerals studied except potassium concentration as shown in appendices 8a, 8b 8c, 8d and 8e.

Table 4.4: Macro mineral composition of dried leaf of *Moringa oleifera* accessions

Origin of accession	Mean Macro Mineral Composition (mg/kg DM) ± SE				
	Ca	**Mg**	**Na**	**K**	**P**
USA	40.3 ± 3.23	22.67 ± 1.19	185.9 ± 8.88	2155 ± 175.21	0.183 ± 0.017
ER	44.5 ± 4.08	26.87 ± 2.12	128.1 ± 3.25	1868 ± 194.13	0.396 ± 0.006
GR	48.2 ± 2.39	26.70 ± 1.21	**253.1 ± 14.06**	2131 ± 279.31	**0.438 ± 0.033**
India	44.2 ± 2.14	20.60 ± 1.69	191.7 ± 1.59	2074 ± 62.65	0.179 ± 0.004
NR	**79.3 ± 1.98**	**48.13 ± 1.85**	184.4 ± 10.17	1700 ± 211.81	0.271 ± 0.039
UER	53.8 ± 4.89	23.57 ± 1.17	192.2 ± 3.13	1549 ± 35.04	0.304 ± 0.009
UWR	41.3 ± 1.21	20.8 ± 1.65	167.2 ± 13.10	**2272 ± 394.42**	0.183 ± 0.010
VR	50.3 ± 4.27	19.23 ± 1.04	87.5 ± 6.51	1162 ± 93.72	0.267 ± 0.024
L.s.d (p< 0.05)	**9.84**	**4.88**	**39.12**	**ns**	**0.1174**
%CV	**11.2**	**10.7**	**12.9**		**24.1**

Note: SE = standard error of the mean, ns = not significant, minimum values are underlined whilst maximum values are bolded. ER = Eastern region, GA = Greater Accra region, NR = Northern region, UER = Upper East region, UWR = Upper West region, VR = Volta region and USA = United States of America.

4.2.3 Percentage crude protein and percentage dry matter of dried leaf samples of *Moringa oleifera* accessions.

Percentage nitrogen of *Moringa oleifera* accessions studied ranged from 2.06% to 4.21%. The lowest percentage nitrogen was observed in the accession from USA and the highest in the accessions from Eastern region (figure 4.2). Significant differences existed between the provenances studied for percentage nitrogen (appendix 9a). The percentage crude protein of *Moringa oleifera* accessions studied showed that

accessions from the eastern region contained the highest crude protein value of 26.3% whilst the lowest value of 12.9% occurred in accessions from USA (figure 4.2). However, the accessions were not significantly different from each other with regard to percentage crude protein (appendix 9b). The percentage dry matter content of the accessions ranged from 14.63% to 25.37% (figure 4.2). The lowest was recorded in accessions from the Upper West region of Ghana and the greatest from the accessions from USA. There were significant differences in percentage dry matter of the accessions studied (appendix 9c).

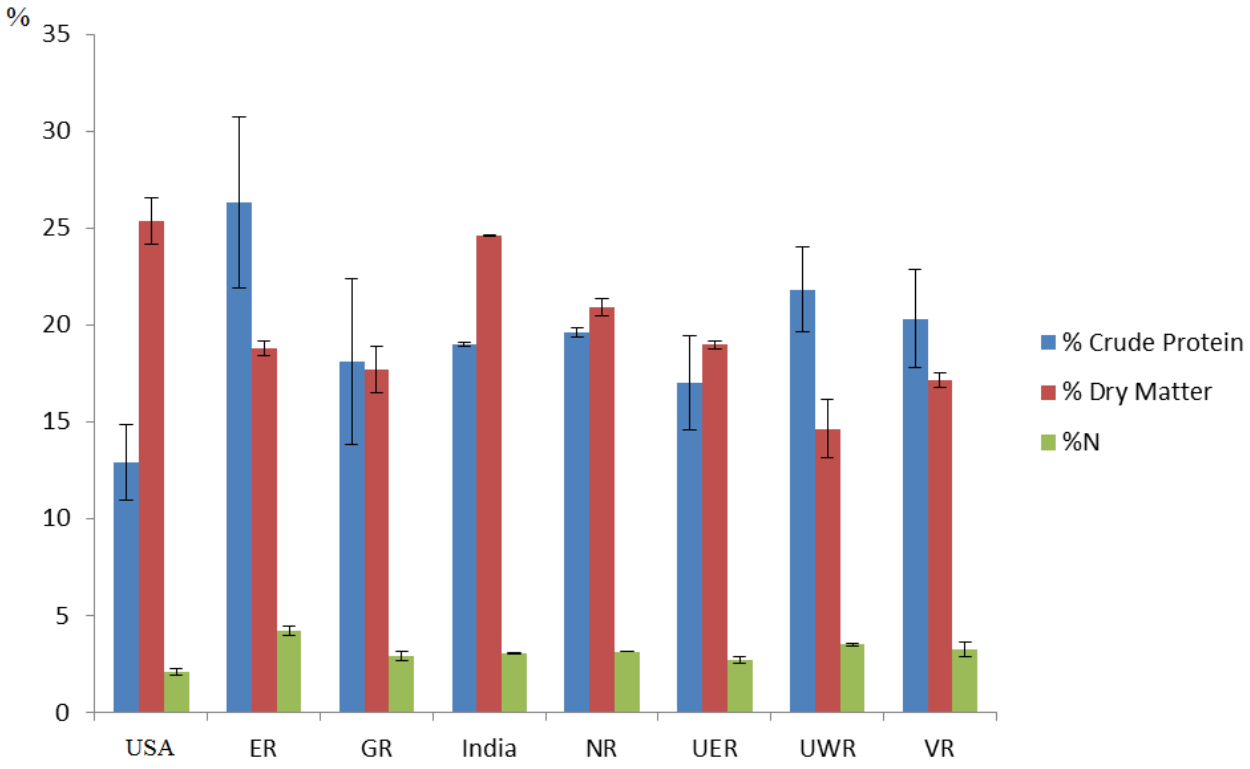

Fig 4.2: Percentage crude protein and dry matter of dried leaves of *M. oleifera* accessions.

Table 4.5: Nutrient composition per 100 g of dried Moringa leaves and USDA-RDI

Origin of Accession		Nutrient per 100g of dried Moringa leaves (mg)						
		Fe	Mg	Mn	P	Zn	K	Ca
USA		3.30	2.27	1.10	0.018	0.60	215.50	4.03
ER		3.90	2.69	0.70	0.040	3.10	186.80	4.45
GR		4.40	2.67	0.80	0.044	0.60	213.10	4.82
India		73.60	2.06	9.70	0.018	11.10	207.40	4.42
NR		2.70	4.81	0.70	0.027	0.50	170.00	7.93
UER		4.40	2.36	0.60	0.030	0.70	154.90	5.38
UWR		1.40	1.92	0.70	0.018	0.50	227.20	4.13
VR		2.60	2.08	1.00	0.027	0.80	116.20	5.03
Mean conc.		12.04	2.61	1.91	0.028	2.24	186.39	5.02
RDI	Male (mg/d)	8.0	400.0	2.3	700.0	11.0	4700.0	1000.0
	Female (mg/d)	18.0	310.0	1.8	700.0	8.0	4700.0	1000.0

Source: USDA National Nutrient Database for Standard Reference, Release 24

Note: ER = Eastern region, GA = Greater Accra region, NR = Northern region, UER = Upper East region, UWR = Upper West region, VR = Volta region and USA = United States of America.

4.3 Molecular characterisation of *Moringa oleifera* leaf samples

Forty (40) *Moringa oleifera* accessions from Upper East, Upper West, Northern, Eastern, Volta and Greater Accra regions of Ghana, United States of America (USA) and India were electrophoresed to determine the variation existing at the biochemical and genotypic level. Variations were observed in the accessions studied for both total protein and RAPDs markers.

4.3.1 Total Protein

A total of eleven (11) prominent bands were resolved. Two out of the forty accessions, namely E40 and E41 showed all the eleven bands. The bands ranged from a distance of 0.1cm to 3.9cm with a single band at distance of 1.2 cm which was common to all the forty (40) accessions electrophoresed (Figures 4.3a, 4.3b, 4.3c, 4.3d). A sample photograph of the gel is shown in plate 1.

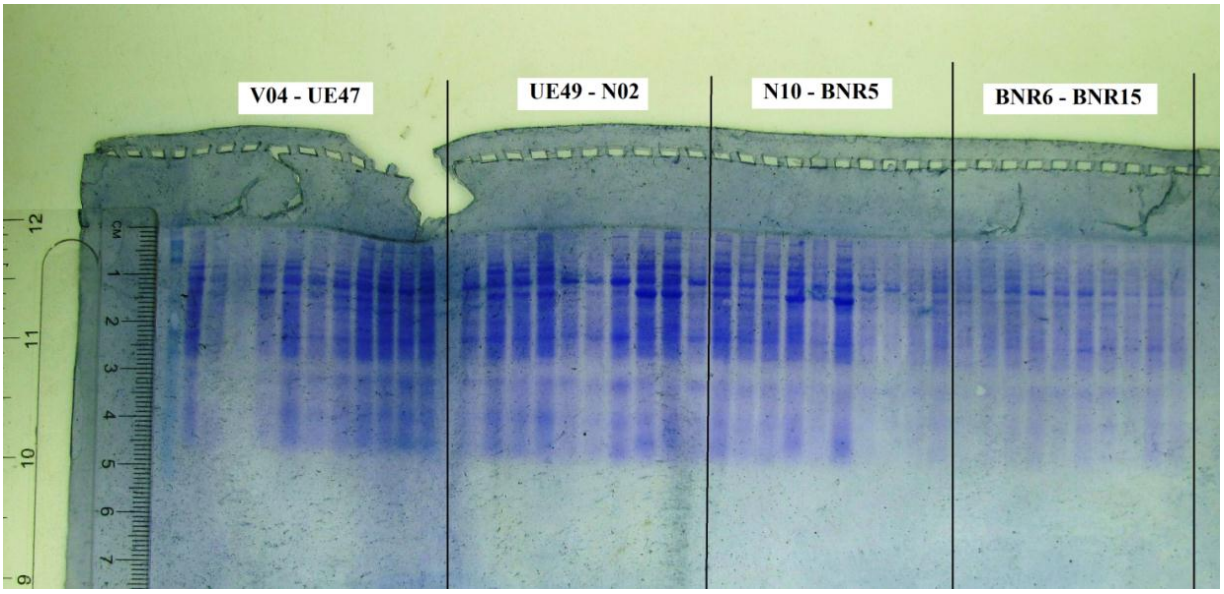

Plate 1: Total Protein Gel of 40 *Moringa oleifera* accessions collected from different parts of the country.

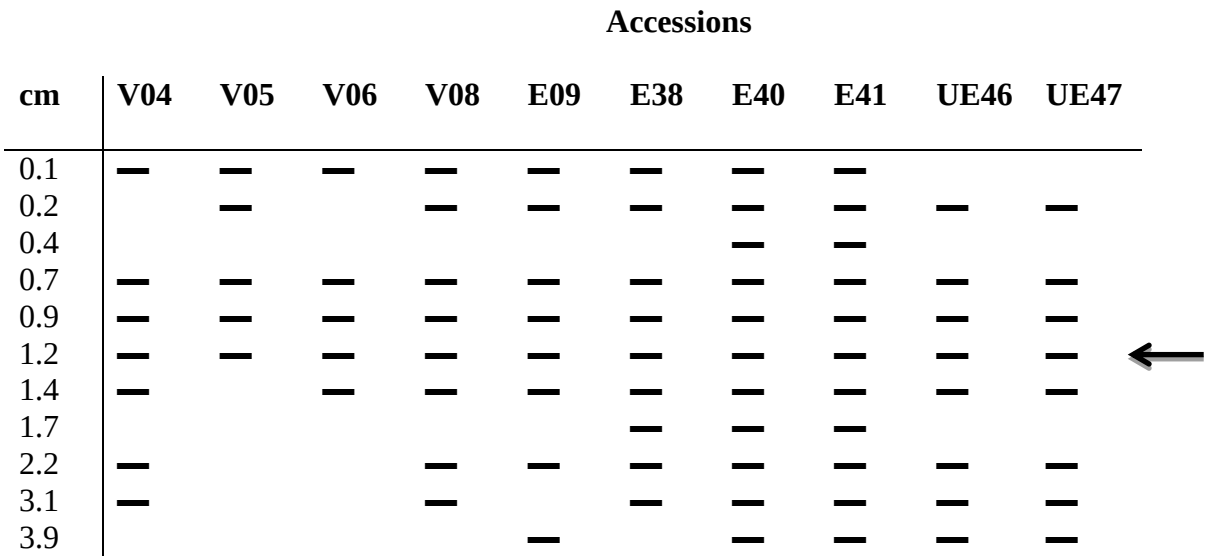

Fig 4.3a: Schematic diagram showing the total protein banding pattern of the 10 Moringa oleifera accessions from Volta, Eastern and Upper east region.

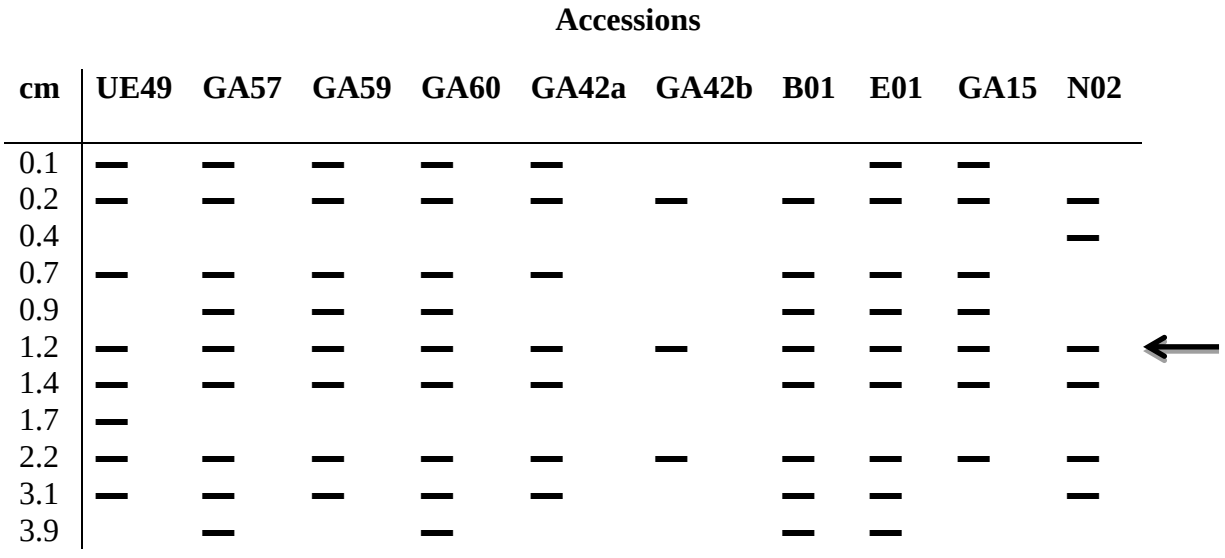

Fig 4.3b: Schematic diagram showing the total protein banding pattern of the 10 *Moringa oleifera* accessions from Upper East, Greater Accra, Brong Ahafo, Eastern and Northern region.

