CHARACTERIZATION OF FINGER MILLET (*Eleusine Coracana* L. Gaertn.) GENOTYPES USING MORPHOLOGICAL TRAITS AND SIMPLE SEQUENCE REPEAT MARKERS

BY

AYORO FABIAN OLANG'O

REG. NO.: AGR/PGB/04/08

A THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE REQUIREMENT OF THE MASTER OF SCIENCE DEGREE IN PLANT BREEDING AND BIOTECHNOLOGY, DEPARTMENT OF BIOTECHNOLOGY, UNIVERSITY OF ELDORET, KENYA

AUGUST, 2013

DECLARATION

Declaration by the candidate

This thesis is my original work and has not been presented for a degree in any other University. No part of this thesis may be reproduced without prior permission of the author or University of Eldoret.

Fabian Ayoro Olang'o (AGR/PGB/04/08) ……..... Date …….....

School of Agriculture and Biotechnology,

Department of Biotechnology,

University of Eldoret

Approval

This thesis has been submitted for examination with our approval as the University Supervisors.

Prof. Miriam Kinyua ……..…….…… Date …..……..……

School of Agriculture and Biotechnology,

Department of Biotechnology,

University of Eldoret

Dr. Oliver Kiplagat …..……..... Date…….

School of Agriculture and Biotechnology,

Department of Biotechnology,

University of Eldoret

DEDICATION

To the God Almighty who created me

ABSTRACT

Finger millet is a crop with largely unexplored and unexplained potentially valuable genetic variability. The knowledge and understanding of the extent of genetic variation of finger millet germplasm is important for conservation and improvement. The study aimed at estimating the genetic divergence of finger millet genotypes using morphological and simple sequence repeat (SSR) markers. Fourteen finger millet genotypes were evaluated under field conditions in 3 sites replicated three times, for 37 morphological traits recorded by the International Board for Plant Genetic Resources (IBPGR) as the minimum that ideally should be used in the identification of any accession and in the laboratory using 12 SSR markers. The SSR markers had several advantages over other molecular markers: microsatellites allowed the identification of many alleles at a single locus, they were evenly distributed all over the genomes, they were co-dominant, little DNA was required and the analysis c be semi-automated and performed without the need of radioactivity .Findings from this study showed that The interaction between genotypes and site were significant ($p \leq 0.05$) for finger length and leaf blade density of hairs on upper side and ($p \leq 0.001$) for productive tillers, days to 50% flowering, days to physiological maturity, number of tillers, a thousand grain weight (g), grain yield per plant (g) and yield in Kg per hectare. Also the principal component analysis (PCA) indicated that the morphological traits principal component (PC) 1, 2 and 3 accounted for 16.98%, 14.17% and 12.45% of total variance, respectively, that is, 43.6% of the total variation. The dendrogram based on UPGMA cluster analysis revealed that SSR and morphological data indicated the existence of high divergence among the accessions. The cophenetic matrices obtained with morphological, SSR and combined data marker types were high, 0.81, 0.90 and 0.78, respectively. The consensus fork index (*CIc)* obtained from morphological and SSR data was 60% identical (*CIc*= 0.60). Both morphological descriptors and SSR markers were able to group the genotypes into distinct clusters. At certain stages, the morphological traits used were able to distinguish between cultivars and the molecular markers complemented the data obtained to detect genetic differences and were able to distinguish them into distinct genotypes with some located in different clusters. These results support the idea of combining both methods, to obtain a more accurate idea of the genotypes under study. I recommend that the findings of this research be used in the collection, conservation and documentation of finger millet genotypes and that this information is used for crop improvement and exploitation of the crop genotype in order to raise the standards of living of the consumers of the crop.

TABLE OF CONTENTS

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ACRONYMS

AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
CIAT	International Centre for Tropical Agriculture
CMD	Cassava mosaic disease
DArTs	Deletion Amplified Regions Tags
DNA	Deoxyribonucleic acid
EST	Expressed sequence tags
FAO	Food Agriculture Organization
Gy	Gray
HCN	Hydrogen cyanide
IITA	International Institute of Tropical Agriculture
MAJRUL	Majority rule
MAP	Months after planting
PCoA	Principal Coordinate Analysis
QTL	Quantitative trait loci
RAPD	Random amplified polymorphism
RCBD	Randomized Complete Block Design
RFLP	Restriction fragment length polymorphism
SIMQUAL	Similarity qualitative
SNP	Single Nucleotide Polymorphism
SSR	Simple sequence repeat
CGIAR	Consultative Group on International Agricultural Research

ACKNOWLEDGEMENT

First I thank Almighty God who has showered his boundless blessings on me enabling the successful completion of this research work. My sincere gratitude, appreciation and thanks also to Alliance for Green Revolution in Africa (AGRA) Foundation who funded this study. I take my immense pleasure to express my deep sense of gratitude to my supervisors Professor Miriam Kinyua and Dr. Oliver Kiplagat for having given me their intimous guidance, constant encouragement, constructive criticism, incessant inspiration, sustained patience and untiring attention at each stage of the research work. I also thank Kenya Seed Company for allowing me to undertake my study.

I express my heartfelt thanks to my dear classmates for their timely support, valuable suggestions for their utmost cooperation rendered at times of need. I am grateful to the affection and support the farmers who gave me their fields for my research. I also thank my colleagues at work for their encouragement and support they gave me.

I owe a great deal to my beloved parents, my family and my relatives for their blessings, continuous encouragement, overwhelming interest and guidance which they showered on me throughout my study. I humbly dedicate my thesis at the feet of my parents and God whose grace and blessings, love, continuous support and encouragement made it possible for me.

CHAPTER ONE

INTRODUCTION

1.1 Background

Finger millet (*Eleusine coracana* L. Gaertn.) is an important traditional food crop in many parts of Africa and Asia (Rao *et al.,* 1986; Ekwamu, 1991). It is a major staple food for millions of resource poor people in the semiarid tropics of Eastern Africa and is produced in a number of countries such as Uganda (500,000 ha), Kenya (65,000 ha) and Ethiopia (1 M ha). This cereal plays an important role in the dietary habits and economy of subsistence farmers. Staple foods prepared from the grain are major sources of minerals and nutrients and are especially important for pregnant women, nursing mothers and children (Oduori, 2000).

Finger millet in Kenya is in high demand for feeding infants, special dishes for the sick and for other special purposes among some people and, therefore, generally sold at several times the price of other cereals (Oduori, 2000). It has excellent storage and drought tolerant qualities. The grain is also used in different forms for food and the straw for feed, fuel and thatching. However, the national average grain yield of finger millet is low, 1.0 ton/ha, although it has a potential to yield up to 3 ton/ha (Mulatu *et al*., 1995).

Finger millet crop has wide diversity and variability that would benefit breeding programs. Plant breeding involves exploiting the genetic variability of specific traits for improvement . Over 2,500 accessions of finger millet have been collected in East and Southern Africa (Attere, 1993); Zimbabwe with over 600, Ethiopia with 1,318, Kenya with 1,136 and Uganda with 2000 accessions (Mushonga *et al*., 1993; Tadesse and Kebede, 1993; Oduori, 1993; Odelle, 1993).

The genetic development finger millet as a crop is still very low, it is about where wheat was in the 1890s (National Research Council, 1996). Since the 1890s, average yields of wheat have risen from about 500 kg/ha to more than 4,000 kg/ha; finger millet's could rise similarly and much more quickly because of the facts that it is a C_4 compared to wheat, a C_3 photosynthesizer and advanced breeding methodologies developed on other crops already exist. Compared to other cereal crops such as wheat, maize, barley, rice and sorghum, comprehensive study on finger millet diversity using morphological or molecular markers are limited (Bezaweletaw *et al.*, 2006; Upadhyaya *et al.,* 2006). This study was initiated, therefore, to assess the patterns of genetic variability, identify major traits contributing to variations among the genotypes for further utilization in the breeding programs and for germplasm collection and conservation.

1.2 Statement of the problem

Finger millet cultivation and production in Kenya is constrained by biotic and abiotic factors (FAO, 2009). Finger millet farmers face numerous challenges, including declining land fertility, high labor requirements for weeding, lack of high-yielding, well adapted varieties, diseases, and unavailability of seed of improved varieties, credit, marketing, weeds, pests and diseases. The problems also include competition from other crops, low government priority and limited research attention, and lack of processing equipment. Despite these challenges, finger millet is still widely used and valued; and new food products such as bread, malt fodder, feed, foods for babies and convalescents, have industrial potential. With more research and an enabling environment, the crop has great potential for expansion.

Finger millet is almost entirely self-pollinating crop and because of the small floret size, cross breeding with different genotypes is limited or can be done with difficulty . Indeed, only a few varieties with stability and wide adaptability persist over years. Data on multilocational testing of genotypes under diverse agro-ecological conditions for evaluation of yield potential, adaptability and stability is lacking, while necessary before recommending a genotype for release as variety. Selection of genotypes suitable for a specific use and place is little or no information on which a plant breeder can rely. A careful characterization is needed to define the existing genetic variability to improve the crop, either for consumption, industry or breeding programs. While breeding suitable varieties for Kenyan farmers, a major problem is to obtain information regarding the exploitation diversity of finger millet (Oduori, 2000).

1.3 Justification

In Kenya, finger millet grain is used as food and in brewing beer. But it can be used in a variety of other ways. Flour can be used for baking bread and various other products with good flavor and aroma; several brands of finger millet flour produced by different companies are available in Kenyan supermarkets. Due to the high traditional values attached to finger millet, the crop will continue to be grown (Obilana *et al.,* 2002). Because it is often grown in favorable production environments yields can be competitive with those of rice and other 'green revolution' cereals (CGIAR, 2001), especially if research efforts are increased (NRC, 1996).

Finger millet germplasm need to be characterized since knowledge of genetic diversity is a must for use in a breeding programme. The trials will enable testing suitability of varieties under different environments (Yang *et al.,* 2005). Identification of the finger millet genotypes adapted

to a particular place will be of benefit and save farmers from food insecurity. The study was aimed at estimating the genetic divergence of finger millet genotypes, in order to provide information that could be used in selection for desirable..

1.4 Objectives:

1.4.1 Main objective:

- To determine the variability of selected finger millet genotypes using both morphological and molecular markers.

1.4.2 Specific objectives:

- To determine the diversity of finger millet genotypes using morphological and agronomic traits.
- To determine the diversity of finger millet using SSR makers.
- To assess relatedness of phenotypic and molecular characterization of finger millet in determining their genetic variability.

1.5 Hypothesis:

H_O: Finger millet varieties do not reveal diversity morphologically and agronomically.

H_A: Finger millet varieties do reveal diversity morphologically and agronomically.

H_O: Simple sequence repeat makers do not reveal genetic diversity in finger millet

H_A: Simple sequence repeat makers reveal genetic diversity in finger millet.