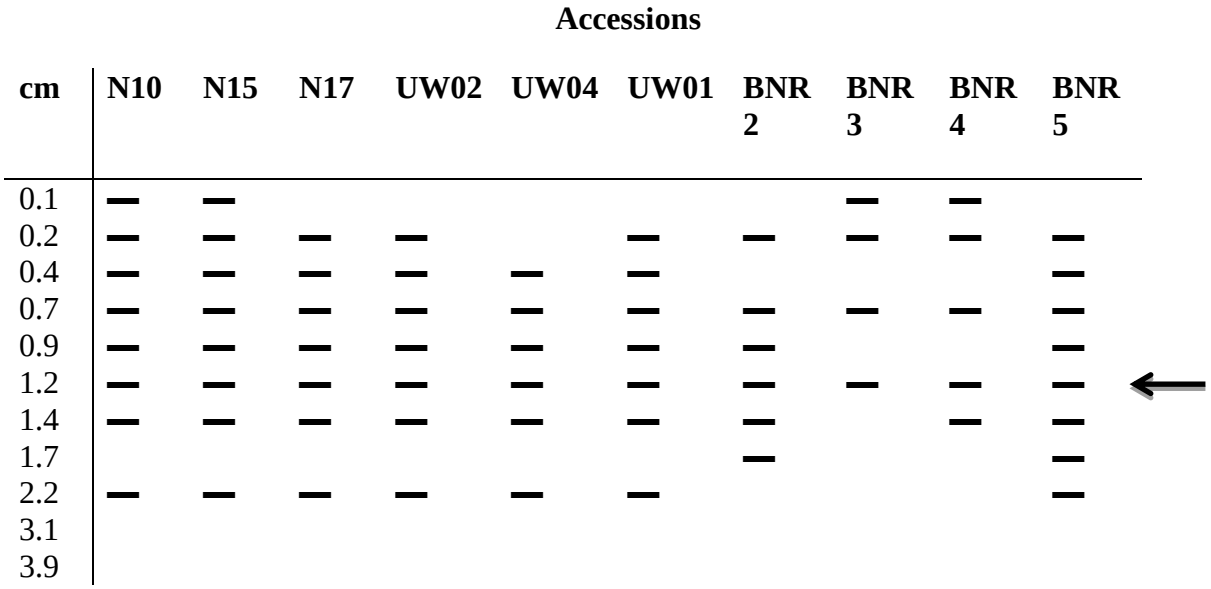

Fig 4.3c: Schematic diagram showing the total protein banding pattern of the 10 *Moringa oleifera* accessions from Northern region, Upper West region and BNARI.

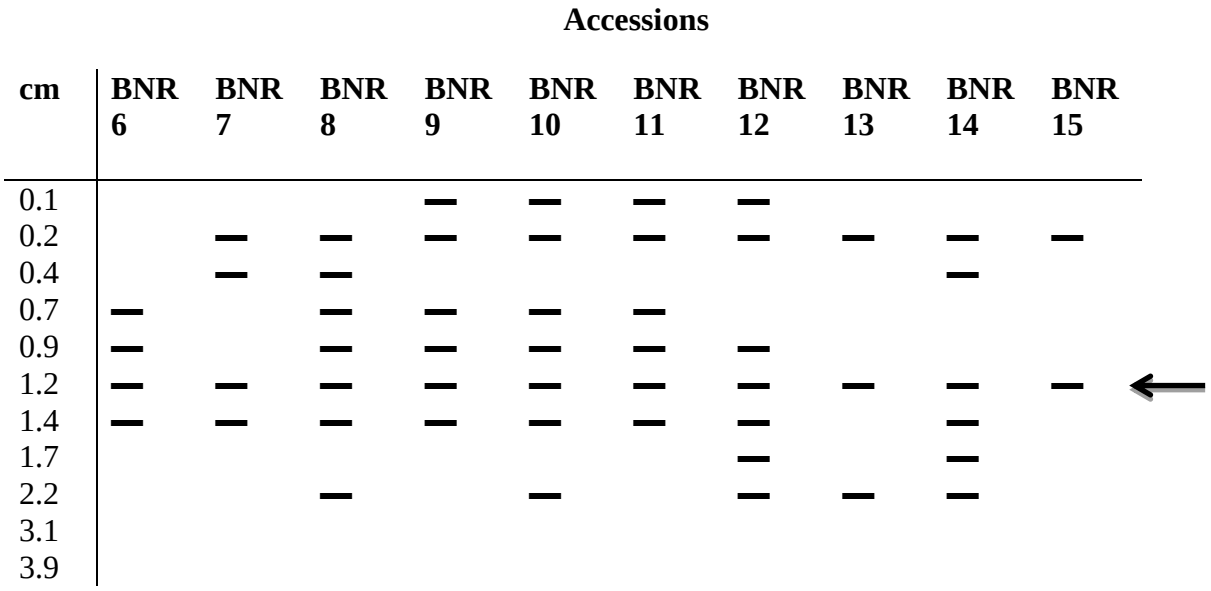

Fig 4.3d: Schematic diagram showing the total protein banding pattern of the 10 *Moringa oleifera* accessions from BNARI.

← Monomorphic locus

4.3.2 Total protein cluster analysis

The dendrogram generated from total protein profile for the forty (40) *Moringa oleifera* accessions is shown in figure 4.4. The cluster analysis resolved the forty accessions into two major clusters (i.e. A and B) at a similarity level of 54%. Major cluster A contained thirty four (34) accessions; V04, V08, GA59, E38, UE49, GA42a, E09, GA57, GA60, E01, UE46, UE47, B01, E40, E41, N17, UW02, UW01, BNR8, UW04, N10, N15, BNR5, BNR2, BNR12, V05, BNR3, BNR4, BNR9, BNR11, GA15, BNR10, V06 and BNR6. Cluster A further separated into two sub-clusters at 73% similarity level with accessions V04, V08, GA59, E38, UE49, GA42a, E09, GA57, GA60, E01, UE46, UE47, B01, E40, E41, N17, UW02, UW01, BNR8, UW04, N10, N15, BNR5, BNR2 and BNR12 in one set and V05, BNR3, BNR4, BNR9, BNR11, GA15, BNR10, V06 and BNR6 in the other set.

Major cluster B contained; N02, BNR14, BNR7, GA42b, BNR13 and BNR15. It further separated at 61% similarity level into two sub-clusters containing N02, BNR14 and BNR7 in one group and GA42b, BNR13 and BNR15 in the other. The following groups of accessions from major cluster A could not be separated from each other at 100% similarity V08 and GA59; GA57, GA60 and E01; UE46, UE47 and BO1; E40 and E41; N17, UW02, UW01 and BNR 8; N10 and N15; BNR 9 and BNR 11; GA15 and BNR10. Similarly accessions GA42b and BNR 13 in major cluster B could not separate at 100% similarity level suggesting that they are very closely related or duplicate accessions.

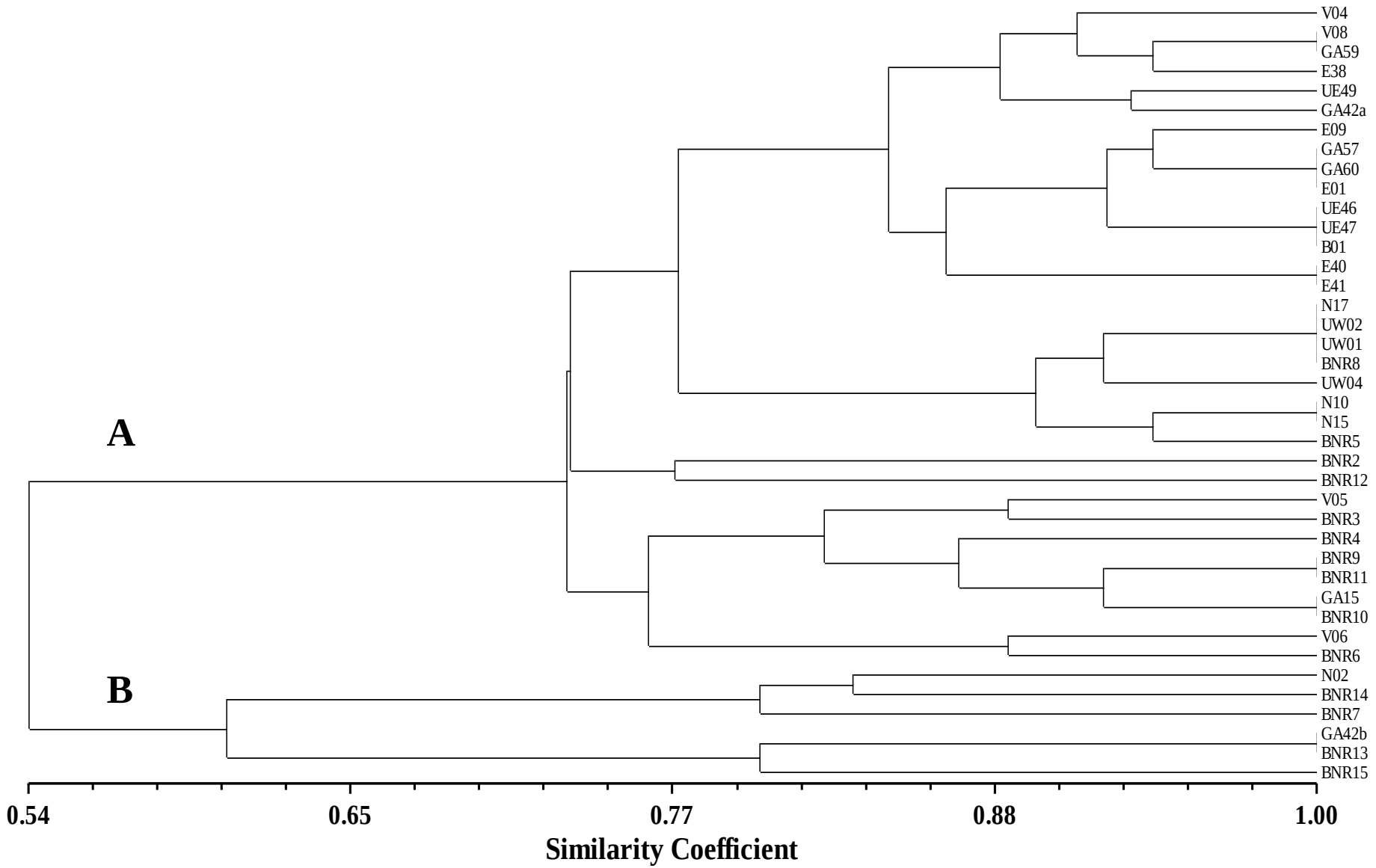

Fig 4.4: Dendogram generated by UPGMA cluster analysis showing the relationship among forty (40) Moringa accessions based on Dice similarity from Total protein data

4.3.3 RAPD markers

10 out of the 12 RAPD markers used generated visible bands. A total of 82 prominent polymorphic RAPD fragments were generated. The number of amplified loci ranged from 7 to 10. Of the 10 primers used, the number of RAPD loci generated was highest for the primers OPC-07 and OPC-06 which amplified 10 fragments each as shown in table 4.5. The lowest number of bands was produced by the primers OPC-08, OPC-04, OPC-16 and OPC-19 with seven loci each (table 4.5). The average number of fragments and percentage polymorphic bands produced per primer were 8.2 and 95.1 respectively. A representative profile of RAPD products amplified with primer OPC-08 from all 40 *Moringa oleifera* accessions is shown in plate 2.

Table 4.6: List of primers and their sequences used for the RAPD analysis and corresponding number of bands generated.

Primer No.	Primer code	Sequence (5' – 3')	Total No. of bands	Polymorphic bands		Monomorphic bands	
				No.	%	No.	%
1	OPC-08	TGGACCGGTA	7	7	100	_	_
2	OPC-04	CCGCATCTAC	7	7	100	_	_
3	OPC-07	CTCCCGACGA	10	9	90	1	10
4	OPC-16	CACACTCCAG	7	7	100	_	_
5	OPC-06	GAACGGACTC	10	8	80	2	20
6	OPC-13	AAGCCTCGTC	9	9	100	_	_
7	OPC-18	TGAGTGGGTG	8	8	100	_	_
8	OPC-15	GACGGATCAG	8	8	100	_	_
9	OPC-01	TTCGAGCCAG	9	9	100	_	_
10	OPC-19	GTTGCCAGCC	7	6	85.7	1	14.3
Total	_	_	**82**	**78**	**95.1**	**4**	**4.9**

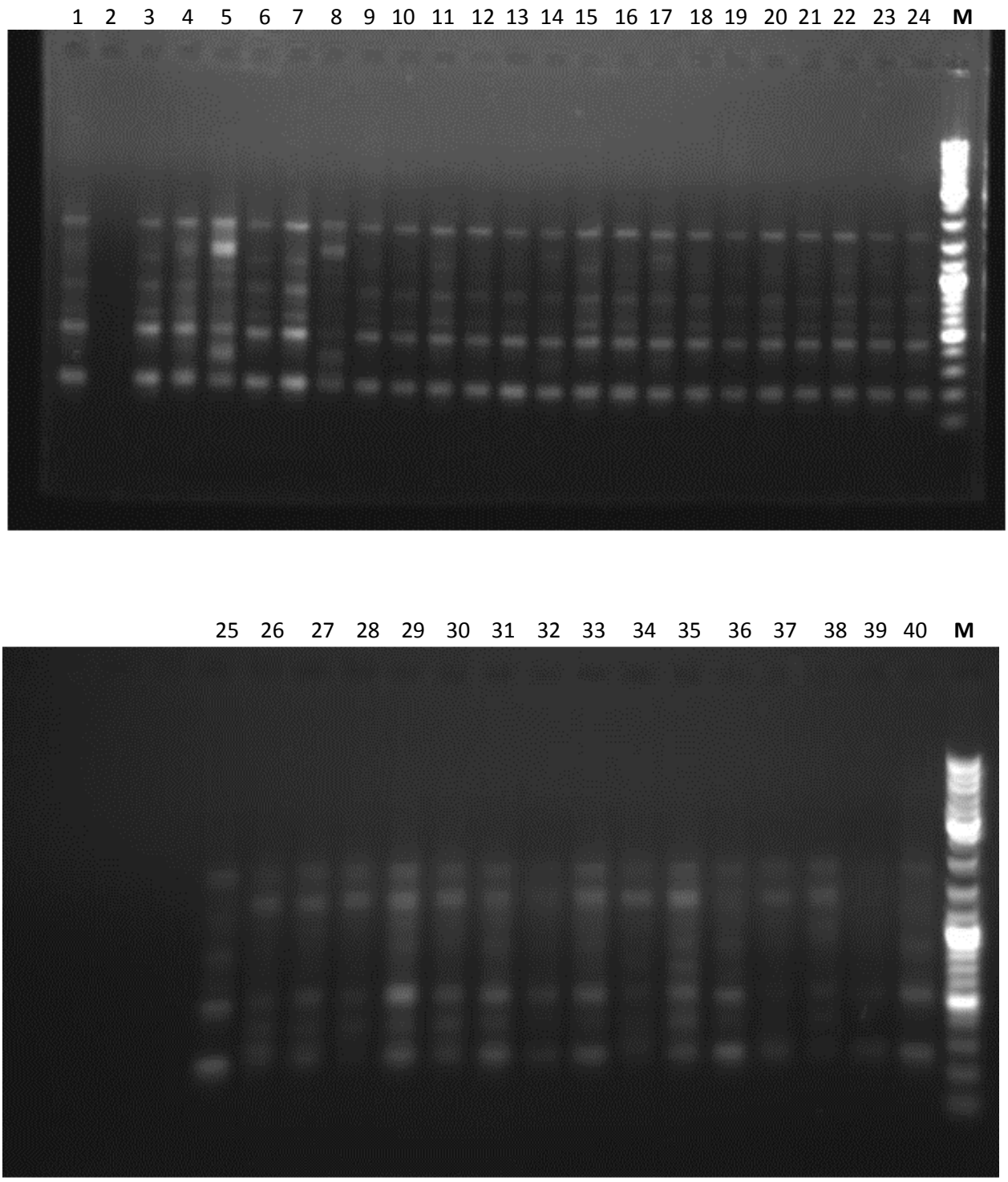

Plate 2: Sample of DNA profile generated by OPC-01; lanes 1-40 corresponding to the code of the 40 *Moringa oleifera* accessions in appendix 2. M - GeneRuler DNA ladder Mix (Fermentas)

4.3.4. RAPD analysis of 40 *Moringa oleifera* accessions

The dendrogram generated for the RAPD data for the *Moringa oleifera* accessions was resolved into two major clusters A and B at a similarity coefficient of 30% as shown in figure 4.5. Major cluster A contained all the accessions analysed except BNR 4 which was in cluster B. Major cluster A further separated into two sub-clusters at 40% similarity level with accessions N17 separating itself from the rest of the accessions. Accessions E41 and UE49 in major cluster A could not separate at 91% similarity coefficient suggesting that they may be very closely related or the same.

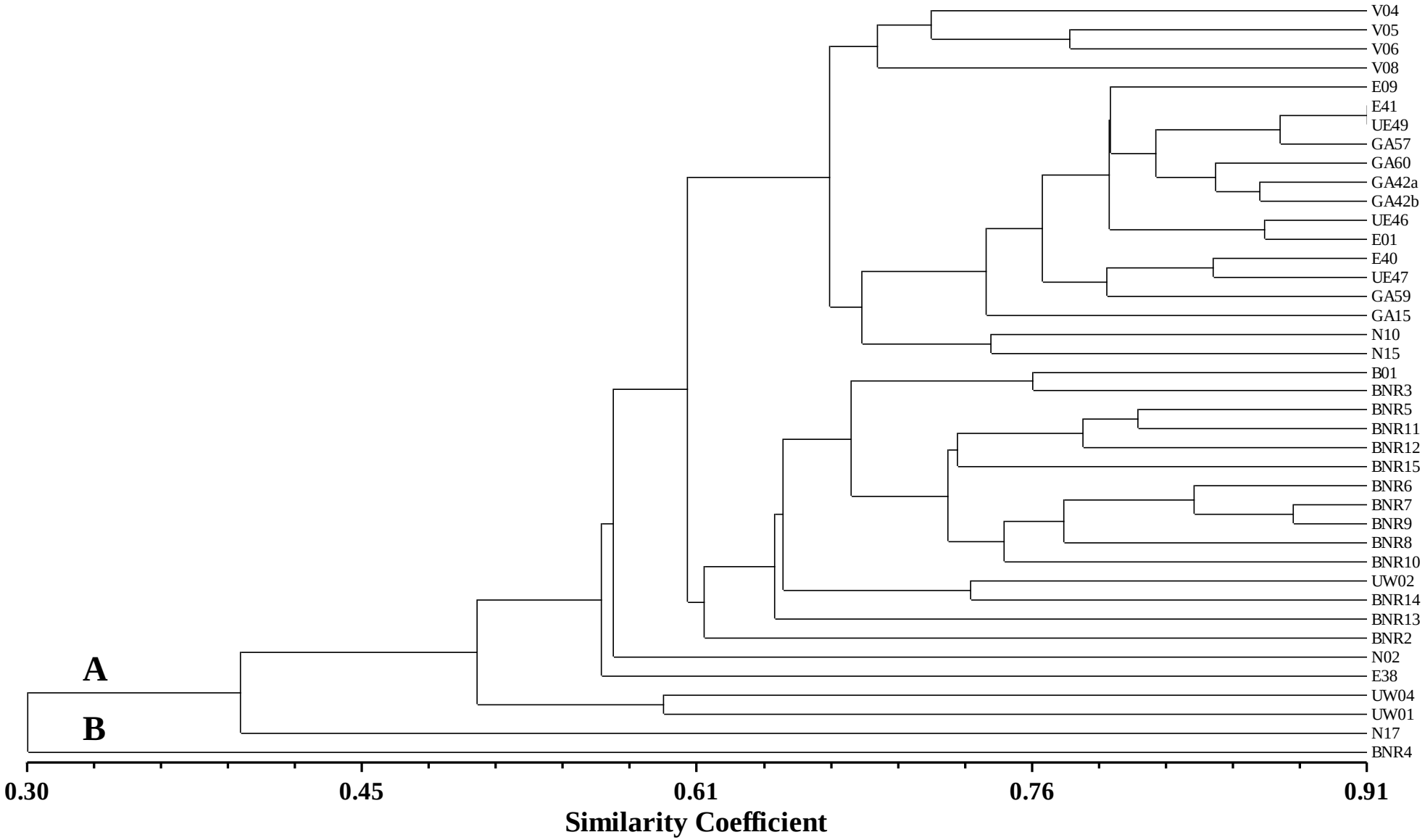

Fig 4.5: Dendrogram generated by UPGMA cluster analysis showing the relationship among 40 Moringa accessions based on Dice similarity from RAPD data

4.3.5 Combination of Total protein and RAPD analysis of 40 *Moringa oleifera* accessions

The cluster analysis generated for the combination of Total protein and RAPD data for the *Moringa oleifera* accessions was resolved into two major clusters A and B at a similarity coefficient of 37% (figure 4.6). Major cluster A comprised all the accessions apart from accession BNR 4 which was in major cluster B. Major cluster A further separated into two sub-clusters at 46% similarity level with accessions N17 separating itself from the rest of the accessions. Accessions E41 and UE49 in major cluster A could not separate at 90% similarity coefficient suggesting that they may be closely related.

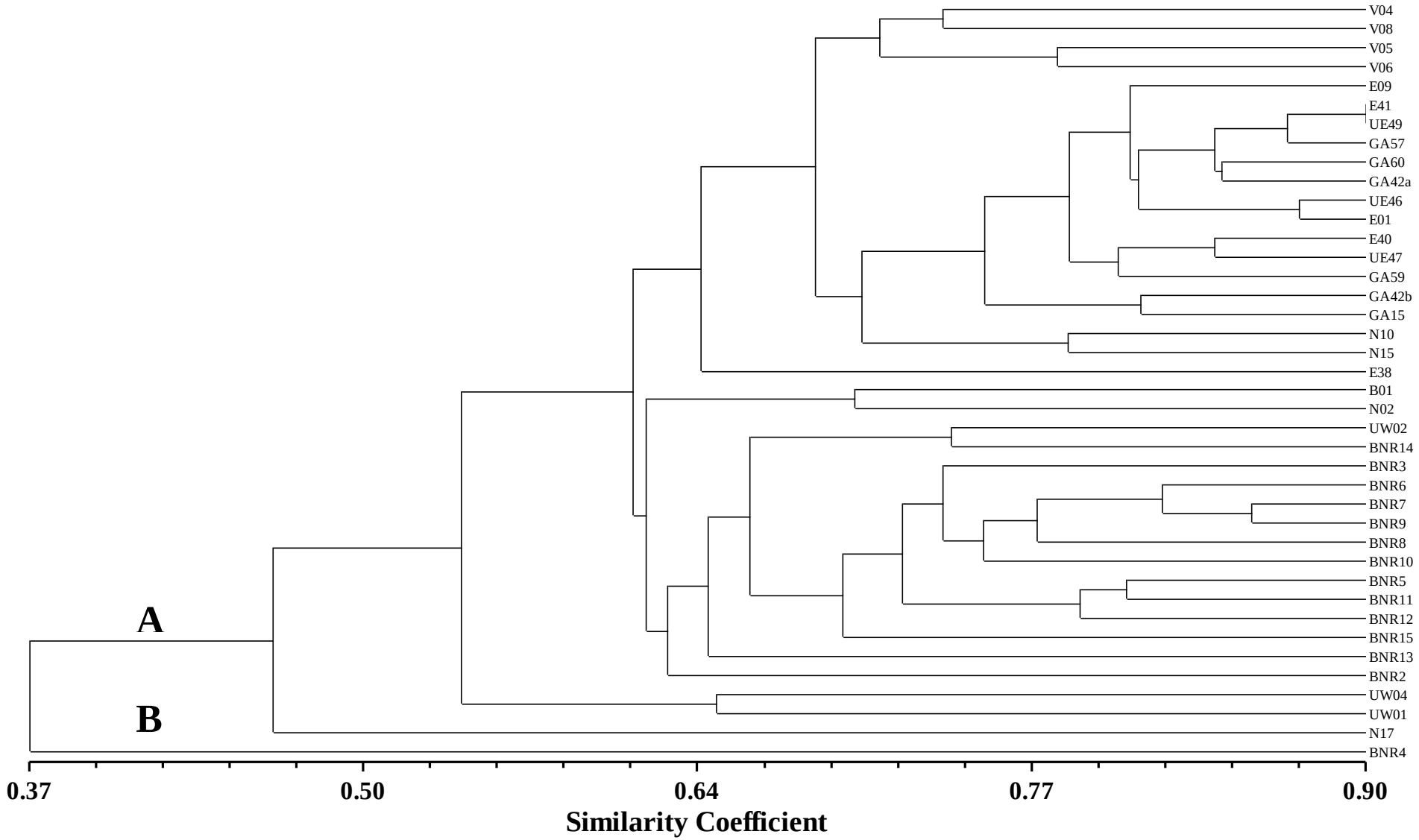

Fig 4.6: Dendrogram generated by UPGMA cluster analysis showing the relationship among 40 Moringa accessions based on Dice similarity from the combination of Total protein and RAPD data

4.3.6 Combination of Molecular and Morphological analyses of *Moringa oleifera* accessions

Cluster analysis generated from the combination of Molecular and Morphological data for the *Moringa oleifera* accessions was resolved into two main clusters, A and B at a similarity coefficient of 6% (figure 4.7). Main cluster A consisted of AS01, N03, AS02, N05, N08, E04, GA04, E02, GA03, GA02, BA01, V01, E05, GA07, GA10, V02, BA02, E10, UE02, E11, UE05 and UW01. It further separated into two sub-clusters at 67% similarity level with accessions AS01, N03, AS02, N05, N08, E04, GA04, E02, GA03 and GA02 forming one sub-clusters and accessions BA01, V01, E05, GA07, GA10, V02, BA02, E10, UE02, E11, UE05 and UW01 making up the other half. Main cluster B comprised of V04, V08, V05, V06, E09, E41, UE49, GA57, GA60, GA42a, GA42b, UE46, E01, E40, UE47, GA59, N10, N15, GA15, E38, UW02, BNR10, BNR5, BNR11, BNR12, BNR6, BNR7, BNR9, BNR8, BNR15, BNR13, BNR14, BNR2, B01, BNR3, N02, UW04, UW01, N17 and BNR4. Main cluster B further separated into two sub-clusters at 39% similarity level with accession BNR4 separating itself from the remaining accessions. These groups of accessions AS01 and N03; AS02, N05 and N08; E02 and GA03; V01, E05, GA07 and GA10 in main cluster A could not separate at 100% similarity coefficient.

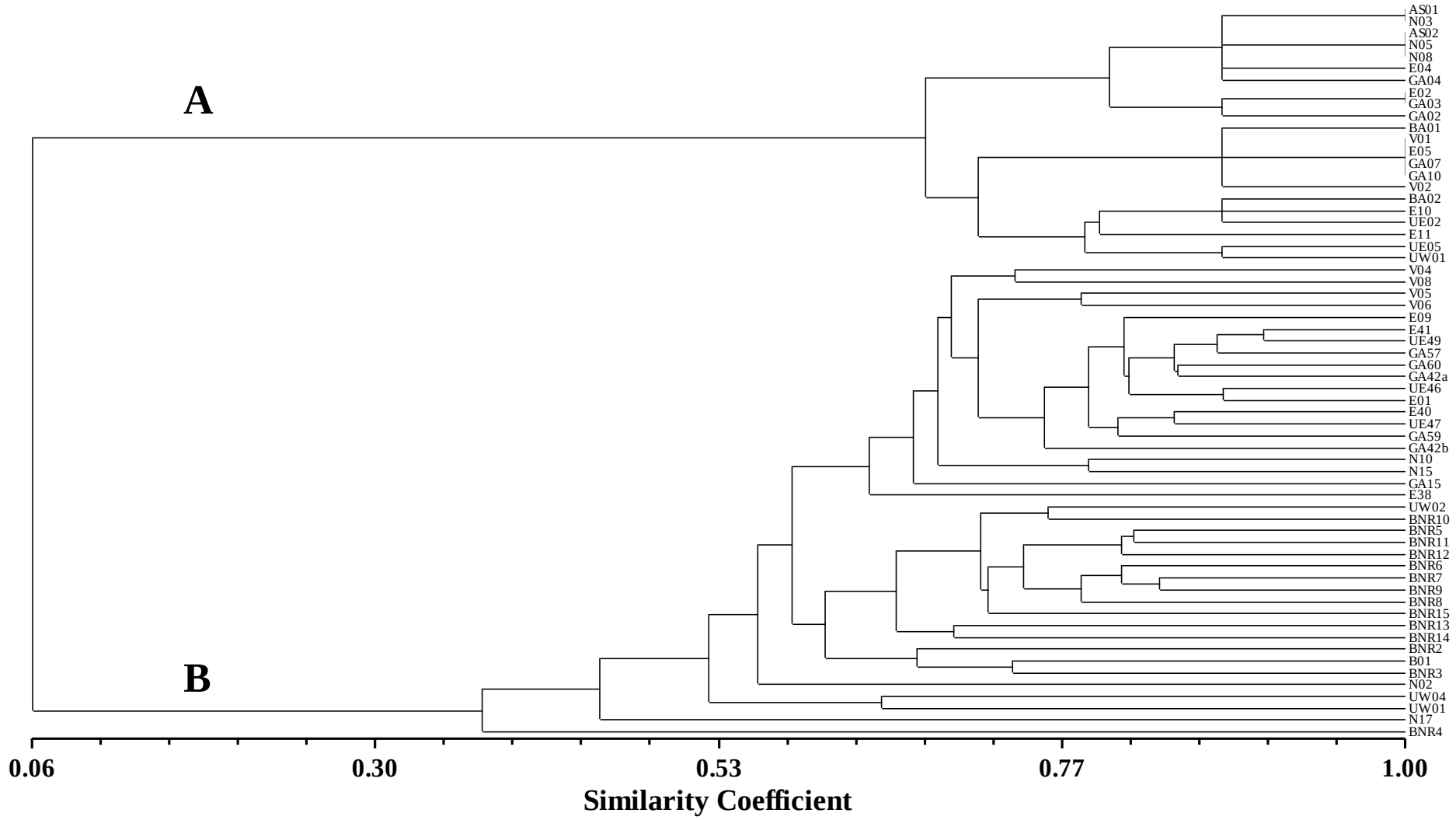

Fig 4.7: Dendrogram generated by UPGMA cluster analysis showing the relationship of Moringa accessions based on Dice similarity from the combination of molecular and morphological data

CHAPTER FIVE

DISCUSSION

5.1 Variability among accessions for morphological characteristics

The provenances of *Moringa oleifera* Lam. from the ten different locations, eight in Ghana and two from United State of America and India, studied exhibited variations with respect to the eight morphological traits studied. These were tripinnatum leaf length (at 6MAP), tripinnatum leaf width (at 6MAP), tripinnatum leaflet length (at 6MAP), tripinnatum leaflet width (at 6MAP), leaf colour, petiole pigmentation, growth habit and stem bark colour. In germplasm management, morphological characterisations are usually employed as markers either within or between species, varieties or accessions (Acquaah, 2007). These markers called descriptors give the description of taxonomy of the crop (Hilu, 1989). The information though basic is vital and provides the breeder with the information on genetic closeness of the accessions. Information acquired from these markers aid breeders in the selection of accessions with desirable traits for both farmers and consumers (Gitonga *et al.*, 2008).

Diversity in morphological characteristics such as tripinnatum leaf length, tripinnatum leaf width, tripinnatum leaflet length and tripinnatum leaflet width give important information to Moringa plant breeders in selecting accessions with large leaf area. This is because the leaves are the most consumed part of the plant especially in Ghana. In this instance larger leaf area will yield larger amounts of dried leaf powder which will mean more income for farmers and business persons who process the leaves into powder for sale. Variability in the qualitative information such as leaf

colour, petiole pigmentation and stem bark colour as well as growth habit recorded in the study was desirable. Edwards *et al.,* (2000) also observed variations in these characters in another species, *Moringa stenopetala* in their study. Diversity provides the breeder with the genetic material for selection, improvement as well as conservation of the plants (Acquaah *et al.*, 1992; Brothers and Kelly, 1993). For instance, the choice of a dark coloured leaf over light coloured one will be desired by the breeder because of the high amount of chlorophyll which will enable the plant to produce more photosynthate for effective growth. Also, dark leaf colour will be preferred for beverage especially when fresh leaves are used.