H_O: Phenotypic and SSR characterization of finger millet do not give same results.

H_A: Phenotypic and SSR characterization of finger millet give same results.

CHAPTER TWO:

LITERATURE REVIEW

2.1 Origin and distribution of finger millet

Eleusine coracana is found in warm temperate regions of the world from Africa to Japan and also in Australia. It is present in archaeological records of early African agriculture in Ethiopia that date back 5000 years, and it probably originated somewhere in the area that today is Uganda (NRC, 1996). It is an important staple crop in many parts of Africa and has been cultivated in eastern and southern Africa since the beginning of the Iron Age. Before maize was introduced it was the staple crop of the southern African region. Finger millet (*Eleusine coracana*) is one of the most important millets worldwide which was originated and domesticated in the eastern African sub-humid uplands (NRC, 1996; Hilu *et al*., 1979).

2.2 Global Economic importance of finger millet

Archaeological excavations show that improved forms of finger millet were once the staple grain diet of southern Africa. In East Africa, where it is cultivated as a cereal, 4 races (*Elongata, Plana, Compacta and Vulgaris*) are distinguished based on inflorescence compactness and shape (Gibbs-Russell *et al*., 1989). It is a versatile grain that can be used in many different types of food. It is eaten by grinding the grains for porridge. Sometimes it is ground into flour and used for bread or various other baked products. The sprouted seeds are nutritious and easily digested food that is recommended for infants and the elderly (NRC, 1996). The grain may be left to germinate to make malt, which is very popular in Southern Africa due to the sweetness of the malt (Van Wyk and Gericke, 2000). Its ability to convert starch to sugar is surpassed only by

barley (NRC, 1996). Finger millet straw makes good fodder and contains up to 61% total digestible nutrients (NRC, 1996).

It is used in traditional medicine as an internal remedy for leprosy or liver disease (Van Wyk and Gericke, 2000). Parts of the plant (the leaves and culms) are used to plait bracelets (Gibbs-Russell *et al.*, 1989). A chemical hydrocyanic acid can be obtained from the plant. It is probably the most important weed in cultivated lands in southern Africa due to its exceptionally strong root system that makes it difficult to control mechanically. It is also listed as a weed in many other parts of the world, especially in North America. In Africa, the wild form (*E. coracana* subsp. *africana*) is considered to be a weed where the cultivated form (*E. coracana* subsp. *coracana*) is grown and is especially problematic since they are so similar in appearance.

2.3 Taxonomy

Finger millet (*Eleusine coracana* L. Gaertn.) is a member of the family Poacea and the tribe Chlorideae. It is considered to be a native crop of Eastern Africa. East Africa is believed to be one of the centres of origin and it has a long history of finger millet cultivation, and there is extensive variability among landraces, especially for panicle type (compactness and shape) (Rachie and Peters, 1978; de Wet *et al.*, 1984).

2.4 Genetics

The cultivated *Eleusine coracana* is a tetraploid (2n=4x=36). Chromosome base number, $x = 9$. *E. coracana* subspecies *coracana* a tetraploid is derived from the wild diploid subspecies *africana* (NRC, 1996). It has morphological similarity to *E. indica* (L) Gaertn (2n=18) and *E. Africana* (2n=36). The cytological evidences indicate that the A genome of the cultivated

Eleusine coracana (AABB) was contributed by *E. indica*, a ubiquitous weed of tropical and subtropical regions. Both *Eleusine tristachya* and *E. xoccifolia* have been considered potential B genome donors to *E. coracana* based on rDNA restriction patterns and genomic *in situ* hybridization (GISH) respectively (Hilu and Johnson 1992; Bisht and Mukai 2001). The latter study demonstrated that *E. tristachya* DNA hybridized to the same subset of *E. coracana* chromosomes as did *E. indica*, suggesting that *E. tristachya* is a B genome species. GISH patterns of *E. Xoccifolia* and *E. indica,* on the other hand, did not overlap. Subspecies *coracana* and africana hybridize readily, with more than 80% of the meiotic cells in the hybrid forming bivalents, suggesting that the two genomes are highly homoeologous (Hiremath and Salimath, 1992).

2.5 Morphology

Plate 1: Finger millet at grain at milk stage

Source: Author, 2011

Eleusine coracana is a tufted annual grass that grows to a height of 210-620 mm tall. The leaf blades are shiny, strongly keeled and difficult to break and are 220-500 mm long and 6-10 mm

wide. The leaves and culms are typically green in colour. It has an exceptionally strong root system and it is difficult to pull out of the ground by hand (Van Wyk and Van Oudtshoorn, 1999). The culms and the leaf sheaths are prominently flattened. The ligule is a fringed membrane. Inflorescence consists of spike-like main branches that are open or contracted and are digitate or sub-digitate. The spikelets are 5-8 mm long and 3-4 mm wide. The spikelets do not disarticulate (break apart at the joints) at maturity. The grains are globose. There are two subspecies of African finger millet, the wild form (*E. coracana* subsp. *africana*) and a cultivated form derived from it (*E. coracana* subsp. *coracana*). Wild African finger millet (*E. coracana* subsp. *africana*) is similar to Indian goose grass (*E. indica*) and may be confused with it, but the latter has smaller spikelets and oblong, not rounded, grains. The grains of the latter are unusual in that the outer layer (pericarp) is not fused and can be easily removed from the seed coat (Van Wyk and Gericke, 2000).

2.6 Agronomy and climatic requirements of finger millet

The wild form is found in areas with rainfall as low as 300 mm per annum in South Africa, but the cultivated form more commonly requires 500-1000 mm of rainfall per year. This should be well-distributed throughout the growing season and with an absence of prolonged droughts (NRC, 1996). The altitude limits of the species are unknown, but most of the cultivated finger millet in the world is found from 500-2400 m elevation (NRC, 1996). It tolerates cool climates, but thrives under hot conditions and can grow where temperatures are as high as 35°C (NRC, 1996). *E. coracana* appears to be photoperiod sensitive, the optimum photoperiod being 12 hours, which is considered to be relatively short.

2.7. Genetic diversity of finger millet

Development of crop varieties with high yield potential is a major plant breeding objective and a large number of high-yielding varieties of different crops have been released for cultivation. But a large chunk of released varieties disappear out of cultivation in a few years due to unstable performance over wide range of environmental conditions. There are only a few varieties with stability and wide adaptability persists over years. Selection of finger millet genotypes adapted to different agro-ecological conditions demands presence of genetic variability with regard to genotypic adaptation in the base population.

2.8 Characterization of finger millet germplasm

2.8.1 Morphological characterization

Phenotypic identification of plants has been used as a powerful tool in the classification of genotypes and to study taxonomic status, based on morphological traits recorded in the field. Most important agronomic characteristics are controlled by multiple genes and are subjected to varying degrees of environmental modifications and interactions (CGIAR, 2001).

2.8.2 DNA fingerprinting

Among the several methods used to assess genetic variability, DNA based molecular marker technique is most efficient in detecting genetic variability among genotypes. PCR-based markers such as RAPD (Random Amplified Polymorphic DNA) have been extensively used to study genetic diversity (Williams *et al.,* 1990; Welsh and McClelland, 1990; Salimath *et al.,* 1995; Kumari and Pande, 2010). Before the development of molecular markers, morphological markers were found to be a source in varietal identification and assessing genetic diversity, but they have certain limitations. Later markers based on protein differences were widely used. Iso-electric

variants of proteins, referred to as isozymes, were found to be markers for specific chromosomes/ chromosomal regions. Many studies have aimed at assessing the genetic diversity of different crops using allozyme markers (Hilu and Johnson 1992; Werth *et al.,* 1994; Muza *et al.,* 1995; Salimath *et al.,* 1995). However, the ultimate difference between individuals lies in the nucleotide sequences of their DNA. Detection of such differences employing various molecular biology techniques has led to the development of DNA-based molecular markers.

Molecular markers follow simple Mendelian inheritance. Unlike morphological markers, molecular markers are stable and not influenced by environmental factors. DNA-based molecular markers are based on two techniques: 1) hybridization (Southern, 1975; Jaccoud *et al*., 2001) and 2) the polymerase chain reaction (PCR) (Mullis *et al*., 1986). Hybridization-based restriction fragment length polymorphisms (RFLP) (Wyman and White, 1980) were the first DNA-based molecular marker system, and their application to genome mapping was conceived and developed by Botstein *et al*. (1980). Later, various types of molecular markers based on the PCR, such as randomly amplified polymorphic DNA (RAPD) (Williams *et al*., 1990), amplified fragment length polymorphism (AFLP) (Vos *et al*., 1995), sequence-tagged site (STS) Edwards *et al,* 1991), and simple sequence repeat (SSR) (Litt and Lutty, 1989; Tautz, 1989; Weber and May, 1989; Jacob *et al*., 1991) markers have been developed and used in assessing the genetic diversity of crop plants.

2.9 Production constraints for finger millet

Farmers face a range of constraints to finger millet production. These differ in different regions of the country but generally the main ones include: declining land fertility, high labor

requirements for weeding, lack of high-yielding, well adapted varieties, diseases, and unavailability of seed of improved varieties. Blast disease is gaining economic importance in almost all regions where finger millet is produced (Takan *et al*., 2002). About 75% of finger millet farmers use own-saved seed of mostly traditional varieties. About 10% get their seed from/through research institutions, church organizations and NGOs; while 10% buy seed from unregistered seed growers. Only 5% purchase commercial seed. As research and development efforts are aimed at developing improved technologies, it is worth noting that women are responsible for the greater share of finger millet production tasks.

In Kenya, production constraints of finger millet include low research priority, limited uses, difficulty in management, lack of improved varieties, poor crop husbandry, competition from other crops with better economic returns, and lack of commercial food products (Mitaru *et al*., 1993 and Oduori, 1993). The small size of finger millet seeds contributes to some of the problems of cultivation which necessitates planting in well made and fine seedbeds at higher plant densities (NRC, 1996). The difficulty in weeding becomes a problem making cultivation of the crop labor intensive and also complicated by wild relatives of the crop (e.g. *Eleusine indica*) that look like finger millet at the time of weeding. The problem of seed size carries over into processing (NRC, 1996). Because the seeds are small, it takes skill and much effort to mill finger millet, especially by hand. Hammer mills have to be fitted with very fine screens and run at high speed, but the NRC, 1996, reported the development of a special mill for millet.

Blast caused by the fungus *Pyricularia grisea* (a close relative of rice blast) is the most serious disease of finger millet (NRC, 1996; CGIAR, 2001). The crop has few pests, but shoot fly and

stem borers, which can be controlled by insecticides. Birds are also a pest, especially, the notorious *Quelea quelea* and other small grain feeding birds.