5.2 Variations in Biochemical Analysis

Variations in nutrient composition among *Moringa oleifera* accessions could be from genetic source, environmental source or cultivations method (Broin 2006; Dechasa, 2006). In this study, the leaf samples from cuttings of the various accessions from the different location were used. The environmental conditions as well as cultivation methods were the same for all accessions studied. Therefore the only source of variation affecting the biochemical variations namely variation in the amount of micro and macronutrients may be the genotypic differences of provenances used in the study.

Minerals are required for normal growth, activities of muscles and skeletal development (such as calcium), cellular activity and oxygen transport (copper and iron), chemical reaction in the body and intestinal absorption (magnesium), fluid balance and nerve transmission (sodium and potassium), as well as the regulation of acid-base balance (phosphorus). Iron is useful in prevention of anemia and other

related diseases (Oluyemi *et al.*, 2006). Manganese plays a role in energy production and in supporting the immune system (Muhammad *et al.*, 2011). Zinc is useful for protein synthesis, normal body development and recovery from illness (Muhammad *et al.*, 2011). The minerals determined are very essential macro minerals since they make the edible parts of Moringa useful for food or feed (Dechassa *et al.*, 2003). The average concentration of Ca observed was 50.24 mg/kg. This was lower compared to the findings of authors like Aslam *et al.*, (2005) and Oduro *et al.*, (2008) who reported 229.3 mg/kg and 200.9 mg/kg respectively. However, the value compared well with those reported by Council of Scientific and Industrial Research., (1962); Verma *et al.*, (1975) and Owusu-Ansah, (2010) viz 44 mg/kg, 44 mg/kg and 73.4 mg/kg respectively. The average amounts of Mg (26.07 mg/kg) and P (0.2776 mg/kg) obtained was lower than those reported by Owusu-Ansah, (2010) who obtained average concentrations of Mg and P of 26.6 mg/kg and 0.59 mg/kg respectively. The mean concentration of K recorded in the study was 1863.9 mg/kg. This was lower than the concentration reported by Aslam *et al.*, (2005) who had 2098.2 mg/kg. The quantity of K detected was less than the USDA-RDI (4700 mg/d) for an adult human being.

Regarding the micro-nutrients, Fe is an essential trace element for haemoglobin formation, normal functioning of the central nervous system and in the oxidation of carbohydrates, proteins and fats (Adeyeye and Otokiti, 1999). The mean value of Fe in the dried Moringa leaf samples was 0.012% (i.e. 120 mg/kg). This is similar to values reported Magat *et al.*, (2009) of 100 mg/kg but higher than that reported by Oduro *et al.*, (2008) for Fe composition in dry leaf power of Moringa. The micronutrients Mn and Zn had 1.9 mg/kg and 2.2 mg/kg respectively. These were

lower than values obtained by Magat *et al.*, (2009) who reported 340mg/10kg for Mn and 100mg/10kg for Zn in Moringa leaf.

In plants, the critical levels of the heavy metal Pb is 10-20 ppm and higher concentrations than these are very likely to be toxic (Sauerbeck 1982). According to the FAO/WHO standard (Codex Alimentarius Commission, 2001) the safe level of lead (Pb) in vegetables is 0.2 mg/kg. The recorded average Pb level of the accessions studied was 0.0019% (i.e. 1.9 mg/kg), which is above the accepted safe level of 0.2 mg/kg in vegetables but below the critical level of 10-20 ppm in plants. The observed lead level in the accessions studied is considered toxic for human or animal consumption but not toxic to the plant. Authors like Garba *et al.*, (2010) of Nigeria and Magat *et al.*, (2009) of Philippines also reported high lead levels in Moringa species taken as vegetables in their countries. They reported 321 mg/kg and 90 mg/kg respectively. The amount of Pb in the dried leaf samples of Moringa probably may be due to the area or location of collection especially in the local collections which included collections from the road side and near filling stations. Lead (Pb) pollution has been shown to be commensurate with population and vehicular density (Afolami *et al.*, 2010). Also high levels of Pb in some plants are attributable to pollutants in irrigation water, farm soil or due to pollution from the highways traffic (Qui *et al.*, 2000). Like the Chinese cabbage which has been reported to pick up and accumulate higher Pb concentrations (Wong *et al.*, 1996; Chove *et al.*, 2006), *Moringa oleifera* may also behave likewise especially when exposed to lead pollutants.

The lowest percentage dry matter among the accessions was observed in the accessions from the Upper West, meaning they had the highest moisture content and

hence may be more prone to deterioration since foods with high moisture content are more prone to perishability (Fennema and Tannenbaum, 1996). Inferably, the accessions from USA will be less prone to deterioration since they have the least amount of moisture content. Similar observations of variability in dry matter of the leaves from different accessions have been reported by Fuglie, (2001); Magat *et al.*, 2009 and Oduro *et al.*, (2008).

With the exception of the accessions from USA which had 12.9% crude protein, all the Moringa accessions had 17% protein or more with those from the Eastern region of Ghana recording the highest amount of 26.3%. The results obtained for the range of protein levels in the accessions are similar to levels documented by Fuglie, (2001), Elkhalifa *et al.,* (2007) and Oduro *et al.*, (2008). This makes the leaves of Moringa a good source of protein since plant food that provides more than 12% of its calorific value from protein are considered good source of protein (Pearson, 1976). The crude protein levels observed in the study were higher than the levels reported in *Cucurbita moschata, Trianthema portulacastrum, Spinacia oleracea, Abelmoschus esculentus, Praecitrullus fistulosus, Luffa acutangula and Cucumis sativus* with protein percentage of 10.77±0.23, 19.63±0.9, 20.82±0.07, 16.17±0.26, 15.34±.30, 13.47±0.05 and 10.57±0.23 respectively (Hussain *et al.,* 2010). Percentage nitrogen corresponds with percentage protein. The average nitrogen level of the accessions of 3.1% is lower than those observed by Magat *et al*., (2009) who recorded an average of 4.737%.

The results of the study showed that all the nutrients with the exception of Fe occurred in minute quantities compared to the USDA- RDI. The average quantity of 12 mg/100g of iron detected was higher when compared to the USDA- RDI for an adult male but less

than that needed by an adult female (that is, about two thirds of the USDA- RDI of required by an adult female). This may be as a result of the drying process since drying foodstuffs under high temperature over a period of time can contribute to nutrient loss.

5.3 Molecular Analysis

Biochemical markers assess accurate genetic diversity index (Akhtar, 2001; Rabbani *et al.*, 2001). Protein electrophoresis is a powerful tool for population genetics (Parker *et al*., 1998). Irwin *et al.,* (1998) reported that closely related accessions are normally located within 80–90% similarity. Crosses between accessions with similarity indices of 80–100% may, therefore, not be recommended. The results of the study on total protein marker revealed that considerable variation was available in the accessions. This is because a sizeable number of the accessions studied had a similarity index of 54 – 77% (figure 4.4). Following the recommendation of Irwin *et al*. (1998), crosses between the accessions BNR 7 and BNR 15 may provide superior genotypes from which useful agronomic types can be selected. In spite of the variation existing among the accessions, some accessions from Ghana were similar which probably may be attributable to a common parent or ancestry. This is likely because most Moringa planting materials in Ghana are collected from cuttings or seeds of plants from relatives, friends and loved ones who recommend the crop due to its numerous benefits. Large scale production from seed is not yet established.

RAPD technique is an effective method of screening for nucleotide sequence polymorphisms randomly distributed throughout the genome of an organism (Subudhi and Huang, 1999) which provides adequate information on the variability of

accessions studied. For the RAPD marker analysis, there was a high level of polymorphism (95.1%) among the accessions of *Moringa oleifera* studied. This may be due to the fact that the variations observed may be variations that exist at the genotypic level. Accession pair E41 and UE49 in cluster A could not be resolved at 91% similarity (figure 4.5) suggesting that they could be closely related or be the same. Accession BNR04 from USA was resolved from the rest of the accessions, forming cluster B whilst accession N17 was also resolved from the other accessions in cluster A showing their uniqueness among the accessions studied (figure 4.5). The observed similarity level of 30% (that is 70% genetic variability) shows that there is a high genetic variability among the 40 Moringa accessions analysed. This confirms the ability of RAPD markers to detect variations in the samples. The study compares well with Abubakar *et al.,* (2011) who reported high degree of polymorphism (74%) among 75 Moringa accessions from Sudan and Guinea savannah zones in Nigeria. Fu *et al.,* (2003) obtained 51.2% genetic differentiation coefficient in *Changnium smyrnioides* using RAPD marker suggesting high genetic diversity among the populations of these plants. The result from the present study indicates the ability of RAPD to discriminate among *Moringa oleifera* accessions.

In analysing the electrophoresis data, an assumption made is that differences in banding patterns among accession reflect differences in their genetic make-up (Shecter and de Wet, 1975). A combination of two marker systems shows much diversity or variability among the accession studied since they cover a large section of the genome. This confirms that the use of different marker systems tend to give better picture of the genetic relatedness than single marker. The combined dendrogram of total protein and RAPD markers suggest high level of genetic variability since a

similarity coefficient of 37% (that is 63% genetic variability) was observed. Accession BNR 4 was resolved from the rest of the accessions studied forming cluster B. This shows the level of distinctiveness of the accession from the rest of the accessions used in the study. Similarly in cluster A of the RAPD dendrogram, accession N17 was resolved from the rest of the accessions for the combined data of total protein and RAPD analysis. The accessions E41 and UE49 were similar at 90% similarity coefficient suggesting that they were either duplicates or samples with common parents (figure 4.6).

The combined dendrogram of morphological and molecular analysis generated very high variability among the accessions used in the study. A similarity coefficient of 6% (that is 94% variability) was observed in the study (figure 4.7). Again, accession BNR4 in major cluster B was resolved from the other accessions studied showing its uniqueness. Accession pairs AS01 and N03; AS02, N05 and N08; E02 and GA03 were 100% similar and could not be resolved. These tied accessions are the ones that were subjected to morphological analysis but not molecular analysis. From the cluster analysis of RAPD data, combined data from total protein and RAPD as well as combined data from molecular and morphological data, the accessions N17 and BNR04 are the most divergent genotypes among the accessions studied hence crosses between them will provide superior genotypes from which useful agronomic traits can be selected for any Moringa breeding programme (Irwin *et al.*, 1998).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From the study, it may be concluded that;

- *Moringa oleifera* Lam. accessions studied have considerable amount of variability in morphological characteristics such as leaf colour, petiole pigmentation, stem bark colour and growth habit.

- Cluster analyses of both total protein and RAPD markers revealed variations in the accessions studied.
  - The total protein profile of the accessions studied revealed a moderate level of diversity since almost half of the total accessions analysed could not be resolved at 100% similarity.
  - On the other hand, the RAPD profile distinguished between most of the accessions, resolving almost all the accessions except the pair E41 and UE49 at 91% similarity.
  - A combination cluster analysis of RAPD and total protein markers could still not resolve accessions pair E41 and UE49. These accessions are therefore closely related.

- Regarding the relationship between the accessions, it may be concluded that accessions BNR04 and N17 from United States of America and Northern region of Ghana respectively were the most distinctive accessions in the study.

- These two accessions are the most diverse. A cross between them will generate recombinants from which genotypes with superior and useful agronomic traits could be selected.

- A high variability existed in the micro as well as macronutrient composition of the leaves. This variation offers a good opportunity for exploitation in plant improvement programmes.
  - The minerals Ca, Mg, Na, K, P, Fe, Mn and Zn in Moringa accessions studied were in appreciable quantities and showed marked variation in their concentrations but were less than the USDA- RDI.
  - The crude protein content was high compared to leafy vegetable such as "Kontomire" (*Xanthosoma sagittifolia*) and "Bokoboko" (*Talinum triangulare*) which are commonly consumed locally. The high protein with a fairly high concentration of potassium and iron; make it a potential leafy vegetable that is suitable for fortification of foods.
  - Among the local accessions studied, those from Greater Accra, Northern and Upper East regions exhibited the highest levels of certain macro-nutrients and crude protein. Crosses between these have the potential to concentrate the superior characteristics in new varieties.

- The high concentration of lead detected in the accessions studied suggests that *Moringa oleifera* Lam could have high levels of the metal Lead.

6.2 Recommendations

- Studies on agro-morphological characteristics such as yield, 100 seed weight, pod colour, pod length, pod width and number of seeds/pod and reproductive traits such as flower colour, flower aromatic, Days to flowering (from sowing to 50% with inflorescence) should be considered in the future to further ascertain the variability that exist at the phenotypic level.

- Further investigation into lead accumulation in *Moringa oleifera* Lam. as well as the ability of the plant to pick up and accumulate other heavy metals such as cadmium, arsenic, mercury, nickel and chromium in the edible parts of the plant is very necessary and should be carried out.

- The use of more modern molecular markers such as microsatellites or single nucleotide polymorphic (SNP) that easily detect polymorphism even among close relations could be adopted to further investigate the relationships between the accessions especially E41 and UE49 at the genotypic level.

- The accessions that have not been subjected to morphological analysis including accessions E41 and UE49 should be studied to confirm the relationships between them.

REFERENCE

Abdulkarim, S. M., Long K., Lai O. M., Muhammad, S. K. S., Ghazali, H. M. (2005). Some physio-chemical properties of *Moringa oleifera* seed oil extracted using solvent and aqueous enzymatic methods. *Food Chemistry* 93: 253–263.

Abubakar, B. Y., Wusirika, R., MuA'zu, S., Khan, A. U. And Adamn, A. K. (2011). Detection of genetic variability using random amplified polymorphic DNA markers in some accessions of *Moringa oleifera* lam. from Northern Nigeria. *International Journal of Botany* 7(3): 237-242

Acquaah, G. (2007). Principles of plant genetics and breeding. 1st Ed. Blackwell Publishing Ltd. Malden, USA, pp 199

Acquaah, G., Adams, M. W. and Kelly, J. D (1992). A factor analysis of plant variables associated with architecture and seed size in dry beans. *Euphytica* 60: 171-177

Adams, C. D. (1972). Flowering plants of Jamaica. Mona, Jamaica; University of the West Indies. Pp 848

Adeyeye, E. I. and Otokiti, M. K. O. (1999). Proximate composition and some nutritionally valuable minerals of two varieties of *Capsicum annum*. (Bell and Cherry peppers). *Discovery and Innovation*, 11: 75-81.

Afolami. I., Anyakora, C., Ebuehi, O. and Bolawa, O. (2010). Lead levels in some edible vegetables in Lagos, Nigeria. *Scientific Research and Essays*. 5(8): 813-818,

Akhtar, M. (2001). Phylogenetic relationship among Vigna species based on agronomic and biochemical analysis. M. Phil. Thesis, Department of Biological Sciences, Quaid-I-Azam University, Islamabad, Pakistan, 99pp.

Akinnibosun, F. I, Akinnibosun, H. A and Ogedegbe, D. (2009). Investigation on the antibacterial activity of Aqueous and ethanolic extracts of leaves of Boerhavia diffusa. *Science World Journal* 4(2):15-18.

Akinnibosun, H. A. Akinnibosun, F. E. and German, B. E. (2008). Antibacterial activity of aqueous and Ethanolic leaf extracts of *Pegeromic pellucida* (L) H. B and K (Piperaceae) on three Gram negative bacteria isolates. *Science World Journal* 3(4):33-361

Alfred, D. and Patrick, N. (1985). Integrated Food Science and Technology for tropics.3rd ed Mcmillan publishers. London Pp 293-300

Ali, G. H, El-Taweel, G. E. and Ali, M. A. (2004). The cytotoxicity and antimicrobial efficiency of *Moringa oleifera* seeds extracts. *International Journal of Environmental Studies* 61(6):699-708.

Antosiewicz, D. and M. Wierzbicka. (1999). Localization of lead in *Allium cepa* L., cell by electron microscopy. *Journal of Microscopy*, 195: 139-146.

Anwar, F., Ashraf, M. and Bhanger, M. I., (2005). Interprovenance variation in the composition of *Moringa oleifera* oilseeds from Pakistan. *Journal of the American Oil Chemists Society*, 82: 45–51.

Anwar, F. and Bhanger, M. I. (2003). Analytical characterization of *Moringa oleifera* seed oil grown in temperate regions of Pakistan. *Journal of Agricultural and Food Chemistry*, 51: 6558–6563.

Anwar, F. Latif, S. Ashraf, M. and Gilani, A. H. (2007). *Moringa oleifera*: A Food Plant with Multiple Medicinal Uses. *Phytotherapy Research*, 21:17–25.

Aregheore, E. M. (2002). Intake and digestibility of *Moringa oleifera*–batiki grass mixtures by growing goats. *Small Ruminant Research* 46:23–28.

Arnoldo-Broeders, Fr. M. (1967). Handleiding tot het gebruik van inheemse en ingevoerde planten op Aruba, Bonaire en Curacao. Boekhandel "St. Augustinus," Curacao, N.A.

Arnoldo-Broeders, Fr. M (1971). Gekweekte en nuttige planten van de Nederlandse Antillen. Natuurwetenschappelijke Werkgroep Nederlandse Antillen, Curacao, N.A.

Asghar, R., Siddique, T. and Afzal, M. (2003). Inter and intra-specific variation in SDS-PAGE electrophoregrams of total seed protein in chickpea (*Cicer arietinum* L.) germplasm. *Pakistan Journal* of Biological Sciences, 6(24): 1991-1995.

Aslam, M., Anwar, F., Nadeem, R., Rashid, U., Kazi, T. G. and Nadeem, M. (2005). Mineral composition of *Moringa oleifera* leaves and pods from different regions of Punjab, Pakistan. *Asian Journal of Plant Sciences*, 4(4):417-21.

Bachmann, K. (1994). Tanksley review No. 6: Molecular markers in plant ecology. New phytol. 126: 403-418

Baker, E. M. and Griffin, L. (1967). Vitamin C requirements in stress. *American Journal of Science*, 6: 213-214.

Bandana, B., Srivastava, R. C. and Mathur, S. N. (1987). Nodulation and nitrogenase activity in *Vigna mungo* in response to seed-soaking and root-dressing treatments of Moringa leaf extracts. *Indian Journal Plant Physiology* 30(4): 362–367.

Bekele, E. (1983). Some measure of genetic diversity analysis on land race populations of Ethiopian Barely. *Hereditas* 98: 127-143.

Benko-Iseppon, A. M. (2001). Estudos moleculares e citogenéticos no caupi e em espécies relacionadas: Avanços e perspectivas. *Embrapa Docs*. 56: 327-332.

Berger, M. R, M. Hubs, S. A. A. Jahn, and D. Schmald. (1984). Toxicological assessment of seeds from *Moringa oleifera and M. stenopetala*, two efficient primary coagulants for domestic water treatment of tropical raw waters. East African Med. J. Sept.: 712-716.

Beyene, D. (2005). Genetic variation in *Moringa stenopetala* germplasm of Ethiopia by using RAPD as genetic marker. MSc. Thesis. Addis Ababa University, Ethiopia.

Bhatnagar, S. S., Santapau, H., Desai, J. D. H., Yellore, S. and Rao, T. N. S., (1961). Biological activity of Indian medicinal plants. Part 1. Antibacterial, antitubercular and antifungal action. *Indian Journal of Medical Research*, 49: 799–805.

Bhattacharya, S. B., Das, A. K. and Banerji, N., (1982). Chemical investigations on the gum exudates from Sonja (*Moringa oleifera*). *Carbohydrate Research*, 102: 253–262.

Booth, F. E. M. and Wickens, G. E., (1988): Non-timber uses of selected arid zone trees and shrubs in Africa. FAO Conservation Guide 19, Food and Agriculture Organization of the United Nations, Rome.

Brain A. F. and Allan G. C. (1986). Food Science. A Chemical Approach, 15th Ed. Edward Anold publishers Ltd London Pp 210-211

Breting, P. K. and Wildrelechner, M. P. (1995). Genetic markers and plant genetic resource management. *Plant Breeding reviews*, 13: 11-86.

Britton, N. L. and Millspangh, C. F. (1920). The Bahama flora. Authors, New York.

Britton, N. L. (1918). Flora of Bermuda. Charles Scribher's Sons, New York, NY.

Broin, M. (2006). The nutritional value of *Moringa oleifera* Lam. Leaves: what can we learn from figures? Moringa news Workshop. [Poster][Online]Available at: http://www.moringanews.org/doc/GB/Posters/Broin_poster.pdf

Broin, M., Santaella, C., Cuine, S., Kokou, K., Peltier, G. and Joet, T., (2002). Flocculent activity of a recombinant protein from *Moringa oleifera* Lam. seeds. *Applied Microbiology and Biotechnology*, 60: 114– 119.

Brothers, M. E. and Kelly, J. D. (1993). Interrelationship of plant architecture and yield component in pinto bean ideotype. *Crop Science*, 33: 1234-1238

Brush, S. B. (1989). Rethinking crop genetic resource conservation. *Biol Conserv* 3: 1-11

Bureau of Plant Industry. (1915). *Moringa oleifera La*marck (Moringaceae). 40913. Plant Immigrants No. 111-2. Off. of For. Seed & Plant Intro., U.S. Dept. Agr., Washington, D.C.

Caceres, A., Cabrera, O., Morales, O., Mollinedo, P. and Mendia, P. (1991). Pharmacological properties of *Moringa oleifera*. 1: Preliminary screening for antimicrobial activity. *Journal of Ethnopharmacology*, 33: 213–216.

Caceres, A., Saravia, A., Rizzo, S., Zabala, L., De Leon, E. and Nave, F. (1992). Pharmacological properties of *Moringa oleifera* 2: Screening for antispasmodic, anti-inflammatory and diuretic activity. *Journal of Ethnopharmacology* 36(3): 233-237.

Chapman, C. (1989). "Principles of germplasm evaluation". In: Stalker, H. T., Chapman, C. (eds). IBPGR Training Courses: Lecture Series 2. Scientific Management of germplasm: Characterization, Evaluation, and enhancement. International Board for Plant Genetic Resources, Rome, pp 55-64.

Chove, B. E., Ballegu, W. R. and Chove, L. M. (2006). Copper and Lead levels in two popular leafy vegetables grown around Morogoro Municipality, Tanzania. *Tanzania Health Research Bulletin.* 8(1): 37-40.

Clegg, M. T. (1990). "Molecular diversity in plant populations". In: Brown, A. H. D., Clegg, M. T., Kahler. A. L., Weir, B. S. (Eds). Plant population 681 genetics, breeding, and genetic resources. Sinauer Associates Inc., Sunderland, Massachusetts, pp 98-11

Codex Alimentarius Commission, (2001). Food additives and contaminants. Joint FAO/WHO Food Standards programme; ALINORM 01/12A:1-289.

Correll, D. S. and Correll H. B. (1982). Flora of the Bahama Archipelago *(Including the Turks and Caicos).* Germany: A. R. G. Gantner Verlag K.-G FL-9490 Vaduz.

Council of Scientific and Industrial Research, (1962). (Ed.) The Wealth of India: A dictionary of Indian Raw Materials and industrial products. Council of Scientific and Industrial Research, New Delhi, vol. 6: L–M pp. 425–429.

Crapper, D. R., Krishnan, S. S. and Dalton, A. J. (1973). Brain aluminium distribution in Alzheimer's disease and experimental neurofibrillary degeneration. Science 180: 511–513. DOI: *10.1126/science.180.4085.511*

D'souza, J. and Kulkarni, A. R. (1993). Comparative studies on nutritive values of tender foliage of seedlings and mature plants of *Moringa oleifera* Lam. *Journal of Economic and Taxonomic Botany,* 17: 479–485.

Dahot M. U. (1998). Antimicrobial activity of small protein of *Moringa oleifera* leaves. *Journal of Islamic Academy of Sciences* 11(1): 27-32.

Dahot, M. U. (1988). Vitamin contents of flowers and seeds of *Moringa oleifera*. *Pakistan Journal of Biochemistry and Molecular Biology,* 21: 1–24.

Das, S. and Mukerjee, K. K. (1995). Comparative study on seed proteins of *Ipomoea batatas*. *Seed Science and Technology*, 23: 501-509.

Dechassa, N, Schenk, N. M. K., and Steingrobe, N. (2003). Phosphorus Efficiency of Cabbge (*Brassica oleraceae* L. var. capitata), Carrot (*Daucus carota* L.), and Potato (*Solanum tuberosum* L). *Plant Soil* 250: 215- 224.

Dechasa, J., Sonder, K., Alemayehu, L., Mekonen, Y. and Agena (2006). Leaf yield and nutritive value of *Moringa Stenopetala* and *Moringa oleifera* accessions: its potential role in food security in constraint dry farming agroforestry system. Moringa and other highly nutritious plan resources: strategies, standards and markets for a better impact on nutrition in Africa, Accra, Ghana. Workshop presentation. In: http://www.moringanews.org/doc/GB/papers/Dechasa_Jiru_Text_GB.pdf

Dastur, J. F. (1951). Useful plants of India and Pakistan. 2nd Indian ed. D. B. Taraporevala Sons & Co., Ltd., Bombay.

Delaveau, P. and Boiteau, P. (1980). Huiles a interêt pharmacologique, cosmetologique et dietetique. 4. Huiles de *Moringa oleifera* Lamk. et de M. drouhardii Jumelle. *Plantes Medicinales et Phytotherapie* 14(1): 29–33.

Demissie, A. and Bjørnstrand, A. (1996). Phenotypic diversity of Ethiopian Barely in relation to geographic regions, altitudes and agro ecological zones: as an aid to germplasm collection strategy. *Hereditas* 124: 17-29

Dice, L. R. (1945). Measures of the amount of ecologic association between species. *Ecology* 26: 297–302.

Dillard, C. J. and German, J. B. (2000). Phytochemicals: nutraceuticals and human health: A review. *Journal* of the *Science* of *Food* and *Agriculture*, 80: 1744–1756

Doebley, J. (1989). "Isozymic evidence and the evolution of crop plants". In: Soltis DE, Soltis PS (eds) Isozymes in plant biology. Dioscorides Press, Portland, Oregon, pp 165-191

Doer, B. (2005). Field Guides for Emergency water Treatment with *Moringa oleifera*. www.cawst.org/technology/watertreatment/filtrationbiosandanphp

Duke, J. A. (1987). Moringaceae: Horseradish-tree, benzolive-tree, drumstick-tree, sohnja, moringa, murunga-kai, malunggay, p. 19-28. In: M. Benge {ed.} Moringa: A multipurpose vegetable and tree that purifies water. Science and Technololgy / For., Environmental and Natural Resources Agro-Forestation Tech. Ser. 27. US AID, Washington, D.C.

Duke, J. A., (1983). Assorted crop information sheets from *Handbook of Energy Crops* (unpublished). Available at http://www.hort.purdue.edu/newcrop/duke_energy/Genus_species.html

ECHO Network, (2008). Moringa, nature's medicine cabinet. In; http://www.drumsticksindia.com/varities.2008

Edwards, S., Tadesse, M., Demissew, S. and Hedberg, I. (2000). Flora of Ethiopia and Eritrea, Vol 2, Part: Mognoliaceae to Flacourtiaceae. Addis Ababa University, Uppsal, Sweden. Pp: 660

Elkhalifa , A. O., Ahmed, S. A. and Adam, S. (2007). Nutritional evaluation of *Moringa Oleifera* leaves and extracts. *Ahfad Journal* 24: 113 -22.

Estrella, M. C. P., Mantaring, J. B. V. and David, G. Z., (2000). A double blind randomised controlled trial on the use of malunggay (*Moringa oleifera*) for augmentation of the volume of breastmilk among non-nursing mothers of preterm infants. *Philippine Journal of Pediatric* 49: 3–6.

Faizi, S., Siddiqui, B. S., Saleem, R., Aftab, K., Shaheen, F. and Gilani, A. H. (1998). Hypotensive constituents from the pods of *Moringa oleifera*. *Planta Medica*, 64: 225–228.

Fahey, J. W. (2005). *Moringa Oleifera*: A review of the Medical Evidence for Its Nutritional, Therapeutic, and Prophylactic Properties. Part I. *Trees for Life Journal*, 1(5): 1 -19.

FAO/UNESCO (Food and Agriculture Organization / United Nations Educational, Scientific and Cultural Organization) (1990). Soil Map of Ghana, *Land Suitability Section*. Soil Research Institute, CSIR, Ghana.

Fawcett, W. and Rendle, A. B. (1914). Flora of Jamaica. Vol. III. British Mus. (Nat. Hist.), London.

Fennema, R. O. and Tannenbaum, S. R. (1996). Introduction to Food Chemistry. In: Fennema RO, Karel M, Sanderson GW, Tannenbaum SR, Walstra P, Witaker JR (eds). Food Chemistry, Marcel Dekker Inc. New York. pp. 1-64.

Foidl, N., Makkar, H. P. S. and Becker, K. (2001). The potential of *Moringa oleifera* for agricultural and industrial uses. In: L.J. Fuglie (Ed.), The Miracle Tree: The Multiple Attributes of Moringa (pp. 45-76). Dakar, Senegal: Church World Service.

Folkard, G. K., Sutherland, J. P., & Shaw, R. (1993). Water clarification using *Moringa oleifera* seed coagulant. www.iboroac.uk/well.downloaded

Francis, J. K. and Liogier, H. A., (1991). Naturalized exotic tree species in Puerto Rico. Gen. Tech. Rep. SO-82, South. For. Res. Sta., For. Serv., U.S. Dep. Agric., New Orleans, LA, USA.

Fu, C., Qiu, Y. and Kong, H. (2003). RAPD analysis for genetic diversity in Changium smyrnioides (Apiaceae), an endangered plant. Botanical Bulletin of Academia Sinica, 44: 13-18

Fuglie, L. J., (2001). The Miracle Tree. *Moringa oleifera*: Natural Nutrition for the Tropics. CWS, Dakar, Senegal p.115.

Fuglie, L. J., (2005). The Moringa Tree: a local solution to malnutrition. Church World Service in Senegal.

Garba, M. A., Musa, A., Mohammed, L. and Musa, H. A. (2010). The concentrations of lead in some herbs in Makarfi, Nigeria. *Nigerian Journal of Pharmaceutical Sciences*, 9(1): pp 10-14

Gepts, P. (1990). "Genetic diversity of seed storage proteins in plants". In: Brown A. H. D., Clegg, M. T., Kahler, A. L., Weir, B. S. (eds). Plant population genetics, breeding and genetic resources. SinauerAssociates Inc., Sunderland, Massachusetts, pp 64-82

Gepts, P. (1995). "Genetic markers and core collections". In: Hodgkin, T., Brown, A. H. D., Hintum, Th J. L., Morales, E. A. V. (eds). Core collections of plant genetic resources. John Wiley and Sons, Chichester, UK, pp 127-146

Gitonga, L., Kahangi, E., Muigai, A., Ngaman, K., Gichuki, S. Cheluget, W. and Wepukhulu, S. (2008). Assessment of phenotypic diversity in macadamia (Macadamia spp) germplasm in Kenya usin leaf and fruit morphology. *African Journal of Plant Science.* 2 (9): 86-96

Ghafoor, A., Gulbaaz, F. N., Afzal, M., Ashraf, M. and Arshad, M. (2003). Inter-relationship between SDS-PAGE markers and agronomic traits in chickpea (*Cicer arietinum* L.). *Pakistan Journal of Botany.* 35(4): 613-624.