The poor attitude to the crop is also a major constraint to finger millet production (NRC, 1996). For all its importance, finger millet is grossly neglected both scientifically and internationally in terms of research, compared to the research lavished on wheat, rice, and maize. Most of the world has never heard of it, and even many countries that grow it have left it to languish in the limbo of a "poor person's crop," a "famine food," or, even worse, a "birdfeed" (Mnyenyembe and Gupta, 1998). Use of local unimproved, disease susceptible cultivars is partly responsible for the low yields observed in finger millet.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Morphological characterization of finger millet genotypes

3.1.1 Genotypes in the study

A total of 14 genotypes were used in the present study. Seeds of these 14 genotypes of *E. coracana* varieties were obtained from local farmers in various farming centres in Kenya and Uganda and Kenya seed company, Kitale. The 14 genotypes studied are Katumani, Gulu, Kapchorwa, Malaba, Busia, Nanjala Brown, Masindi, Hoima, P221, P224, Lira, Songhor, Bugiri and Jinja.The genotypes Nanjala Brown, Katumani, P224 and P221 were obtained from Kenya Seed Company .Genotypes Lira,Malaba, Kapchorwa, Gulu and Jinja were obtained from farmers in Eastern Uganda.GenotypesMasindi, Hoima, Bugiri and Busia were obtained from Central and Western Uganda while Genotype Songhor was obtained from western Kenya.

3.1.2 Experimental sites

The study was carried out in three locations at lowland areas Ahero and Ugunja (Table 1). These areas receive medium amount of rainfall and have a long history of cultivation of finger millet and cultural diversity in the Lake Victoria catchment area has resulted in large number of landraces of finger millet. Kitale site has a history of plantation cropping and hence genetic diversity of crops in this region is threatened.

Table 1: Description of experimental sites used in the study in the year 2010/2011

Location	Climatic conditions					
Sites	Latitude (N)	Longitude (E)	Altitude (m.a.s.l)	Rainfall mm	Temperature (°C)	Soil types
Ahero	0°05'	34°05'	1130	900 - 1200	21°C - 34°C	Planasol and alluvial
Ugunja	0°21'	34°05'	1164	800 - 1300	22°C - 32°C	Acrisols
Kitale	0°46'	34°05'	1800	900 - 1400	18°C - 27°C	Ferrasols

Sources: Jaetzold and Schmidt, 2006

Ahero is a town in Kenya. It is part of Nyando District of Nyanza Province. Ahero hosts a town council. It has an urban population of 7,891 and a total population of 61,556. The town is located 20 kilometers east of Kisumu.

Ugunja town is a market town in Western Kenya, located in Ugunja Division, Siaya District, and Nyanza Province. It has a population of approximately 17,000 and is rapidly growing

Kitale is an agricultural town in western Kenya situated between Mount Elgon and the Cherangani Hills at an elevation of around 6,300 feet. Its urban population was estimated at 220,000 in 2007

3.1.3 Planting in field plots

The genotypes were planted in plots of 6 x 6 m with three replicates and were spaced at 30cm between rows by drilling in furrows and thinned to 10 cm between plants. The plots were spaced 0.5m apart. A compound fertilizer (diammonium phosphate) was used at planting at the rate of 20kg P_2O_5 and 7.8 kg N ha^{-1} and drilled in furrows before planting at the seed rate of 4 kg ha^{-1}. No additional fertilizers were applied. Two hand weeding were carried out in all the sites. The genotypes were randomized within each block. **3.1.4 Experimental design**

The experimental design for the trials was Randomized Complete Block Design (RCBD). Randomization was done for each location. Uniformity in management was ensured particularly within the blocks.

3.1.5 Morphological characters scored

Data was collected at all sites from all plants per genotype per replication and later 10 plants per genotype selected based on plant height. The scoring scale for all the characters scored was done according to according to the IBPGR, 1985. Data was collected on Productive tillers(number of basal tillers which bore mature ears) counted at dough stage, harvest index (The ratio of weight of plant to weight of harvest), days to 50% flowering(counted days from sowing to days when ears have emerged on 50% of the main tillers), days to physiological maturity (counted days from sowing to the time the grain is hard dough), plant height (measured in cm from the ground level to tip of inflorescence at hard dough stage) , number of tillers,(counted number of basal tillers which bore mature fruit) finger length,(measured in mm from base to tip of longest spike on main tiller at dough stage. a thousand grain weight(Weight of 1000 grains in grams), grain yield per plant(mean of yield of 5 plants), seedling anthocyanin coloration of sheath of first leaf(Color chart), leaf sheath anthocyanin coloration (Color chart), leaf sheath density hairs on both sides just beneath the leaf blade(number of spikelets per cm along middle portion of the rachis of any finger at dough stage), leaf blade anthocyanin coloration(Color Chart), panicle length(measured from the base to the tip), inflorescence anthocyanin coloration(color chart), panicle attitude of branches(scored on erectness, prostrateness or decumbent), seedling coleoptiles anthocynin of and anthocynin coloration of dorsal side of the first leaf (Color chart); leaf sheath: density of hairs on margin, length of hairs of ligule; leaf blade: density of fringe of

hairs on margin of base, density of hairs on upper side; (number of spikelets at flowering) plant growth habit(40 days after sowing, erect, prostrate or decumbent); leaf color (Color chart); leaf width, leaf glaucosity of lower side, time of 50% inflorescence emergence, flag leaf length and width at time of inflorescence emergence measured at flowering from base of leaf to tip, average of 5 plants), stem length when fully expanded and of uppermost internode (measured in mm from base to tip at flowering), panicle shape and number of digits(, level of ploidy and caryopsis color after threshing.(Color chart)

3.1.6 Data analysis

The fourteen genotypes were evaluated using analysis of variance (ANOVA) for all the 37 morphological traits and were computed using GENSTAT statistical software, version 12. The means of genotypes and location were compared based on the mean grouping test according to Fisher's protected least significant difference (PLSD) whenever the genotypes and location effects was significant at 95% confidence level. Factor analysis was performed to know which trait contributed to maximum variability. Principal component analysis of the traits was employed to examine the percentage contribution of each trait to total genetic variation. Coefficient of variation (CV) was used to measure statistically the dispersion of data around the mean. Correlation coefficient was also calculated to determine the relatedness of the finger millet genotypes with respect to the different parameters.

The general linear model for individual location was:

$\mathbf{X_{ijk} = \mu + t_i + \beta_j + e_{ijk}}$

Where: $\mathbf{X_{ijk}}$ = plot observation/ trait

$\boldsymbol{\mu}$ = overall mean

$\mathbf{t_i}$ = genotype effect

$\boldsymbol{\beta_j}$ = block effect

e_{ijk} = error/ residual effect

The general linear model for the three locations was:

$$\mathbf{X_{ijk} = \mu + \pi_i + t_j + \beta_k + \gamma_{ik} + e_{ijk}}$$

Where: $\mathbf{X_{ijk}}$ = plot observation/ trait

$\boldsymbol{\mu}$ = overall mean

$\boldsymbol{\pi_i}$ = Replication effect

$\mathbf{t_j}$ = Environment effect

$\boldsymbol{\beta_k}$ = Genotype effect

γ_{jk} = Genotype × environment interaction

e_{ijk} = Intra-block error effect

Cluster analysis

The morphological observations were scored as binary data and were used to construct a dendrogram. The genetic associations based on the phenotypic data between genotypes were evaluated by calculating the Euclidean coefficient (When variables are on different measurement scales, standardization is necessary to standardize the contributions of the variables in the computation of distance. The Euclidean distance computed on standardized variables is called the standardized

Euclidean distance). A similarity matrix was generated using the (similarity interval) SIMINT programme of Numerical Taxonomy Multivariate Analysis System package (NTSYS-pc) software, version 2.1 (Rohlf, 2000) and the similarity coefficients were used for cluster analysis.

Dendrograms were constructed for morphological data for across the sites by the Unweighted Pair-Group method (UPGMA) (Sokal and Michener, 1958) clustering using the sequential agglomerative hierarchical nested (SAHN) programme and tree plot of NTSYS. The cophenetic correlation coefficient (r) for each dendrogram was computed between the genetic similarity matrix (original distances) and the cophenetic values using the matrix comparison (MXCOMP) programme of NTSYS-pc. The relatedness between two matrices is measured by '*r'*, a product moment correlation coefficient. A higher *'r*' - value indicates a higher degree of similarity and vice versa. The significance of association between distance matrices was tested using Mantel matrix test as described by Mantel, (1967) to test the goodness of fit between the similarity and the cophenetic matrices (Sneath and Sokal, 1973).

3.2 Genetic diversity of finger millet genotypes based on SSR markers.

3.2.1 Plant materials

The plant materials used comprised of 14 genotypes as in Section 3.1.1.

3.2.2 DNA extraction and PCR amplification

The genomic DNA of different accessions of finger millets were isolated by the phenol-chorophorm purification method methods (Murray and Thompson, 1980), and subsequently quantified and analyzed via agarose gel electrophoresis (Maniatis et al., 1989). A total of 14 cultivars (0.1g) was extracted in CTAB, cethyltrimethylammonium bromide, buffer (Murray and Thompson, 1980; Doyle and Doyle, 1990) followed by an RNase-A treatment for 30 min at 37◦C. The quality and quantity of extracted DNA were determined by comparing band sizes and intensities of the test DNA with those of standard λ DNA using spectrophotometer.

SSR markers analyses

A total of 12 SSR primers (Table 2) were used for the polymorphism survey. PCR amplification was performed as per the standard protocol using 50–100 ng of template DNA, 30 ng of primer, 0.1 mM dNTPS, 1.5 U *Taq* DNA polymerase, 1× PCR buffer (10 mM Tris pH 8.0, 50 mM KCl and 1.8 mM $MgCl_2$) in a volume of 25 μL. Amplification was performed with thermal cycler (Eppendorf). The standardized amplification was: initial denaturation 95°C for 5 min followed by 40 cycles of denaturation 94°C for 1 min; primer annealing based on melting temperature value for 1 min; primer extension at 72°C for 2 min; and final primer extension at 72°C for 7 min. The annealing temperatures PCR amplified products of all the primers were subjected to gel electrophoresis using 1.8% agarose gel in 1× TAE buffer at 100 V. The fragment sizes, ranging from 0.3 to 4.0 kb, were detected by comparing the amplicons with a 100-bp DNA ladder and ethidium bromide stained gels were duplicated independent DNA preparations for each sample were done and only major bands consistently amplified were scored.

Statistical analysis

DNA fingerprints were scored for the presence (1) or absence (0) of bands of various molecular weight sizes in the form of binary matrix. Similarity matrices based on these indices were calculated. Correlation between the matrices obtained with three marker types (SSR and morphological) was estimated by means of Mantel matrix correspondence test (Mantel 1967).

Product-moment correlation (*r*) obtained from this test provides one measure of relatedness among the three matrices. In this instance, the matrix correlation corresponds to three independently derived dendrograms. Similarity matrices were utilized to construct the UPGMA (unweighed pair group method with arithmetic average) dendrograms. In order to estimate the

congruence among dendrograms, cophenetic matrices for each marker and index type were computed and compared using Mantel test.