Ghafoor, A., Ahmad, Z. Qureshi, A. S. and Bashir, M. (2002). Genetic relationship in *Vigna mungo* (L.) Hepper and *V. radiata* (L.) R. Wileczek based on morphological traits and SDS-PAGE. *Euphytica*, 123: 378-2002

Ghana Stardards Board, (2009a). GS 998: 2009 – Medicinal Plants – *Specification for Moringa products.* Pp 1-7

Ghana Stardards Board, (2009b). GS 999: 2009 – Medicinal Plants – *Code of practice for the production of Moringa leaf Products. Pp 1-5*

Ghana Stardards Board, (2009c). GS IM 12: 2009 – Inspection Manual – *Instructions for the Inspection of Moringa Leaf Products*. Pp 1-4

Ghana web, 2007
http://www.ghanaweb.com/GhanaHomePage/health/artikel.php?ID=118217

Ghasi, S., Nwobodo, E. and Ofili, J. O. (2000). Hypocholesterolemic effects of crude extract of leaf of *Moringa oleifera* Lam in high-fat diet fed Wistar rats. *Journal of Ethnopharmacology*, 69: 21– 25.

Guha, S. R. D., Dhoundiyal, S. N. and Mathur, G. M. (1968). Mechanical pulps for newsprint grade papers from *Moringa pterygosperma*. *Indian Forester*, 94(8) 634–638.

Hadrys, H., Balick, M. and Schierwater, B. (1992). Application of random amplified polymorphic DNA (RAPD) in molecular ecology. *Molecular Ecology*, 1:55-63.

Hawthrone, W. and Jongking, C. (2006). Woody plants of western African forests: A guide to the forest trees,shrubs and lianas from Senegal to Ghana. Publication of the Royal Botanical Gardens, Kew, UK. Pp 39-46

Henretig, F. M. (2006). "Lead". In Goldfrank, LR. *Goldfrank's Toxicologic Emergencies, 8th edition*. McGraw-Hill Professional. p 1310 ISBN 0071437630

Hilu, K. W. (1989). Taxonomy of cultivated plants. In: Stalker, H. T., Chapman, C. (eds). IBPGR training courses: lecture series 2. Scientific management of germplasm characterization, evaluation and enhancement. International Board for Plant Genetic Resources, Rome, pp 33-40

Huang, J. W. and Cunningham, S. D. (1996). Lead phytoextraction: species variation in lead uptake and translocation. *New Phytologist* 134(1), 75-84.

Hussain, J., Najeeb, Ur., Rehman, N. U., Khan, A. L., Hamayun, M., Hussain, M. S. and Shinwari, K. S. (2010). Proximate and essential nutrients evaluation of selected vegetables species from Kohat Region, Pakistan. *Pakistan* Journal of *Botany*, 42(4): 2847-2855,

Irvine, F. R. (1961). Woody plants of Ghana, with special reference to their uses. Oxford University Press, London.

Irwin, S. V., Kaufusi, P., Banks, K., de la Peña, R. and Cho, J. J. (1998). Molecular characterisation of taro (*Colocasia esculenta*) using RAPD markers. *Euphytica*, 99: 183-189

Iqbal, M. Z. and Shazia Y. (2004). Reduction of germination and seedling growth of *Leucaena leucocephala* caused by lead and cadmium individually and combination. *Ekologia (Braslava),* 23(2): 162-168.

Iqbal, M. Z., Shafiq, M. and Kausar, A. S. (2001). Toxic effects of lead and cadmium individually and in combination on germination and growth of *Leucaena leucocephala*. *Pakistan Journal of Botany*, 33: 551-557.

Jahn, S. A. A. (1989). For food and water purification - selection of clones and growing of annual short stem. Entwicklung + Landlicher Raum, 23 (4) pp22-25.

Jahn, S. A. A. (1991). The traditional Domestication of a multipurpose tree *Moringa stenopetala* (Bak.f.) Cuf. in the Ethiopia Rift valley. *AMBIO* 20: 244-247.

Jahn, S. A. A., Mushad, H. A., and Burgstaller, H. (1986). The tree that purifies water –*Moringa oleifera* cultivating multipurpose Moringaceae in the Sudan. UNASYLVA (*an International Journal of Forestry and Forest Industries*) 38:23-28.

Johnson, B. C. (2005). Clinical Perspectives on the Health Effects of *Moringa oleifera*: Promising Adjunct for Balanced Nutrition and Better Health. KOS Health Publications 466 Foothill Blvd. #251, La Cañada, California 91011

Joseph, L. U., Andrea, L. C. and Mal, T. K. (2002). Effects of lead contamination on the growth of *Lythrum salicaria*. *Environmental Pollution*, 120(2): 319-323.

Kaga, A., Tomooka, N., Hosaka, K. and Kamijima, O. (1996). Species relationship in the subgenus *Ceratotropis* (genus *Vigna*) as revealed by RAPD analysis. *Euphytcia*, 88: 17-24.

Kalkunte, S., Swamy, N., Dizon, D. S. and Brard, L. (2006). Benzyl isothiocyanate (BITC) induces apoptosis in ovarian cancer cells in vitro. *Journal of Experimental Therapeutics and Oncology, 5:287–300.*

Kalogo, Y., Rosillon, F., Hammes, F. and Verstraete, W. (2000). Effect of a water extract of Moringa oleifera seeds on the hydrolytic microbial species diversity of a UASB reactor treating domestic wastewater. *Letters in Applied Microbiology,* 31: 259–264.

Karp, A., Kresovich, S., Bhat, K. V., Ayad, W. and Hodgkin, T. (1997). Molecular tools in plant genetic conservation: A guide to technologies. IPGRC technical bulletin No. 2, Rome, Italy. Pp. 9-42.

Krapovickas, A. (1973). Evolution of genus Arachis. In: Agricultural genetics, Selected topics (Ed. R. Maov). National Council for Research and Development, Jerusalem. Pp. 35-157

Khan, M. K. (1990). Production and utility of chickpea (*Cicer arietinum* L.) in Pakistan. *Progressive Farming*, 10: 28-33.

Kwenin, W. K. J., Wolli, M. and Dzomeku , B. M. (2011). Assessing the nutritional value of some African indigenous green Leafy Vegetables in Ghana. *Journal of Animal & Plant Sciences,* Vol. **10 (2):** 1300- 1305. http://www.biosciences.elewa.org/JAPS

Lagerwerff, J. W. and Specht, A. W. (1970). Contamination of roadside and vegetation with cadmium, nickel, lead and zinc. *Environmental Science and Technology*. 4: 583-586.

Lahjie, A. M. and Siebert, B. (1987). Kelor or horse radish tree (*Moringa oleifera* Lam.). A report from East Kalimantan. German Forestry Group, Mulawarman Univ., GFG report 6: 41–43.

Lalas S., Tsaknis J. and Sflomos K. (2003). Characterisation of *Moringa stenopetala* seed oil variety "Marigat" from island Kokwa. *European Journal of Lipid Science and Technology*, 105: 123–31

Lalas, S. and Tsaknis, J. (2002). Extraction and identification of natural antioxidants from the seeds of *Moringa oleifera* tree variety of Malavi. *Journal of the American* Oil *Chemists' Society*, 79: 677–683.

Lerda, D. (1992). The effect of lead on *Alium cepa* L. *Mutation Research*, 231: 80-92.

Liu, J. and Furnier, G. P. (1993). Comparism of allozyme, RFLP and RAPD markers for revealing genetic variation within and between trembling aspen and bigtooth aspen. Theoretical and Applied genetics 87: 97-105.

Lyman, J. K. (1984). Progress and planning for germplasm conservation of major food crops. *Plant Genetic Resources Newsletter*, 60 : 3-21

Madsen, M., Schlundt, J. and Omer, E. (1987). Effect of water coagulation by seeds of *Moringa oleifera* on bacterial concentration. *Journal* of *Tropical Medicine and Hygiene,* 90: 101–109.

Magat, S. S., Cecilia, M. Raquepo, M. and. Pabustan, D. C. (2009). Mineral Macro-Nutrients, Micro-Nutrients and Other Elements in Leaves of Malunggay Plant (*Moringa Oleifera*).Plant and Soil Analysis Division, Agricultural Research Management Department, R & D, and Extension Branch, Philippine Coconut Authority, Diliman, Quezon City 1101

Mahajan, S. and Sharma, Y. K., (1984). Production of rayon grade pulp from *Moringa oleifera. Indian Forester* 110(3): 303–306.

Mahmood, K. T., Mugal, T. and Huq, I. U. (2010). Moringa oleifera: A natural gift-A review. *Journal of Pharmaceutical Science and Research*, 2(11): 775-781

Makkar, L. M. and Becker, K. (1997). Nutrients and antiquality factors in different Morphological parts of the *Moringa oleifera* tree. *Journal of Agricultural Science*, 128: 311-322.

Makonnen, E., Hunde, A. and Damecha, G. (1997). Hypoglycaemic effect of Moringa stenopetala aqueous extract in rabbits. *Phytotherapy Research,* 11: 147–148.

Maroyi, A. (2006). Resources users' knowledge and overexploitation of plant species in Zimbabwe. In: http://www.jsd_africa.com/jsda/summer_2006/pdf/ARC_utilisation.Moleifera.pdf pp 1-9

Martyn, C. N., Barker, D. J. P., Osmond, C., Harris, E. C., Edwardson, J. A. and Lacey, R. F., (1989). Geographical relation between Alzheimer's disease and aluminum in drinking water. *Lancet* 1: 59–62

Mallevialle, J. Bruchet, A. and Fiessinger, F. (1984). How safe are organic polymers in water treatment. *Journal of American Water Works Association,* 76 (6):431-436

Manzoor, M., Anwar, F., Iqbal, T. and Bhnager, M. I. 2007. Physico-chemical characterization of *Moringa concanensis* seeds and seed oil. *Journal of American. Oil Chemists' Society,* 84: 413-419.

Massal, E. and Barrau. J. (1956). Food plants of the South Sea Islands. Tech. Paper 94. South Pacific Commission, Noumea, New Caledonia.

Mehta, L. K., Balaraman, R., Amin, A. H., Bafna, P. A. and Gulati, O. D., (2003). Effect of fruits of Moringa oleifera on the lipid profile of normal and hypercholesterolaemic rabbits. *Journal of Ethnopharmacology,* 86: 191–195

Merrill, J. C., Morton, J. J. P. and Soileau, S. D. (2007). "Metals". In Hayes, A.W.. *Principles and Methods of Toxicology, 5th edition*. CRC Press. p 862 ISBN 084933778X.

Miller, R. G., Kopfler, F. C., Kelty, K. C., Stober, J. A. and Ulmer, N. S., (1984). The occurrence of aluminum in drinking water. *Jounal of American Water Works Association,* 76: 84–91.

Mondini, L., Noorani, A. and Pagnotta, A. M. (2009). Review: Assessing Plant Genetic Diversity by Molecular Tools. *Diversity* (1): 19-35. doi:10.3390/d1010019
www.mdpi.com/journal/diversity

Morton, J. F. (1991). The horseradish tree, *Moringa pterygosperma* (Moringaceae) – a boon to arid lands. *Economic Botany*, 45(3): 318–333

Muhammad, A., Dangoggo, S. M., Tsafe, A. I., Itodo, A. U. and Atiku, F. A. (2011). Proximate, minerals and anti-nutritional factors of *Gardenia aqualla (Gauden dutse)* fruit pulp. In *Pakistan Journal of Nutrition*, 10(6): 577-581

Muluvi, G. M., Sprent, J. I., Odee, D. and Powell, W. (2004). Estimates of out crossing rates in *Moringa oleifera* Amplified Fragment Length Polymorphism (AFLP). *African Journal of Biotechnology* 3: 146-151.

Muluvi, G. M., Sprent, J. I., Soranzo, N., Provan, J., Odee, D., Folkard, G., McNicol, J.W. and Powell, W. (1999). Amplified fragment length polymorphism (AFLP) analysis of genetic variation in *Moringa oleifera* Lam. *Molecular Ecology*, 8: 463 – 470.

Murphy, R. W., Sites, J. W., Buth, D. G. and Haufler, C. H. (1990). Protein isozymes eletrophoresis. In: *Molecular systematic*. D.H. Hillis and C. Moritz (Eds): *Sinnauer Association, Sunderland*, MA pp. 45-126.

Muyibi, S. A. & Okuofu, C. A. (1995). Coagulation of low turbid surface water with *Moringa oleifera* seeds. *International Journal of Environmental Studies*, 46:263-273. Also available http://cat.inistfr/amodele=afficheN$cpsidt=3141587

Muyibi, S. A. and Evison, L. M., (1995b). Optimizing physical parameters affecting coagulation of turbid water with *Moringa oleifera* seeds. *Water.Resources*, 29: 2689–2695.

Muyibi, S. A. (1994). The potential of Zogale (*Moringa oleifera*) seeds as a water treatment chemical. *Nigeria Society of Engineers*, 29: 27–33.

Nabhan, G. P. (1985). Native crop diversity in Aridoamerica: conservation of regional gene pools. *Economic Botany*, 39: 387-399

Nagajyoti, P. C., Dinakar, N., Prasad, T. N. V. K. V., Suresh, C. and Damodharam, T. (2008). Heavy metal toxicity: Industrial Effluent Effect on Groundnut (*Arachis hypogaea* L.) seedlings. *Journal of Applied Sciences Research*, 4(1): 110-121.

Najm, I., Tate, C. and Selby, D. (1998). Optimizing enhanced coagulation with PAC a case study, *Journal of American Water Works Association*, 90(10):88-95

Nakajima, K. (1994). Biotechnology for crop improvement in Japan. In: *Biotechnology application in agriculture in Asia and Pacific*. *Published by Asian Productivity Organization*. 87-107.

Nautiyal, B. P. and Venhataraman, K. G. (1987). Moringa (Drumstick) – an ideal tree for social forestry: Growing conditions and uses-Part 1. MYFOREST 1987; 23(1):53–58.

Needleman, H. (2004). "Lead poisoning.". *Annual review of medicine*, 55: 209–22. doi:10.1146/annurev.med.55.091902.103653. PMID 14746518.

Nei, M. and Li, W. H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National academy of Sciences of the United States of America 76: 5269–5273.

Ndabigengesere, A. and Narasiah, K. S. (1998). Quality of water treated by coagulation using *Moringa oleifera* seeds. *Water.Resources*, 32: 781–791.

Nisar, M., Ghafoor, A., Rashid Khan, M., Ahmad, H., Qureshi, A. S. and Ali, H (2007). Genetic Diversity and Geographic Relationship among Local and Exotic Chickpea Germplasm. *Pakistan Journal of Botany*, 39(5): 1575-1581, 2007.

Magat, S. S., Ma. Raquepo, C. M. and Pabustan, C. D. (2009). Mineral macro-nutrients, micro-nutrients and other elements in leaves of Malunggay plant (*Moringa Oleifera*) sampled in some locations in the Philippines. Crop Agronomy, Nutrition & Farming Systems Program Technology-Advisory Notes. Plant and Soil Analysis Division, Agricultural Research Management Department, R & D, and Extension Branch, Philippine Coconut Authority, Diliman, Quezon City 1101. http://malunggayherbatea.multiply.com/journal/item/2.

Obioma, U. N. and Adikwu, M. U. (1997). Investigation on some physiochemical antioxidant and toxicological properties of Moringa oleifera seed oil. *Acta Pharm* 47: 287–290.

Oduro, I., Ellis, W. O. and Owusu, D. (2008). Nutritional potential of two leafy vegetables: *Moringa oleifera* and *Ipomoea batatas* leaves. *Scientific Research and Essay*. 3(2): 57–60.

Oke, L. O. and Ojofeitimi, O. (1988). Nutrition for Nurses, tropical 2nd Ed. health series. London group Ltd. Pp 91-92.