Table 2: *Eleusine coracana*: summary of genetic diversity study using SSR primers.

Name of primer	Primer Sequence (5' to 3"	Product Length
UGE 77	(F) TICGCGCGAAATATAGGC	245
	(R) CTCGAAGCACCCACCTTTC	
UGEP68	(F) CGGTCAGCATATAACGAATGG	232
	(R) TCATTGATGAATCCGACGTG	
UGEP106	(F) AATTGATGAATCCGACGTG	175
	(R) TGCTGTGCTCCTCTGTTGAC	
EGEP21	(F) CAATTGATGCATTGGGACAAC	225
	(R) GTATCCACCTGCATGCCAAC	
EGEP8	(F) ATTTTCCGCCATCACTCCAC	297
	(R) AGACGCAATGGGTAAATGTC	
EGEP18	(F) TTGCATGTGTTGCTTTTTGC	318
	(R) TGTTCTTGATTGCAAACTGATG	
EGEP12	(F) ATCCCCACCTACGAGATGC	230
	(R) TCAAAGTGATGCGTCAGGTC	
EGEP81	(F) AAGGGCCATACCAACACTCC	192
	(R) CACTCGAGAACCGACCTTTG	
EGEP104	(F) TCAGCACCACCTGAATAGG	189
	(R) AATAGGGAGGGCGAAGACTC	
EGEP90	(F) GGCCTTTGCAGTCATGTCAG	232
	(R) GGCCTTTTGCAGTCATGTGAG	
EGEP6	(F) AGCTGCAGTTTTCAGTGGATTC	229
	(R) TCAACAAGGTGAAGCAGC	
EGE26	(F) ATGGGGTTAGGGTTCGAGTC	227

(R) TGTCCCTCACTCGTCCTC

Source: Dida *et al.,* 2007

3.3 Comparison of finger millet genetic distance based on morphological and SSR markers.

3.3.1 Genetic similarities and clustering analysis

Data from section 3.3.1 and 3.3.2 was used to calculate the genetic distance of finger millet using Euclidean with the help of the NTSYS-pc, version 2.1 (Rohlf, 2000). Similarity matrices for morphological, SSR and combined morphological and SSR marker types were subjected to UPGMA (Sokal and Michener, 1958) clustering and dendrograms was constructed as previously indicated. The cophenetic correlation coefficient (r) for each dendrogram was computed as previously described in section 3.1.6 (cluster analysis). Consensus tree analysis was used to compare the different dendrogram types using (majority rule) MAJRUL method by estimation of consensus fork index (*CIc*) as presented by Rohlf, (1982) using NTSYS software according to Rohlf, (2000) which provides an indication of similarity of the dendrograms (Duarte *et al.*, (1999).

CHAPTER FOUR:

RESULTS

4.1 Morphological characterization of finger millet genotypes

Thirty seven morphological traits were evaluated and showed marked differences in their distribution and amount of variation. In no cases monomorphic phenotypic classes were observed. At Ahero (Table 3), the genotypes showed highly significant ($P \leq 0.001$) differences for the following traits, productive tillers, finger length, harvest index, days to 50% flowering, days to physiological maturity, plant height, number of tillers, a thousand grain weight, grain yield per plant, seedling anthocyanin coloration of sheath of first leaf and inflorescence anthocyanin coloration, ($P \leq 0.01$) leaf sheath anthocyanin coloration and leaf sheath density hairs on both sides just beneath the leaf blade, and ($P \leq 0.05$) plant height, leaf blade anthocyanin coloration, panicle attitude of branches and panicle length.

The finger millet genotypes showed variation for 18 morphological traits as shown in Table 3. Genotypes suitable for Ahero site based on a thousand grain weight is Busia (6.87g), based on grain yield per plant (54.33g) is Malaba and based on yields per hectare in Kg (515) is P221. The traits leaf sheath anthocyanin coloration and leaf sheath density hairs on both sides just beneath the leaf blade had the highest CV (39.4%) each and days to 50% flowering had the lowest CV (1.5%).

Table 3: Means of quantitative traits of 14 finger millet genotypes at Ahero in the year 2010/2011.

Varieties	Prd til	Fingers	Hi	D50%	DPM	PH	NT	FL	100GW
Malaba	5.33ab	5.3a	0.017a	81.67f	101def	93.67bcd	6.33ab	20.33f	6.57de
Nanjala Brown	6.33ab	6.7bcd	0.163e	61b	91c	90.67abcd	7.67b	20.67f	6.07a
Busia	8.67c	7.3de	0.11cde	71d	91.33c	71.67a	7.67b	17.33e	6.87f
Kapchorwa	5a	7cd	0.053ab	80.33f	98.33d	101d	6.67ab	15.67cde	6.77ef
Katumani	5a	7cd	0.14de	56.67a	73.67a	90abcd	5.67a	16de	6.3abc
Holma	5.33ab	6ab	0.123cde	70.67d	89.67c	73a	6.33ab	14abcd	6.23ab
P221	6ab	7.3de	0.08bc	76e	91.33c	93.3bcd	7.33ab	16de	6.23ab
Masindi	5a	8e	0.087bcd	65c	84.33b	87.3abcd	5.67a	13.33ab	6.33abc
Gulu	5.33ab	7cd	0.157e	93h	119.33g	100d	7.33ab	12a	6.2ab
P224	6.67b	6.7bcd	0.123cde	82f	103ef	92.7bcd	8b	12.67ab	6.7def
Jinja	6.33ab	6.7bcd	0.11cde	81f	117.33g	95.3bcd	7.33ab	13.67abc	6.47bcd
Lira	8.33c	6.3bc	0.113cde	94h	120g	763.ab	10.3c	14.33bcd	6.47bcd
Songhor	6.33ab	6ab	0.127cde	87g	104.67f	98cd	7.67b	13.33ab	6.43bcd
Bugiri	9.33c	7cd	0.023a	77e	100.67de	79.7abc	12c	15.67cde	6.43bcd
CV	7	7	31.9	1.5	2.3	12.9	14.9	8.7	2.5
MEAN	6.36***	6.74***	0.1***	76.88***	98.98***	88.8*	7.57***	15.36***	6.43***

Varieties	GYP	Yield	SACS	LSAC	LSDL	LBAC	IAC	PAB	PL
Malaba	54.33e	419.3cd	1a	7e	7e	3.67bc	6.33e	4.33bcd	5.67bc
Nanjala Brown	41a	521.7ef	5d	1.67a	1.67a	4033c	3abc	5cd	2.33a
Busia	50.67d	290b	1a	1.67a	1.67a	2.33ab	3abc	2.33ab	5cd
Kapchorwa	39.67a	373.3bc	4.33cd	3.67abcd	3.67abcd	2.33ab	1.67a	6.33d	6.33bc
Katumani	44.67b	338.3bc	1a	5cde	5cde	3.67bc	4.33cd	4.33bcd	2.33a
Hoima	41.33a	648.7g	3bc	3.67abcd	3.67abcd	2.33ab	3.67bc	2.33ab	6.33bc
P221	40.67a	515de	1.67ab	2.33ab	2.33ab	3abc	1.67a	3.67abc	5.67bc
Masindi	51d	330bc	1a	3.67abcd	3.67abcd	4.33c	2.33ab	3.67abc	5.67bc
Gulu	51.67de	315.3bc	1a	3.67abcd	3.67abcd	2.33ab	2.33ab	5.67cd	7c
P224	40.67a	370bc	3.67cd	3.67abcd	3.67abcd	1.67a	3abc	5cd	4.33ab
Jinja	49.67cd	620fg	5d	3abc	3abc	2.33ab	3abc	3.67abc	5.67bc
Lira	47.67c	175.3a	3.67cd	2.33ab	2.33ab	3.67bc	4.33cd	1.67a	6.33bc
Songhor	40.33a	500.3d	1a	4.33bcd	4.33bcd	3.67bc	5.67de	2.33ab	5.67bc
Bugiri	47bc	617.7efg	1a	5.67de	5.67de	3.67bc	2.33ab	5cd	4.33ab
CV	3.6	14.4	42.4	39.4	39.4	31.2	34	38.8	23.7
MEAN	45.74***	431.1***	2.38***	3.67**	3.67**	3.1*	3.33***	3.95*	5.19**

*=significant at $p \leq 0.05$, **=significant at $p \leq 0.01$, ***=significant at $p \leq 0.001$; productive tillers (prd til), harvest index (HI), days to 50% flowering (D50%), days to physiological maturity (DPM), plant height (PH), number of tillers (NT), finger length (FL), a thousand grain weight (1000GW), grain yield per plant (GYP), seedling anthocyanin coloration of sheath of first leaf (SACS), leaf sheath anthocyanin coloration (LSAC), leaf sheath density hairs on both sides just beneath the leaf blade (LSDL), leaf blade anthocyanin coloration (LBAC), inflorescence anthocyanin coloration (IAC), panicle attitude of branches (PAB), panicle length (PL) Coefficient of variation (CV). Genotypes means having the same letter are not significantly different at the 5% level of significance according to Fishers PLSD.

In Ugunja (Table 4), the genotypes showed highly significant ($P \leq 0.001$) differences for the following traits, productive tillers, harvest index, days to 50% flowering, days to physiological maturity, number of tillers, grain yield per plant, yields per hectare, seedling anthocyanin coloration of sheath of first leaf and leaf blade density of hairs on upper side and ($P \leq 0.01$) a thousand grain weight and leaf sheath anthocyanin coloration, ($P \leq 0.05$) plant height, finger length and caryopsis colour after threshing.

The finger millet genotypes showed variation for 14 morphological traits as shown in table 4. Genotypes suitable for Ugunja site based on a thousand grain weight is Malaba (6.67g), based on grain yield per plant (58.33g) is Gulu and based on yields per hectare in Kg (769) is Bugiri. The trait seedling anthocyanin coloration of sheath of first leaf had the highest CV (40.3%) and a thousand grain weight trait had the lowest CV (2.2%).

Table 4: Means of quantitative traits of 14 finger millet genotypes at Ugunja in the year 2010/2011.

Varieties	Prd til	HI	D50%	DPM	PH	NT	FL	1000GW	GYP	Yield	SACS	LSAC	LBDU	CCT
Malaba	7.33abc	0.033a	8le	104.7c	97.3bcde	8ab	15.33cd	6.67d	47bcde	486.7bc	La	5d	La	5bc
Nanjala Brown	8.67cde	0.167de	61.33b	83a	90.abcde	9.33bc	14abc	6.27ab	44.7abcd	627cde	4.33c	3abc	2.33ab	5.67c
Busia	8.33bcd	0.113bcd	71.33d	95.5b	72.67a	9.67bc	14abc	6.27ab	48cde	642de	1.67a	1.67a	3b	5.67c
Kapchorwa	7.67abcd	0.08ab	88f	107.7c	108.3e	9abc	14.7abcs	6.53cd	48cde	551cd	4.33c	3.67bcd	2.33ab	4.33abc
Katumani	6.67ab	0.17e	5.33a	84.3a	93bcde	8ab	14.33abc	6.47bcd	48.33de	385ab	1.67a	5d	5 cd	2.67a
Hoima	11.67g	0.17e	72d	96b	85.67abc	8.67abc	14abc	6.17a	45.7abcde	722e	3.67bc	4.33cd	2.33 ab	4.33abc
P221	6.33a	0.107bc	74.33d	105c	86abc	7.33a	14abc	6.5bcd	43a	518bcd	2.33ab	3abc	5cd	5 bc
Masindi	6.33a	0.113bcd	66c	94.7b	89.3abc	7.33a	13.33abc	6.5bcd	49e	385.3ab	1.67a	2.33ab	5cd	4 abc
Gulu	10.67fg	0.167de	93.67g	113d	105.3de	12d	12.33a	6.2a	58.33f	488.3bc	2.33ab	3abc	5cd	4 abc
P224	10.33cfg	0.14cde	74d	104.3c	95.67bcde	12d	12.67ab	6.2a	44ab	652de	3.67bc	4.33cd	3b	4 abc
Jinja	7.67abcd	0.13bcde	82.33e	105.3c	96.3bcde	8.67abc	12.67ab	6.47bcd	46abcde	625cde	5c	3.67bcd	5.67d	4 abc
Lira	9.33def	0.123bcde	92.33g	114.3d	86.3abc	10.33cd	14abc	6.33abc	43.33ab	263a	5c	3abc	3.67bc	5.67c
Songhor	7.67abcd	0.137cde	85ef	107.3c	102cde	8.67abc	13.33abc	6.37abc	43.33ab	546.7cd	1.67a	5d	3b	3.33ab
Bugiri	10.33efg	0.037a	82.67e	104.3c	81.67ab	11.67d	15bcd	6.2a	48cde	769e	La	4.33cd	5cd	3.33ab
CV	13.1.	27.6	3.2	2.5	12	6	10.2	2.2	4.9	16.6	40.3	26.9	24.6	25.3
MEAN	8.5	0.12	77.2	101.4	92.1	16.48	14.05	6.37	46.98	547	2.81	3.67	3.67	4.36

*=significant at p≤0.05, **=significant at p≤0.01, ***=significant at p≤0.001; productive tillers (prd til), harvest index (HI), days to 50% flowering (D50%), days to physiological maturity (DPM), plant height (PH), number of tillers (NT), finger length (FL), a thousand grain weight (1000GW), grain yield per plant (GYP), seedling anthocyanin coloration of sheath of first leaf (SACS), leaf sheath anthocyanin coloration (LSAC), leaf blade density of hairs on upper side (LBDU), caryopsis color after threshing (CCT) Coefficient of variation (CV). Genotypes

means having the same letter are not significantly different at the 5% level of significance according to Fishers PLSD.

In Kitale (Table 5), the genotypes that showed highly significant ($P \leq 0.001$) difference for the following traits, days to physiological maturity, days to 50% flowering, plant height, a thousand grain weight, grain yield per plant, yields per hectare in Kg, seedling anthocyanin coloration of sheath of first leaf, ($P \leq 0.01$) productive tillers, harvest index, finger length, number of fingers, number of tillers and flag leaf length at time of inflorescence emergence and ($P \leq 0.05$) leaf sheath density of hairs on both margin, inflorescence anthocyanin coloration.

The finger millet genotypes showed variation for 15 morphological traits as shown in table 5. Genotypes suitable for Kitale site based on a thousand grain weight is Busia (6.77g), based on grain yield per plant (51g) is Malaba and based on yields per hectare in Kg (894.3) is Hoima. The trait seedling anthocyanin coloration of sheath of first leaf had the highest CV of 43.3% and days to 50% flowering had the lowest CV of 1.4%.

Table 5: Means of quantitative traits of 14 finger millet genotypes at Kitale in the year 2010/2011.

Varieties	Prd til	Fingers	HI	D50%	DPM	PH	NT	FL	1000GW	GYP	Yield	SACS	LSDM	AIC	FLLIE
Malaba	7ab	6.67bcd	0.043a	104f	128.7ef	99bcd	7.67bcd	15.3cde	6.5bcde	51g	483b	1a	3ab	6.33e	5bde
Nanjala	7.33b	5.67ab	0.15bcd	85.3b	106ab	97.3abcd	8.67d	18f	6.33abc	44.3bc	688.3f	5d	2.33a	3.67abcd	4.33abc
Brown															
Busia	7.33b	5.33a	0.12bc	96d	109.3b	82a	8.33cd	16.cdef	6.77f	46cd	678ef	1a	2.33a	3.67abcd	3ab
Kapchorwa	6.33ab	7.33d	0.09ab	111.3g	131.3f	119e	7abc	14.7bcde	6.7ef	42ab	453.3b	4.33d	3ab	1.67a	7d
Katumani	6ab	7cd	0.19d	82a	103a	87.3abc	6.67ab	16.3def	6.5bcde	44.3bc	483.3b	1a	3ab	3.67abcd	5bcd
Holma	6.67ab	6.67bcd	0.16bcd	91.67c	118.3cd	96abcd	7.67bcd	14.7bcde	6.27ab	44.3bc	894.3g	3bc	3ab	4.33bcde	3.67ab
P221	6.33ab	5.67ab	0.12bc	96.3d	117.3c	95.3abcd	6.67ab	16.7ef	6.63def	41a	589.7cde	1.67ab	5c	3abc	3.67ab
Masindi	5.67a	5.67ab	0.123bcd	81.3a	105.3a	97.7abcd	6a	13ab	6.4bcd	48.3def	506.7bc	1a	3.67abc	2.33ab	4.33abc
Gulu	7.33b	6abc	0.177cd	115.7h	137g	107de	8bcd	12.3a	6.1a	50.3fg	481.7b	1.67ab	3.67abc	3abc	3ab
P224	6.67ab	7.33d	0.17cd	100.3e	121.7d	106de	8.67d	13ab	6.33abc	41.67a	690f	3.67cd	4.33bc	3abc	7d
Jinja	6.33ab	7cd	0.13bcd	100.7e	121.7d	101cd	7.33abcd	14.7bcde	6.4bcd	49.3fg	621.7def	5d	4.33bc	5cde	6.33cd
Lira	7.33b	6.67cd	0.14 bcd	116.7h	127e	98.3bcd	10.3e	14abc	6.6def	48.7efg	262.7a	3.67cd	3.67abc	3.67abcd	4.33abc
Songhor	6.33ab	5.33a	0.14 bcd	111g	106.7ab	102cd	8bcd	14.3abcd	6.5bcde	41a	529.3bcd	1a	3.67abc	5.67de	2.33a
Bugiri	10c	6abc	0.043a	96d	120.3cd	84.3ab	11.7e	15.3cde	6.53cdef	46.7cde	700f	1a	4.33bc	2.33ab	6.33cd
CV	12.9	10.8	33.5	1.4	2	9.6	11.8	8.9	2.3	3.3	9.6	43.3	26.5	37.6	30.3
MEAN	69**	6.31**	0.13**	99.2***	118***	98**	8.05**	14.9**	6.5***	45.6***	575.9***	2.43***	3.52*	3.67*	4.67**

*=significant at p≤0.05, **=significant at p≤0.01, ***=significant at p≤0.001; productive tillers (prd til), harvest index (HI), days to 50% flowering (D50%), days to physiological maturity (DPM), plant height (PH), number of tillers (NT), finger length (FL), a thousand grain weight (1000GW), grain yield per plant (GYP), seedling anthocyanin coloration of sheath of first leaf (SACS), leaf sheath density of hairs on both margin (LSDM), flag leaf length at time of inflorescence emergence (FLLIE), inflorescence anthocyanin coloration (IAC), Coefficient of variation (CV). Genotypes means having the same letter are not significantly different at the 5% level of significance according to Fishers PLSD.

In Table 6, most of the genotypes showed significant differences for the morphological traits across all the sites except leaf colour, leaf width, stem length fully expanded and level of ploidy. The interaction between genotypes and site were significant ($p \leq 0.05$) for finger length and leaf blade density of hairs on upper side and ($p \leq 0.001$) for productive tillers, days to 50% flowering, days to physiological maturity, number of tillers, a thousand grain weight (g), grain yield per plant (g) and yield in Kg per hectare. The means for location showed that all the sites did not differ significantly ($p \leq 0.05$) for finger length; ($p \leq 0.01$) for a thousand grain weight (g), grain yield per plant and leaf blade density of hairs on upper side and ($p \leq 0.001$) for productive tillers, days to 50% flowering, days to physiological maturity, number of tillers, and finger length, yield in Kg per hectare and plant growth habit. The coefficient of variation percent ranged from 2.1% for days to 50% flowering to 53.6% for plant growth habit. Genotypes which were best in the three sites combined, in terms of a thousand grain weight is Kapchorwa (6.67g), based on grain yield per plant (53.44g) is Gulu and based on yield in Kg/Ha (754.9) is Hoima.

Table 6: Means of morphological traits of 14 finger millet genotypes across the three sites in the year 2010/2011.