Okuda, T., Baes, A. U., Nishitimas, W. and Okada, M. (2001). Isolation and characterization of coagulant extracted from Moringa Oleifera seed by salt solution. *Water.Resources,* 35(2): 405-41 www.elservier.com/located/waters.downloaded

Oliver-Bever, (2009). Medicinal Plants in Tropical West Africa, 375 pages, Cambridge University Press. Revised Edition, pp. 21, 349-363

Oliveira, J. T. A., Silveira, S. B., Vasconcelos, I. M., Cavada, B. S. and Moreira, R. A., (1999). Compositional and nutritional attributes of seeds from the multipurpose tree *Moringa oleifera* Lamarck. *Journal of the Science* of *Food and Agriculture*, 79: 815–820.

Olsen, A. (1987). Low technology water purification by bentonite clay and *Moringa oleifera* seed flocculation as performed in Sudanese villages: effects on Schistosoma mansoni cercariae. *Water Resources,* 21: 517–522.

Olson, M. E. (2001). Introduction to the Moringa family. In: "The miracle tree/ the multiple attributes of Moringa". Pp. 11-28 (Ed. Lowell J. Fuglie) CTA. USA

Olson, M. E. (2002). Combining data from DNA sequences and morphology for a phylogeny of Moringaceae (Brassicales). *Systematic Botany*, 27: 55–73.

Olugbemi, T. S., Mutayoba, S. K. and Lekule, F. P. (2010). Effect of Moringa (*Moringa oleifera*) Inclusion in Cassava based diets to broiler chickens. In *International Journal of Poultry Science*, 9(4): 363-367.

Oluyemi, E. A., Akilua, A. A., Adenuya, A. A. and Adebayo, M. B. (2006). Mineral contents of some commonly consumed Nigerian foods. *Science Focus,* 11: 153-157.

Onwuliri, F. C. and Dawang, N. D. (2006). Antimicrobial activity of aqueous and ethanolic leaf extract of drumstick plant (*Moringa oleifera* Lam) on some bacteria species associated with gastro intestinal diseases, *Nigerian Journal of Botany*, 9(2): 272-279.

Owusu-Ansah, M. (2010). Investigation into propagation and characterisation of *Moringa oleifera* Lamarck). MPhil. Thesis. University of Ghana, Ghana.

Padmarao, P., Acharya, B. M. and Dennis, T. J. (1996). Pharmacognostic study on stembark of *Moringa oleifera* Lam. *Bulletin of Medico-Ethno-Botanical Research*, 17: 141–151.

Pal, S. K., Mukherjee, P. K. and Saha, B. P. (1995). Studies on the antiulcer activity of *Moringa oleifera* leaf extract on gastric ulcer models in rats. *Phytotherapy Researc*h 9(6): 463-465.

Palada, M. C. and Changl, L. C., (2003). Suggested cultural practices for Moringa. International Cooperators' Guide AVRDC. AVRDC pub # 03–545: 1–5. www.avrdc.org

Pallavi, J. and Dipika, M. (2010). Effect of dehydration on the nutritive value of drumstick leaves. *Journal of Metabolomics and Systems Biology* Vol. 1(1), pp. 5 -9

Palmer, E. and Pitman, N. (1972). Trees of Southern Africa covering all known indigenous species in the republic of South-west Africa, Botswana, Lesotho and Swaziland. A. A. Balkema (Ed.). 2, 202-205. Cape Town pub.

Parker, P. G., Snow, A. A., Schug, M. D., Booton, G. C. and Fuerst, P.A. (1998). What molecules can tell us about populations: choosing and using molecular markers. *Ecology*, 79: 361-382.

Patel, S., Thakur, A. S., Chandy, A. and Manigauha, A. (2010). *Moringa oleifera*: A review of their medicinal and economic importance to health and nation. *Drug investigation today* 2(7): 339-342

Pearson, D. (1976). The chemical Analysis of foods. Churchill livingstone. New York. 7th edn. p. 54.

Plant Resources of Tropical Africa, PROTA foundation, (2008). Plant Resources of Tropical Africa, Medicinal Plants. G. H. Schmelzer and A. Gurib-Fakim (Ed.). 11, 14-11.

Powell, W. (1992). Plant genomes. Gene markers and linkage maps. In: Biotechnology and Crop improvement in Asia (Ed. J. P. Moss). Pp. 297-322.

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S. and Rafalski, A. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding*, 2: 225- 238.

Price, M. L. (1985a). The benzolive tree, *Moringa oleifera*, is called Mother's best friend. ECHO (Educational Concerns for Hunger Org.) Development Notes. Issue No. 10:1.

Proctor, G. E. (1984). Flora of the Cayman Islands. Kew Bull. Additional Ser. XI. Her Majesty's Stationery Office, London.

Qaiser, M. (1973). Moringaceae.In Flora of West Pakistan, Nasir E, Ali SI (Eds). No.38. University of Karachi Press: Karachi, 1–4.

Qui, X. X., Huang, D. F., Cai, S. X., Chen, F., Ren, Z. G. and Cai, Y. C. (2000). Investigations on vegetables pollution and pollution sources and its control in Fuzhou, Fujian Province. *Fujian Journal of Agriculture and Science*, 15: 16-21

Rabbani, M. A., Qureshi, A. A., Afzal, M., Anwar, R. and Komatsu. S. (2001). Characterization of mustard (*Brassica junncea* (L.) Cezern. and Cross) germplasm by SDS-PAGE of total seed protein. *Pakistan Journal of Botany*, 33(3): 173-179.

Rabouam, C., Comes, A. M., Bertagnolle, V. Humbert, J. F., Periquet, G. and Bigot, Y. (1999). Features of DNA fragments obtained by random amplified polymorphic DNA (RAPD) assay. *Molecular Ecology*, 8:439-503.

Ramachandran, C., Peter, K. V. and Gopalakrishnan, P. K. (1980). Drumstick *(Moringa oleifera): a* multipurpose Indian vegetable. *Economic Botany*, 34:276- 283.

Rajangam, J., Azahakia, M. R. S., Thamgaraj, T., Vijayakumar, A. and Muthukrishan, N. (2001). Status of production and utilization of Moringa in Southern India. In: "*The Miracle Tree/ The Multiple Attribute of Moringa* " Fugile L. J. (Ed.) CTA. USA. Pp 84

Rashid, P. and Mukhirji, S. (1993). Effect of foliar application of lead on the growth and yield parameters of wheat. *Pakistan Journal of Scientific and Industrial Research*, 36: 473-475.

Reddy, V., Urooj, A. and Kumar, A. (2005). Evaluation of antioxidant activity of some plant extracts and their application in biscuits. *Food Chemistry*, 90(2): 317–321.

Resmi, D. S., Celine, V. A. and Rajamony, L. (2005). Variability among drumstick (*Moringa oleifera* Lam.) accessions from Central and Southern Kerala. *Journal of Tropical Agriculture* 43 (1-2): 83-85.

Reyes, S. N. (2006). *Moringa oleifera* and *Cratylia argentea*: potential fodder species for ruminants in Nicaragua. Doctoral thesis, Swedish University of Agricultural Sciences, Uppsala.

Rossi, E. (2008). "Low level environmental lead exposure—a continuing challenge.". *The Clinical biochemist. Reviews / Australian Association of Clinical Biochemists*, 29 (2): 63–70. PMC 2533151. PMID 18787644.

Ruckmani, K., Kavimani, S., Anandan, R. and Jaykar, B. (1998). Effect of *Moringa oleifera* Lam on paracetamol-induced hepatoxicity. *Indian Journal of Pharmaceutical Sciences*, 60: 33–35.

Saleem, R. and Meinwald, J. (2000). Synthesis of novel hypotensive aromatic thiocarbamate glycosides. *Journal of the Chemical Society-Perkin Transactions*, 1: 391-394.

Sánchez-Machado, D. I., Núñez-Gastélum, A. J., Reyes-Moreno, C., Ramírez-Wong, B and López-Cervantes, J. (2009). Nutritional Quality of Edible Parts of *Moringa oleifera*. *Springer Science*, 3:175–180

Satija, D. R., Adrash, B., Gupta, S. K. and Bala, A. (2002). Genetic diversity in relation to protein and protein fractions in chickpea (*Cicer arietinum* L.). *Crop-Improvement*, 29(2): 122-135; 21

Satyan K. S., Swamy N., Dizon D. S., Singh R., Granai C. O. and Brard L. (2006). Phenethyl isothiocyanate (PEITC) inhibits growth of ovarian cancer cells by inducing apoptosis: role of caspase and MAPK activation. *Gynecologic Oncology*, 103:261–270.

Sauerbeck, D. (1982). Welche Schwermetallgehalte in Pflanzen dürfen nicht überschritten werden, um Wachstumsbeeinträchtigungen zu vermeiden? Landw. Forsch. *Sonderheft, Kongressband.* 39: 108-129.

Shafiq, M. and Iqbal, M. Z. (2005). The toxicity effects of heavy metals on germination and seedling growth of *Cassia siamea*. Lamark. *Journal of New Seeds*, 7: 95-105.

Sharma, G. K. and Raina, V. (1982). Propagation techniques of *Moringa oleifera* Lam. In: Improvement of Forest Biomass, Indian Society of Tree Scientists, Solan, HP, India, 175–181.

Sharma, P., Kumari, P., Srivastava, M. M. and Srivastava, S. (2006). Removal of cadmium from aqueous system by shelled *Moringa oleifera* Lam. seed powder. *Bioresource Technology*, 97: 299–305.

Shaukat, S. S., Mushtaq, M. and Siddiqui, Z. S. (1999). Effects of cadmium, chromium and lead on seed germination, early seedling growth and phenolic contents of *Parkinsonia aculeate* L. and *Pennisatum americanum* (L.) Schumann. *Pakistan Journal of Biological Sciences,* 2: 1307-1313.

Shecter, Y. and deWet, J. M. (1975). Comparative electrophoresis and isozyme analysis of seed protein from cultivated races of sorghum. *American Journal of Botany*, 62: 254-261

Siddhuraju P. and Becker K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agro-climatic origins of drumstick tree (*Moringa oleifera* Lam.). *Journal of Agriculture and Food Chemistry,* 15: 2144–2155.

Singh, U. and Singh, B. (1992). Tropical grain legumes as important human food. *Economic Botany*, 46(3): 310-2.

Singh, U., Wadhwani, A. M. and Johri, B. M., (1983). Dictionary of Economic Plants in India. 2nd ed., Indian Counc. Agric. Res., New Delhi.

Smith, J. S. C., Smith, O. S. (1989). The Description and Assessment of Distance between Inbred Lines of Maize: II. The Utility of Morphological, Biochemical,Genetic Descriptors and a Scheme for the Testing of Distinctiveness Between Inbred Lines. Maydica, 34:151-161

Sokal R. R. and Michener C. D. *(*1958*).* A Statistical Method for Evaluating Systematic Relationships. The University of Kansas Scientific Bulletin 38: 1409-1438.

Subudhi, K. P. and Huang, N. (1999). RAPD mapping in a doubled haploid population of rice *(Oryza sativa* L.). *Hereditas* 130: 41 -49.

Sumalia, P. (Food and Drugs board of Ghana. Introduction to Moringa into Ghana) (personal communication; August, 2008).

Szolnokim T. W. (1985). Food and fruit trees of the Gambia. Bundesforschungsanst. für Forst-und Holzwirtschaft, Hamburg.

The Wealth of India (A Dictionary of Indian Raw Materials and Industrial Products). (1962). Raw Materials, Vol. VI: L-M; Council of Scientific and Industrial Research: New Delhi, 425–429.

Tanksley, S. D. (1983). Molecular Markers in Plant Breeding. *PI. Mol. BioI. Rep*., 1: 3-8.

Tanksley, S. D. (1993). Mapping polygenes. *Annual Review of Genetics*, 27 : 205-233.

Trees for Life, (2005). *Moringa Book* [Brochure]. Balbir Mathur; Wichita, Kansas: http://www.treesforlife.org/documents/moringa/presentation/Moringa%20Presentation%20%28General%29%20screen.pdf

Tingey, S. V. and Tufo, P. (1993). Genetic analysis with Random amplified polymorphic DNA markers. *Plant physiology*, 101: 349-352.

Tsaknis, J., Lalas, S., Gergis, V., Dourtoglou, V., and Spiliotis, V. (1999). Characterization of *Moringa oleifera* variety 'Mbololo' seed oil of Kenya. *Journal of Agriculture and Food Chemistry*, 47: 4495-4499.

United State Department of Agriculture, Agricultural Research Service (2011). USDA National Nutrient Database for Standard Reference, Release 24. Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/ba/bhnrc/ndl

Vasconcelos, I. M., Maia, A. A. B., Siebra, E. A., Oliveira, J. T. A., Carvalho, A. F. F. U., Melo, V. M. M., *et al.*, (2001). Nutritional study of two Brazilian soybean (Glycine max) cultivars differing in the contents of antinutritional and toxic proteins. *Journal of Nutritional Biochemistry*, 12(1): 55-62.

Verma, S. C., Banerji, R., Misra, G. and Nigam, S. K. (1976). Nutritional value of Moringa. *Current Science*, 45(21): 769–770.

Vivien, J. (1990). Wild fruit trees of Cameroon. Fruits (Paris), 45(2); 149–160.

Vlahov, G., Chepkwony, P. K. and Ndalut, P. K. (2002). 13C NMR characterization of triacylglycerols of *Moringa oleifera* seed oil: an "oleic-vaccenic acid" oil. *Journal of Agriculture and Food Chemistry,* 50, 970–975.

Von Maydell H. J. (1986). Trees and Shrubs of Sahel, Their Characterization and Uses. Deutsche Gesellschaft fur Technische Zusammenarbeit, Germany: Eschborn, 334–337.

Welsh, J. and McClelland, M. (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research,* 18, 7213-7218.

Wierzbicka, M. and Obidzinska, J. (1998). The effect of lead on seed imbibition and germination in different plant species. *Plant Science,* 137: 155-171.

Williams. J. G. K., Kubelik, A. R.., Livak, K. J., Rafalski, J. A. and Tingey, S. V. (1991). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research,* 18: 6531-6535.

WHO (1995). Lead. Environmental Health Criteria, Geneva. 165.

Wong, J. W., Li, G. X. and Wong, M. H. (1996). The growth of *Brassica chinensis* in heavy metal contaminated sludge compost from Hong Kong. *Bioresource Technology,* 58: 209-313.

Woolf, A. D., Goldman, R. and Bellinger, D. C. (2007). "Update on the clinical management of childhood lead poisoning.". *Pediatric Clinics of North America*, 54(2): 271–94 doi:10.1016/j.pcl.2007.01.008. PMID 17448360.

World Agroforestry Center. Agroforestry database. http://www.worldagroforestry.org/Sites/TreeDBS/AFT/SpeciesInfo.cfm?SpID=1169

Wormadey, A. (2007). Moringa: Most nutrient- rich plant on earth. *The Weekly Mirror*. Graphic Cocrperation Group, Pub. 9th May, 2007 edition. Pp 8

Zimmerer, K. S. and Douches, D. S. (1991). Geographical approaches to crop conservation: the partitioning of genetic diversity in Andean potatoes. *Economic Botany,* 45: 176-189

APPENDICES

Appendix 1: Passport data of 36 accessions (seeds) of *M. oleifera* used for morphological studies

Acc. Code	Town Collected	Region Collected	Acc. Collector
AS01	Kwadaso	Ashanti	Mr. K. Opare-Obuobi
AS02	Adiebra TUC	Ashanti	Mr. K. Opare-Obuobi
BA01	Bomaa Ward B	Brong Ahafo	Mr. E. W. Anyomi
BA02	Akrudwa	Brong Ahafo	Mr. D. Opare-Obuobi
E02	Akusa	Eastern	Mr. K. Opare-Obuobi & Mr. P. Mireku
E04	Mampong	Eastern	Mr. K. Opare-Obuobi
E05	Nsawam prison	Eastern	Mr. A. Bajo
E10	Kpong	Eastern	Mr. K. Opare-Obuobi & Mr. P. Mireku
E11		Eastern	Mr. K. Opare-Obuobi & Mr. Ankara
GA02	Amasaman	Greater Accra	Mr. K. Opare-Obuobi & Mr. Ankara
GA03	Dzorwulu (Methodist church)	Greater Accra	Mr. K. Opare-Obuobi
GA04	Tifa Daavi	Greater Accra	Mr. K. Opare-Obuobi
GA07	Association sch. (Airport residential area)	Greater Accra	Mr. K. Opare-Obuobi
GA10	Patrice lumuber road (Opp. Ent. Insurance)	Greater Accra	Mr. K. Opare-Obuobi
GA15	ARS- Nungua	Greater Accra	Mr. K. Opare-Obuobi
N03	Kpalsogu (House no. 23) Tolon Kumbungu	Northern	
N05	Shishegu (House no. 861) Tamale metro	Northern	
N08	Shishegu (House no. 916) Tamale metro	Northern	
UE02	Manga (CSIR- SARI station)	Upper East	Mr. F. Kusi
UE05	Bawku central	Upper East	Mr. F. Kusi
UW01	Tumu (Agric village)	Upper West	Mr. A. Haruna
UW02	Sissala east dist. -Tumu (Market square)	Upper West	Mr. A. Haruna
V01	Juapong	Volta	Mr. K. Opare-Obuobi & Mr. P. Mireku
V02	Dzodze	Volta	Mr. E. Sapei
BNR04	USA	Abroad	Department of Horticulture, KNUST
BNR05	Wa	Upper West	Mr. Arhin, ADRA-Ghana
BNR06	USA	Abroad	Department of Horticulture, KNUST
BNR07	South Volta	Volta	Mr. Nii Marcus Arday
BNR08	Wa	Upper West	Mrs. Rebecca Gyedu Karikari

BNR09	South Volta	Volta	Miss Peace Gbecko-Kove
BNR10	Bolgatanga	Upper East	Mrs Grace Tsema
BNR11	Bolgatanga	Upper East	Mrs Grace Tsema
BNR12	Bolgatanga	Upper East	Mrs Grace Tsema
BNR13	Bolgatanga	Upper East	Mrs Grace Tsema
BNR14	India	Abroad	Mr. Pascal/ Amaglo, India
BNR15	CSRPM- Mampong-Akwapim	Eastern	CSRPM- Mampong-Akwapem

Appendix 2: Passport data of 40 accessions (stem cutting) of *M. oleifera* used for biochemical and molecular studies

Acc. Code	Town Collected	Region Collected	Acc. Collector
E01	Koforidua	Eastern	Mr. K. Opare-Obuobi & Mr. D. Djan
E09	Asikuma	Eastern	Mr. K. Opare-Obuobi & Mr. Ankarah
E38	Aburi	Eastern	Mr. K. Opare-Obuobi & Mr. Ankarah
E40	Yaw Adepa	Eastern	Mr. Opare-Obuobi & Mr. E. O. Ankrah
E41	Ahwodjo	Eastern	Mr. Opare-Obuobi & Mr. E. O. Ankrah
GA15	ARS Nungua	Greater Accra	Mr. K. Opare-Obuobi
GA42a	Pokuase 1	Greater Accra	Mr. A. Bajo
GA42b	Pokuase 2	Greater Accra	Mr. A. Bajo
*GA54	Dzorwulu (Mehodist Church)	Greater Accra	Mr. K. Opare-Obuobi
GA59	Tifa	Greater Accra	Mr. K. Opare-Obuobi
GA60	Airport Res. Area	Greater Accra	Mr. K. Opare-Obuobi
N02	Kasalga Tamale metro (Shea butter village)	Northern	Mr. Agyare
N10	Nyankpala (SARI quarters 8) Tolon-Kumbungu	Northern	Mr. Agyare
N15	Kpalsogu (house no. 27) Tolon-Kumbungu	Northern	Mr. Agyare
N17	Kpalsogu (house no. 9) Tolon-Kumbungu	Northern	Mr. Agyare
UE46	Nyorego	Upper East	Mr. F. Kusi
UE47	Manga-SARI station	Upper East	Mr. F. Kusi
UE49	Bolga- Kumbosko	Upper East	Mr. F. Kusi
UW01	Sissala West dist. Pulma village	Upper West	Mr. A. Haruna
UW02	Sorbelle village Dam site	Upper West	Mr. A. Haruna
*UW03	Sorbelle village - Near Techno-serve farm stores	Upper West	Mr. A. Haruna

UW04	Funsi- Near Police station	Upper West	Mr. A. Haruna
V04	Kpong	Volta	Mr. K. Opare-Obuobi & Mr. P. Mireku
V05	Sokode Gbogame	Volta	Mr. K. Opare-Obuobi & Mr. P. Mireku
V06	Sokode Lokoe	Volta	Mr. K. Opare-Obuobi & Mr. P. Mireku
V08	Kpeve	Volta	Mr. K. Opare-Obuobi & Mr. P. Mireku
BNR 2	USA	Abroad	Department of Horticulture, KNUST
BNR 3	Legon	Greater Accra	Agric. Science Research farm, UG
BNR 4	USA	Abroad	Department of Horticulture, KNUST
BNR 5	Wa	Upper West	Mr. Arhin, ADRA-Ghana
BNR 6	USA	Abroad	Department of Horticulture, KNUST
BNR 7	South Volta	Volta	Mr. Nii Marcus Arday
BNR 8	Wa	Upper West	Mrs. Rebecca Gyedu Karikari
BNR 9	South Volta	Volta	Miss Peace Gbecko-Kove
BNR 10	Bolgatanga	Upper East	Mrs Grace Tsema
BNR 11	Bolgatanga	Upper East	Mrs Grace Tsema
BNR 12	Bolgatanga	Upper East	Mrs Grace Tsema
BNR 13	Bolgatanga	Upper East	Mrs Grace Tsema
BNR 14	India	Abroad	Mr. Pascal/ Amaglo, India
BNR 15	CSRPM - Mampong-Akwapem	Eastern	CSRPM- Mampong-Akwapem

* Accession not included in Total Protein studies

Appendix 3: Buffer Systems and Electrophoresis Solutions

a. Sample Buffer (SDS reducing buffer)

Chemical Composition	**Quantity (ml)**
Deionised water	3.55
0.5M Tris-Citric HCl, pH 6.8	1.25
Glycerol	2.5
10% (w/v) SDS	2.0
0.5% (w/v) bromophenol blue	0.2
*β-mercaptoethanol	0.5
Total volume	**9.5**

*added to solution prior to use.

b. Staining Solution

Chemical Composition	Quantity (ml)
Coomassie blue	0.25g
50% Methanol	90ml
Glacial acetic acid	10ml

c. Destaining Solution

Chemical Composition	Quantity (ml)
Methanol	150ml
Glacial acetic acid	50ml

APPENDIX 4: Composition of solutions for Gel Preparation

Stock Solutions and Buffers

a. Acrylamide/Bis (30% T, 2.6% C)

Chemical Composition	Quantity
Acrylamide (29.2g/100ml)	87.6g
N'N'-bis-methylene-acrylamide (0.8g/100ml)	2.4g

b. 10% (w/v) SDS

10g SDS dissolved in 90ml distilled water and topped up to the 100ml mark.

c. Gel buffer; 1.5M Tris-HCl, pH 8.8

Chemical Composition	Quantity
Tris base (18.15g/100ml)	27.23g
Deionised water	80ml

d. Stacking gel buffer; 0.5M Tris-HCl, pH 6.8

Chemical Composition	Quantity
Tris base	6g
Deionised water	60ml

e. 10x Electrode (running) buffer, pH 8.3

Chemical Composition	Quantity
Tris base	30.3g
Glycine	144.0g
SDS	10.0g

f. 10% Ammonium persulfate
100mg of ammonium persulfate dissolved in 1ml of deionised water

g. Separating gel Preparation (10%)

Chemical Composition	Quantity
Distilled H_2O	4.1ml
30% Acrylamide stock	3.3ml
Gel buffer stock (pH 8.8)	2.5ml
10% Ammonium per Sulphate (fresh)	50µl
TEMED	10µl

h. Stacking gel Preparation

Chemical Composition	Quantity
Distilled H_2O	5.7ml
30% Acrylamide stock	1.7ml
Gel buffer stock	2.5ml
10% SDS	0.1ml
10% Ammonium per Sulphate (fresh)	50µl
TEMED	5µl

APPENDIX 5: Microprep buffer

Chemical Composition	**Quantity**
DNA extraxct buffer	
Nuclei lysis buffer	
5% Sarkosyl	
Sodium Bisulfite*	0.4g/100mls

*added prior to use.

Appendix 6a: ANOVA for tripinnatum leaf length of *Moringa oleifera* accessions

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_I stratum	4	323.41	80.85	0.99	
Region	9	4901.47	544.61	6.67	<.001
Residual	36	2939.41	81.65		
Total	49	8164.30			
L.s.d p< 0.05	11.59				

Appendix 6b: ANOVA for tripinnatum leaf width of *Moringa oleifera* accessions

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_I stratum	4	243.53	60.88	0.70	
Region	9	4048.98	449.89	5.20	<.001
Residual	36	3112.04	86.45		
Total	49	7404.56			
L.s.d p< 0.05	11.93				

Appendix 6c: ANOVA for tripinnatum leaflet length of *Moringa oleifera* accessions

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_I stratum	4	46.50	11.62	0.53	
Region	9	972.56	108.06	4.96	<.001
Residual	36	785.11	21.81		
Total	49	1804.17			
L.s.d p< 0.05	5.99				

Appendix 6d: ANOVA for tripinnatum leaflet width of *Moringa oleifera* accessions

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_I stratum	4	84.55	21.14	0.88	
Region	9	528.07	58.67	2.45	0.027
Residual	36	863.57	23.99		
Total	49	1476.18			
L.s.d p< 0.05	6.282				

Appendix 7a: ANOVA for iron composition of dried leaf powder of *Moringa oleifera* accessions

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_I stratum	2	7.702E-06	3.851E-06	1.84	
Region	7	1.304E-02	1.862E-03	890.30	<.001
Residual	14	2.928E-05	2.092E-06		
Total	23	1.307E-02			
L.s.d p< 0.05	0.0025				

Appendix 7b: ANOVA for manganese composition of dried leaf powder of *Moringa oleifera* accessions

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_I stratum	2	1.703E-06	8.517E-07	2.41	
Region	7	2.102E-04	3.002E-05	84.80	<.001
Residual	14	4.957E-06	3.540E-07		
Total	23	2.168E-04			
L.s.d p< 0.05	0.0010				

Appendix 7c: ANOVA for zinc composition of dried leaf powder of *Moringa oleifera* accessions

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_I stratum	2	5.403E-06	2.701E-06	1.14	
Region	7	2.832E-04	4.046E-05	17.01	<.001
Residual	14	3.330E-05	2.379E-06		
Total	23	3.219E-04			
L.s.d p< 0.05	0.0027				

Appendix 7d: ANOVA for lead composition of dried leaf powder of *Moringa oleifera* accessions

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_I stratum	2	1.351E-06	6.754E-07	3.26	
Region	7	5.555E-05	7.935E-06	38.27	<.001
Residual	14	2.903E-06	2.073E-07		
Total	23	5.980E-05			
L.s.d p< 0.05	0.0008				

Appendix 8a: **ANOVA for calcium composition of dried leaf powder leaf of *Moringa oleifera* accessions**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_I stratum	2	67.29	33.64	1.06	
Region	7	3334.71	476.39	15.08	<.001
Residual	14	442.37	31.60		
Total	23	3844.36			
L.s.d p< 0.05	9.84				

Appendix 8b: **ANOVA for magnesium composition of dried powder of *Moringa oleifera* accessions**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_I stratum	2	4.331	2.165	0.28	
Region	7	1830.316	261.474	33.72	<.001
Residual	14	108.563	7.754		
Total	23	1943.21			
L.s.d p< 0.05	4.877				

Appendix 8c: **ANOVA for sodium composition of dried leaf powder leaf of *Moringa oleifera* accessions**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_I stratum	2	4119.0	2059.5	4.13	
Region	7	50364.6	7194.9	14.42	<.001
Residual	14	6985.8	499.0		
Total	23	61469.4			
L.s.d p< 0.05	39.12				

Appendix 8d: **ANOVA for phosphorus composition of dried powder of *Moringa oleifera* accessions**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_I stratum	2	0.002461	0.001230	0.27	
Region	7	0.203541	0.029077	6.47	0.002
Residual	14	0.062880	0.004491		
Total	23	0.268882			
L.s.d p< 0.05	0.1174				

Appendix 8e: **ANOVA for potassium composition of dried leaf powder leaf of *Moringa oleifera* accessions**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_I stratum	2	149113	74557	0.16	
Region	7	2959814	422831	0.93	0.512
Residual	14	6344706	453193		
Total	23	9453634			
L.s.d p< 0.05	1178.9				

Appendix 9a: **ANOVA for nitrogen composition of dried powder of *Moringa oleifera* accessions**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_I stratum	2	0.9884	0.4942	1.13	
Region	7	27.8557	3.9794	9.13	<.001
Residual	14	6.0990	0.4356		
Total	23	34.9431			
L.s.d p< 0.05	1.156				

Appendix 9b: ANOVA for crude protein composition of dried leaf powder of *Moringa oleifera* accessions

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_I stratum	2	147.20	73.60	0.86	
Region	7	312.63	44.66	0.52	0.805
Residual	14	1202.88	85.92		
Total	23	1662.71			
L.s.d p< 0.05	ns				

Appendix 9c: **ANOVA for dry matter composition of dried powder of *Moringa oleifera* accessions**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep_I stratum	2	0.133	0.067	0.01	
Region	7	285.200	40.743	5.26	0.004
Residual	14	108.454	7.747		
Total	23	393.787			
L.s.d p< 0.05	4.874				

Appendix 10: AVRDC- GRSU CHARACTERIZATION RECORD SHEET

Crop: **Moringa** Accession No. _____

Sowing Date _____ Plot No. _____

Transplanting Date _____ Name _____

Location _____ Species _____

Origin _____

SEEDLING DATA

1. Germination period (no. of days from sowing to first germination)
2. Cotyledon Colour

1=light green 3=green 5=purplish green 7= purple 9=dark purple

3. Hypocotyl colour

1=light green 3=green 5=purplish green 7= purple

VEGETATIVE DATA

4. Growth habit

1= compact 2= intermediate 3= tall (erect)

5. Plant height (cm) (at flowering stage)
6. Tripinnatum leaf length (cm)
7. Tripinnatum leaf width (cm)
8. Leaf type

1= simple 2= compound

9. Leaf arrangement

1= opposite 2= alternate 3=pinnate 4=palmate

10. Leaflet shape

1=simple 2=compound

11. Leaflet arrangement

1= opposite 2= alternate 3=pinnate 4=palmate

12. Number of secondary rachis

13. Leaf Colour

1=Light green 3=Green 5= Dark green

14. Leaf flavour

1=bitter 3=normal 5=strong

15. Tripinnatum leaflet length (cm)

16. Tripinnatum leaflet width (cm)

17. Petiole pigmentation

1=Green 3=Dark Green 5= Purple 7=Dark Purple

18. Stem bark colour

1=Whitish 3=Green 5= Brown 7=Dark Brown

INFLORESCENCE DATA

19. Days to flowering (from sowing to 50% with inflorescence)

20. Flower Colour

1= white 2= cream

21. Flower aromatic

1=weak 5=Intermediate 7=strong

22. No. of Petals

23. Petal length

24. Petal width

25. No. of Sepals

26. Sepal length

27. Sepal width

28. Height of Pistil

29. Height of Stamen

FRUIT DATA

30. Pod Colour

1= green 2=greenish brown 3=brown

31. Pod length (cm)

32. Pod width (cm)

33. Number of seeds/pod

SEED DATA

34. Seed Colour

1=brown 2=dark brown

35. 1000-seed weight (g)

36. Pollen fertility