Varieties	Prd til	HI	D50%	DPM	PH	NT	FL	1000GW	GYP	Yield kg	SACC	SACD	SACS	LSAC	LSDM	LSDL
Malaba	6.56abc	0.03a	88.89f	111.4hi	96.7defg	7.33bcd	17f	6.58cd	50.78f	463bc	3abc	2.78ab	1a	5.67f	3.44cde	3.67bcd
Nanjala Brown	7.44cd	0.16f	69.22b	93.3b	92.8cdef	8.56ef	18.56g	6.22a	43.33b	612.2e	3.4bcd	3.89c	4.78d	2.33ab	2.33ab	2.56a
Busia	8.11d	0.11cd	79.44c	98.7c	75.44a	8.56ef	15.78ef	6.63d	48.22de	536bc	3.4bcd	3.67bc	1.22a	2.11a	1.89a	2.78ab
Kapchorwa	6.33ab	0.04b	93.22g	112.4i	109.3h	7.56bcd	15de	6.67d	43.22ab	459.2b	3abc	3.44bc	4.33cd	3.67cd	3.44cde	4.11cd
Katumani	5.89ab	0.17f	65a	87a	90.1bcde	6.78ab	15.56e	6.42b	45.78c	402.2b	3.2abc	3.89c	1.22a	5ef	3.44cde	2.56a
Hoima	7.89d	0.152ef	78.11c	101.3d	84.9abc	7.56bcd	14.22cd	6.22a	43.44b	754.9f	4.78e	3.89c	3.22b	3.44bcd	3bc	3.67bcd
P221	6.22ab	0.10bc	82.22d	104.6e	91.6bcdef	7.11abc	15.56e	6.46bc	41.56a	540.9d	3.67cd	3abc	1.89a	2.78abc	4.11de	4.11cd
Masindi	5.67a	0.11bc	70.78b	94.8b	91.4bcdef	6.33a	13.22abc	6.41b	49.44ef	407.3b	2.33a	2.33a	1.22a	3.22abcd	3.67cde	3.89cd
Gulu	7.78bc	0.17f	100.78h	123.1l	104.2gh	9.11fg	12.22a	6.7a	53.44g	428.4b	4.33de	3.44bc	1.67a	3.44bcd	4.11de	3.89cd
P224	7.89d	0.146def	85.44e	109.7gh	98.1efg	9.56gh	12.78ab	6.41b	42.11ab	570.6de	2.56ab	3abc	3.67bc	3.67cd	3.89cde	4.33d
Jinja	6.78bc	0.124cde	88f	114.8j	97.4efg	7.78cde	13.67bc	6.44bc	48.33de	622.3e	4.33de	3.67bc	5d	3.22abcd	3.89cde	3.89cd
Lira	8.33d	0.124cde	101h	120.4k	87bcd	10.33h	14.11cd	6.47bc	46.89cd	233.7a	2.56ab	3.44bc	4.1bcd	2.78abc	3.22bcd	4.33d
Songhor	6.78bc	0.134cdef	94.33g	106.2ef	100.7fgh	8.11de	13.67bc	6.43b	41.56a	525.4cd	2.33a	3abc	1.22a	4.33de	3.67cde	3.22abc
Bugiri	9.89e	0.034a	85.22e	108.4fg	81.9ab	14.11i	15.33de	6.39b	47.22cd	695.4f	3.67cd	2.33a	1a	4.33de	4.33e	3.44abcd
MS (site)	55.1***	0.01*	6865.1***	4566.8***	917.6***	1054***	18.5***	0.19**	24.7**	246850***	1.14	2.89	2.32	1.14	0.51	1.18
MS(genotype)	11.9***	0.02***	1119.0***	964.9***	731.9***	893.8***	26.3***	0.11***	123.7***	160650***	5.39***	2.6**	21.7***	8.87***	4.2***	3.38***
MS(genotype* site)	3.2***	0.0002	21.1***	99.6***	52.4	690.13***	3.2*	0.07***	18.6***	13950***	0.425	0.325	0.3	1.59	1.19	1.18
MS (error)	0.9139	0.001	3.28	5.63	110.7	1.01	1.87	0.02	3.587	4915	1.103	1.068	1.105	1.7	1.3	1.08
SE	0.55	0.02	1.05	1.4	6.075	0.58	0.79	0.087	1.1	40.48	0.61	0.6	0.6	0.75	0.66	0.6
CV(%)	13.2	30.7	2.1	2.2	11.3	9.4	9.3	2.3	4.1	13.5	31.5	31.6	41.4	36.5	32.9	28.9

*=significant at p≤0.05, **=significant at p≤0.01, ***=significant at p≤0.001;productive tillers (prd til), harvest index (HI), days to 50% flowering (D50%), days to physiological maturity (DPM), plant height (PH), number of tillers (NT), finger length (FL), a thousand grain weight (1000GW), grain yield per plant (GYP), seedling anthocynin coloration of sheath of first leaf (SACS), leaf sheath density of hairs on both margin (LSDM), Seedling anthocyanin coleoptile colouration (SACC), seedling anthocyanin dorsal side colouration (SACD), Leaf sheath density of hairs on both sides (LSDL), means square (MS), Standard Error (SE), Coefficient of variation (CV). Genotypes and sites means having the same letter are not significantly different at the 5% level of significance according to Fishers PLSD.

Table 6: Cont.'

Varieties	LSLL	LBAC	LBDM	LBDU	PGH	LGL	T50%IE	IAC	FLUE	FLWIE	SLU	PS	PDN	PAB	PL	CCT
Malaba	5de	4.11d	3abcde	2.11a	3.89cd	4.11c	68.11a	5.67f	5.44defg	3.67abc	6.11de	2.22cde	3.67abcd	3.67bcd	5.22cde	4.78defg
Nanjala Brown	3.89bcd	4.11d	3.44cde	2.56ab	3.44bcd	2.78ab	70.44ab	3.67cde	4.33bcde	5.22ef	3.89ab	2.11cd	2.11a	4.33cde	3.44ab	5.67g
Busia	3.67abcd	2.56ab	3.22bcde	3.44bcd	1.44a	3.89bc	73.11bc	3.44bcde	3ab	3.44ab	6.33e	2.22cde	3.89bcd	2.56ab	4.78cd	5.44fg
Kapchorwa	3.89bcd	2.33a	2.56abcd	1.67a	3bcd	2.11a	67.33a	1.89a	6.44g	5.89f	5bcde	2bcd	3.67abcd	4.56cde	5.22cde	4.33bcde
Katumani	5de	3.22abcd	2.11ab	3.67bcd	3.89cd	4.11c	69.67ab	4.11e	4.56cdef	3.44ab	5bcde	1.33ab	3ab	4.33cde	3.22a	3.11a
Hoima	3.67abcd	2.78abc	3.89e	2.56ab	3bcd	4.33cd	68.89a	4.11e	3.89abc	4.78de	4.1abc	1.67abc	3.44abc	2.33ab	5.67cde	3.89abcd
P221	2.33a	3abcd	1.89a	3.89cd	2.33ab	4.11c	68.11a	2.33ab	4.11abcd	4.78de	5.2bcde	2bcd	3.22ab	3.44abc	5.22cde	5.11efg
Masindi	4.78cde	3.89cd	3.67de	2.78abc	4.11d	3.67bc	68.89a	2.56abc	4.33bcde	3.67abc	3.44a	1.11a	4.56bcd	3.44abc	4.78cd	4.22bcde
Gulu	3.22ab	2.56ab	3.67de	4.11d	2.56abc	4.56cd	73bc	2.78abcd	2.78a	4.33bcde	4.3abc	1.78abc	3.67abcd	5de	6.33e	4.56cdef
P224	3.44abc	2.11a	3.89e	3.67bcd	2.56abc	3.67bc	68.11a	3.22bcde	6.33g	4.56cde	5.2bcde	1.67abc	4.11bcd	5.44e	4.56bc	4abcd
Jinja	5.89e	2.56ab	3.89e	4.33d	2.33ab	4.78cd	76.56c	3.67cde	5.89fg	4.33bcde	5.4cde	2.89e	5cd	3.67bcd	5.22cde	4abcd
Lira	3.22ab	3.67bcd	3abcde	2.56ab	2.33ab	4.11c	68.11a	3.89de	4.33bcde	4.11abcd	4.78abcd	2.11cd	3.22ab	2.11a	5.89de	5.11efg
Songhor	4.11bcd	3.89cd	3.67de	3.67bcd	3.67bcd	5.44d	66.56a	5.67f	3.22abc	3.89abcd	5bcde	2.67de	5.22d	2.33ab	5.44cde	3.56abc
Bugiri	2.78ab	3.67bcd	2.33abc	3.44bcd	3bcd	4.33cd	68.11a	2.56abc	5.67fg	3.22a	5.4cde	1.78abc	3ab	4.56cde	3.44ab	3.44ab
MS (site)	0.98	2.13	1.65	8.8**	32.89***	0.095	11.45	1.365	3.3	3.3	0.98	0.056	0.98	2.41	2.9	0.03
MS(genotype)	8.4***	4.46***	4.3**	5.8***	5.26*	5.9***	68.86***	11.54***	12.9***	12.9***	5.79**	2.01***	6.21*	10.18***	7.98***	5.38***
MS(genotype*site)	0.95	0.69	0.45	2.9*	2.12	0.95	10.67	1.023	0.87	0.87	0.642	0.17	0.92	1.18	1.93	0.6
MS (error)	2.7	1.44	1.73	1.65	2.54	1.719	18.93	1.904	2.28	2.28	2.329	0.52	3.23	2.38	1.98	1.4
SE	0.94	0.69	0.76	0.74	0.92	0.76	2.5	0.8	0.87	0.87	0.88	0.41	1.04	0.89	0.81	0.68
CV(%)	41.6	37.7	41.6	40.4	53.6	32.8	6.2	39	32.9	32.9	31	36.5	48.6	41.7	28.8	27.1

*=significant at p≤0.05, **=significant at p≤0.01, ***=significant at p≤0.001; (LSLL) Leaf sheath length of hairs ligule, (LBAC) Leaf blade anthocyanin colouration, (LBDM) Leaf blade density of hairs on margin base, (LBDU) Leaf blade density of hairs on upper side, Plant growth habit (PGH), (CCT) Caryopsis colour after threshing, (T50%IE) Time to 50% inflorescence emergence, (FLWIE) Flag leaf width at inflorescence emergence, (SLU) Stem length upper most internode, (PS) Panicle shape, (PDN) Panicle number of digits, (PAB) Panicle attitude of branches, (PL) Panicle length, (CCT) Caryopsis colour after threshing, (FLLIE) flag leaf length at time of inflorescence emergence, (IAC) inflorescence anthocyanin coloration, Coefficient of variation (CV). Genotypes and sites means having the same letter are not significantly different at the 5% level of significance according to Fishers PLSD.

Factor and principal component analysis (PCA)

Principal component analysis showed that the first three principal components (PC) were important and explained that morphological traits accounted for 43.6% of the total variation (Table 7). The first three PCA factors were selected on the basis of highest factor loadings. Morphological traits, PC1 accounted for 16.98% of the variation with major contribution from days to physiological maturity, days to 50% flowering, panicle length, leaf sheath density of hairs margin, a thousand grain weight (g) and panicle number of digits, PC2 explained 14.17% with major contribution from seedling anthocyanin dorsal side colouration, leaf colour, harvest weight, time to 50% inflorescence emergence, caryopsis colour after threshing, seedling anthocyanin coleoptile colouration and PC3 explained 12.45% with major contribution from grain yield per plant, seedling anthocyanin sheath colouration, stem length fully expanded, plant height, level of ploidy and seedling anthocyanin dorsal side colouration.

Table 7: Principal component analysis of 14 finger millet genotypes contributed by morphological traits across three sites to total variation.

Variables	PC1	PC2	PC3
Yield – kg/ha	-0.051	0.13	-0.08
1000 Grain Weight	0.23	-0.11	-0.05
Caryoptsis colour after threshing	0.008	0.22	0.1
Days to 50% flowering	0.33	-0.1	-0.02
Days to physiological maturity	0.34	-0.09	0.02
Finger length	-0.29	0.13	-0.05
Flag leaf length at inflorescence emergence	-0.02	-0.21	0.13
Flag leaf width at inflorescence emergence	0.02	0.07	0.39
Grain yield per plant	0.12	-0.07	-0.08
Harvest weight	0.01	0.23	0.11
Inflorescence anthocyanin colouration	-0.04	0.03	-0.17
Leaf blade anthocyanin colouration	-0.22	-0.08	-0.14
Leaf blade density of hairs on margin base	0.14	0.08	0.13
Leaf blade density of hairs on upper side	0.17	0.1	-0.17
Leaf colour	0.14	0.3	0.05
Leaf gaulosity of lower side	0.16	0.02	-0.3
Level of ploidy	0.19	-0.15	0.18
Leaf sheath anthocyanin coloration	-0.07	-0.3	-0.15
Leaf sheath density of hairs margin	0.26	-0.18	0.15

Leaf sheath density of hairs on both sides	0.15	-0.29	-0.04
Leaf sheath length of hairs ligule	-0.04	-0.05	0.09
Leaf width	-0.01	-0.05	0.02
Number of tillers	0.07	-0.04	-0.23
Panicle attitude of branches	-0.02	-0.17	0.13
Panic number of digits	0.22	-0.12	-0.01
Plant growth habit	-0.25	-0.25	0.05
Plant height	0.12	-0.2	0.28
Panicle length	0.31	0.004	0.09
Panicle shape	0.17	0.15	-0.06
Productive tillers	0.09	0.09	-0.22
Seedling anthocyanin coleoptiles colouration	0.09	0.21	-0.01
Seedling anthocyanin dorsal side colouration	-0.01	0.33	0.16
Seedling anthocyanin sheath colouration	0.06	0.15	0.33
Stem length fully expanded	-0.19	-0.13	0.31
Stem length upper most internote	0.1	0.02	-0.26
Time to 50% inflorescence emergence	0.15	0.23	0.03
Eigen value	**6.114**	**5.103**	**4.482**
Percentage variation	**16.98**	**14.17**	**12.45**

Principal component 1 (PC1), Principal component 2 (PC2), Principal component 3 (PC3)

The cluster analysis (Figure 4.1) constructed from morphological traits based on Euclidean revealed that the closest genotypes were Katumani and Masindi while the longest distance was observed between Katumani and Lira. Using the mean similarity as cutoff, the genotypes were clustered in three groups; the first consisted of 11 genotypes; Malaba, Kapchorwa, Gulu, Katumani, Masindi, Nanjala brown, Jinja, Busia, P221, Songhor and P224; the second Hoima and Buguri; and the third only with Lira. The cophenetic correlation coefficients (r) of the traits studied showed that the dendrogram were 0.81.

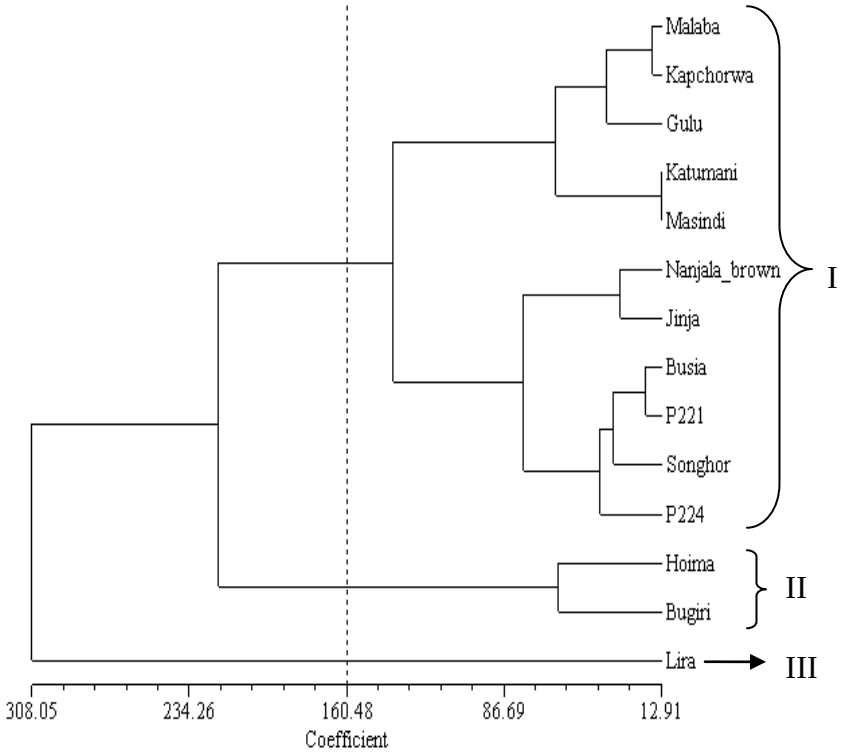

Figure 4.1: Dendrogram generated from 14 finger millet genotypes based on Euclidean, UPGMA clustering using morphological traits.

Correlation analysis

The relationship between 10 quantitative traits observed among 14 finger millet genotypes were estimated by correlation analysis as presented in Table 8. A thousand grain weight was positive and significantly correlated with yield; ($p \leq 0.05$) days to 50% flowering, grain yield per plant, number of tillers and ($p \leq 0.01$) yield. Days to 50% flowering was positively significantly correlated to ($p \leq 0.001$) grain yield per plant. Grain yield per plant was positively significantly correlated to ($p \leq 0.05$) plant height and yield in Kg. Number of tillers was positively significantly correlated ($p \leq 0.05$) yield in Kg and to ($p \leq 0.001$) productive tillers. Plant height was positively significantly correlated to ($p \leq 0.05$) yield in Kg. Yield per hectare in Kg was positively significantly correlated to ($p \leq 0.001$) productive tillers.

Table 8: Correlation among some quantitative traits of finger millet genotypes across the three

	100GW	D50%	Dpm	Fl	Gyp	Hi	Nt	Ph	Prd til	Yield kg/ha
1000GW	-									
D50%	0.56*	-								
DPM	0.51	0.95***	-							
FL	0.24	-0.45	-0.47	-						
GYP	0.55*	0.55*0.2	0.32	-0.17	-					
HI	-0.3	-0,23	-.0.24	-0.28	-0.11	-				
NT	0.04*	0.34	0.37	-0.004	0.08	-.027	-			
PH	0.29	0.42	0.42	-0.29	0.05*	-0.01	-0.31	-		
Prd_til	-0.09	0.31	0.34	0.03**	0.09	-0.1	0.9**	-046	-	
Yield - kg/ha	0.51**	-0.29	-0.21	0.17	0.32*	-0.05	0.21*	0.22*	0.34**	-

Sites (combined for locations) in the year 2010/2011.

Productive tillers (prd til), harvest index (HI), days to 50% flowering (D50%), days to physiological maturity (DPM), plant height (PH), number of tillers (NT), finger length (FL), a thousand grain weight (1000GW), grain yield per plant (GYP).

4.2 Genetic diversity of finger millet genotypes using SSR markers

A total of 9 out of the 12 pairs of primers gave polymorphic bands; the remaining primers failed to amplify any product or were monomorphic and therefore were not considered for further analysis. A total number of 21 alleles were detected with the 9 SSR primer pairs with a mean of 3 alleles per locus among the fourteen finger millet genotypes (Table 9) (Plate 2). The polymorphic information content (PIC) values also ranged from 0.09 in UGEP77 to 0.65 in UGEP8 with an average of 0.29.

The most polymorphic primers were UGEP106, UGEP21 and UGEP21 based on PIC values. Gene diversity was high ranging from 0.1 in UGEP77 to 0.56 in UGEP18 with a mean value of 0.34. The observed heterozygozity (Ho) calculated for each primer ranged from 0.32 (UGEP18) to 0.62 (UGEP68) with the mean of 0.45.

Table 9: SSR marker, number and frequency of alleles, gene diversity, heterozygozity and polymorphic information content (PIC) values generated from 9 SSR data.

SSR Marker	Allele Number	Allele Frequency	Gene Diversity	Heterozygosity	PIC
UGEP77	2	0.75	0.1	0.37	0.1
UGEP68	4	0.65	0.36	0.62	0.85
UGEP106	3	0.5	0.5	0.59	0.38
UGEP21	3	0.58	0.48	0.55	0.42
UGEP8	2	0.4	0.6	0.45	0.65
UGEP18	2	0.63	0.56	0.32	0.52
UGEP12	3	0.82	0.31	0.43	0.28
UGEP81	4	0.68	0.43	0.36	0.64
UGEP104	2	0.73	0.4	0.35	0.32
Mean	2.7	0.64	0.41	0.45	0.46

In Figure 4.2, the genotypes were clustered in to two groups; (I) consisted of Malaba, Busia, Kapchorwa, Bugiri, Katumani, Jinja, Hoima, Lira and Songhor and (II) consisted of P221, Masindi, Nanjala Brown, Gulu and P224. The cophenetic correlation coefficients (r) of the SSR dendrogram were 0.90.

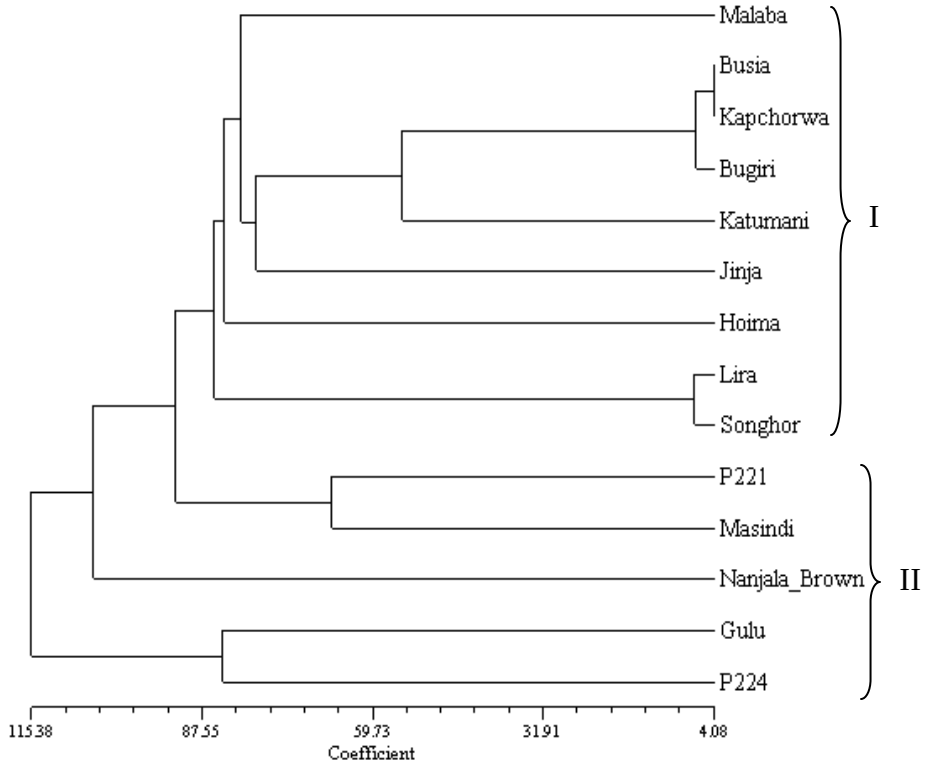

Figure 4.2: Dendrogram of 14 finger millet genotypes generated by UPGMA clustering based on 9 SSR markers using Euclidean coefficient.

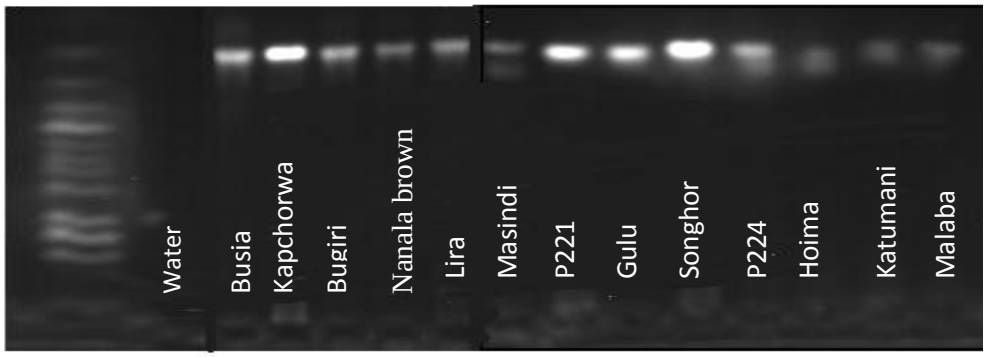

Plate 2: SSR markers profile of 14 finger millet genotypes generated by primer UGEP21.

Source: Author, 2011

4.3 Comparison of finger millet genetic distance based on morphological and SSR markers.

Morphological and SSR dendrograms were produced from binary data using the Euclidean similarity coefficient using UPGMA clustering method. From the results morphological (Figure 4.1) and Figure 4.2) SSR dendrograms revealed two and three clusters respectively. The comparison of morphological versus SSR dendrograms revealed two main clusters and some genotypes clustered together for both analyses. Malaba genotype was observed positioned at the top in SSR and morphological dendrograms.

The genetic distance estimated by combined morphological and SSR dendrogram revealed that the closest genotypes were Busia and Kapchorwa, while the longest distance was observed between genotypes P224 and Busia. The fourteen finger millet genotypes were grouped into two groups; the first consisted of 5 genotypes Malaba, Katumani, Busia, Kapchorwa and Bugiri and the second consisted of 9 genotypes Jinja, P221, Masindi, Lira, Songhor, Nanjala Brown, Hoima, Gulu and P224.

The difference between Figure 4.2, morphological dendrogram and Figure 4.3, SSR dendrogram is that the genotypes Bugiri, Katumani, Hoima, P221, Masindi and Nanjala Brown changed their positions in the dendrogram while the rest of the genotypes maintained their positions in the dendrogram.

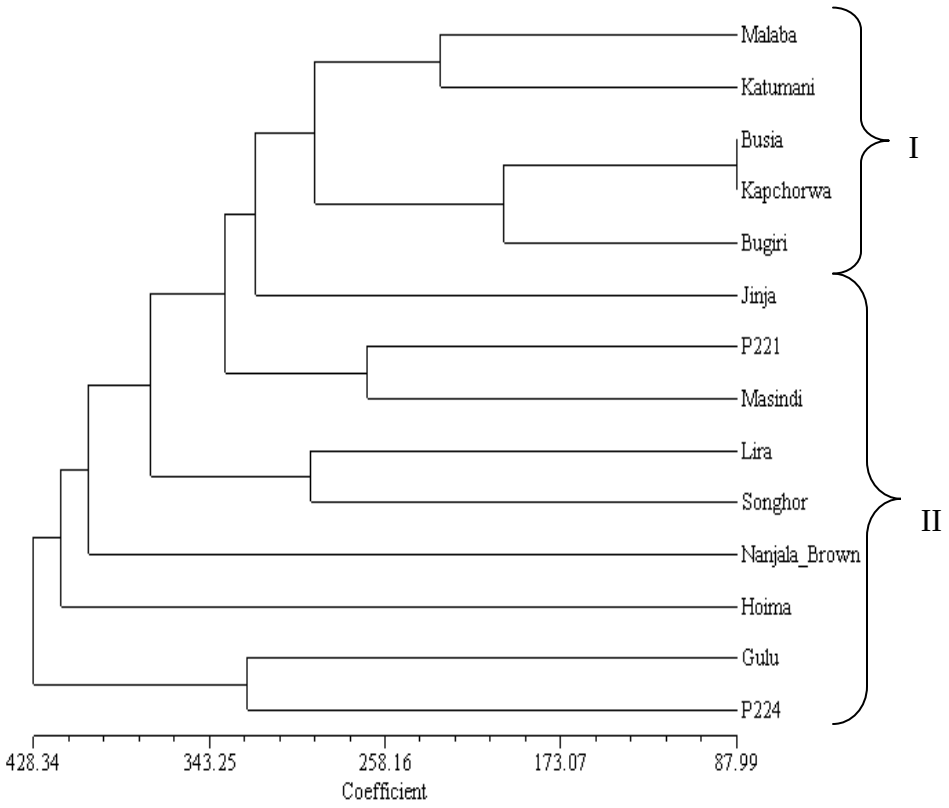

Figure 4.3: Dendrogram of 14 finger millet genotypes generated by UPGMA clustering based on 9 SSR markers using Euclidean coefficient.

Genotype Malaba maintained its positioning, for both the SSR and the morphological dendrograms. Gulu and P224 genotypes were also clustered close at the bottom together in SSR

Mantel test was used objectively to compare matrices generated by cophenetic correlation coefficients (r). The cophenetic correlation coefficients (r) between the similarity matrices and cophenetic matrices obtained with, morphological versus SSR, , SSR versus combined morphological and SSR dendrogram was , 0.26, . When the dendrograms (SSR and morphological data) were compared using consensus tree analysis it was 60% identical (*CIc*= 0.60). When the consensus fork index *CIc* equals one the dendrograms is considered identical.

CHAPTER FIVE:

DISCUSSION

5.1 Morphological characterization and performance of finger millet genotypes

Finger millet germplasm identification and characterization is an important link between conservation and utilization of plant genetic resources. The significant difference between genotypes for most of the morphological traits observed from the analysis of variance in the three sites indicated that most of the genotypes are highly variable. This is an opportunity for plant breeder to undertake further breeding activities to clearly identify suitable varieties for specific environment. Several authors have also reported that the mean square due to location and genotypes were highly significant some quantitative traits considered in their study (Adnew, 2002; Lule *et al.,* 2008; Misra *et al.*, 2009; Naveed *et al.*, 2007).

The thirty seven morphological traits evaluated across the three sites showed that only 18 traits in Ahero, 14 traits in Ugunja and 15 traits in Kitale and 32 traits for the three sites showed significant variation. The significant differences observed in the three locations, could be attributed to the differences in environmental conditions (temperature, soil or rainfall) and this would serve as a criteria for selecting suitable finger millet varieties for their ecological zones. The genotypes selected for each site was based on a thousand grain weight (g), grain yield per plant (g) and yield in hectares (Kg) which varied significantly for the three sites meaning that environmental conditions influences the performance of the genotypes.

Morphological traits studied which showed significant variation in the individual locations and common to all locations include productive tillers, harvest index, days to 50% flowering, days to

physiological maturity, plant height, number of tillers, finger length, a thousand grain weight, grain yield per plant, yield per hectare and seedling anthocyanin coloration of sheath of first leaf. Contrasting responses were observed between locations which would mean that multilocational testing should be continued in order to identify stable genotypes and investigation into the genetics and physiological specialization of finger millet. Principal component analysis was important and explained that morphological traits contributed considerable diversity and this agrees with Reddy *et al.*, (2009.) Genotypes adapted for each location was selected based on a thousand grain weight, grain yield per plant and yields per hectare in Kg and Malaba was common to all the locations. Selection of genotypes or parents based on grain yield should be marched with the knowledge about relationship between yield and its contributing characters needed for an efficient selection strategy.

From the cluster analysis, cluster I comprised of 11 genotypes; Malaba, Kapchorwa, Gulu, Katumani, Masindi, Nanjala brown, Jinja, Busia, P221, Songhor and P224. Cluster II comprised of Hoima and Bugiri and cluster III comprised of Lira.. The cophenetic correlation obtained for dendrogram showed that the dendrogram was good fit and in agreement between the graphical representation of the distances and the original matrices, which enables more accurate visual inferences to be drawn.

Correlation provides information on the nature and extends of relationship among characters. Grain yield is a complex trait controlled by many genes. In the present study, grain yield was significantly correlated to productive tillers, plant height, number of tillers, grain yield per plant and a thousand grain weight and this means that it contributed to economic yield which confirms the reports as described by Bedis *et al.*, (2006) in finger millet and is evident that traits that are positively correlated with grain yield indicates that the selection for any of these yield attributing traits will

lead to improvement of the other related traits and thereby finally enhancing the yield. Bendale *et al.,* (2002) found that grain yield per plant was significantly influenced by days to emergence of finger, days to 50% flowering, finger length, finger width, and weight of grains of main ear head. Ganapathy *et al.,* (2011) reported that productive tillers per plant and finger length are the important yield contributing traits and are important when selecting for grain yield improvement in finger millet. Correlations among different traits suggested associations that can be used to facilitate finger millet genetic improvement through traditional selection.

5.2 Genetic diversity of finger millet genotypes based on SSR markers.

From the results 9 out of 12 primers produced 21 alleles with an average of 3.0 alleles per locus. This result shows the ability of SSR to discriminate among genotypes and their application for cultivar identification. The polymorphic information content (PIC) values ranged from 0.1 to 0.85 comparing favourably with the results obtained by Panwar *et al*., (2010) who used RAPD markers to evaluate the genetic diversity of finger millet. The PIC was calculated to characterize the capacity of each primer to detect polymorphic loci and results showed that most of the primers were found to be highly informative and can be used to study phylogenetic relationship and genetic diversity studies in future. The allele frequency of all the primers was generally below 0.95 indicating that they were all polymorphic in character. Gene diversity was significantly high ranging from 0.1 to 0.56. The differences in distribution of different microsatellite sequences in genomes determine the possibility of using this method for DNA fingerprinting. The observed heterozygozity (Ho) mean was 0.45 which suggests a diverse set of considerable genetic heterogeneity among the finger millet genotypes that could be useful for improving finger millet diversity (Dida *et al*., 2007; 2008).

The genetic distance between the populations was studied using Euclidean analysis. The genotypes genetic showed that similarity exists between the some germplasms of finger millet. Inspite of their different sources of collection, these two germplasms fall under one subgroup (similarity coefficient). Some genotypes also showed dissimilarity between them and fall in different groups. In plant breeding programmes, the most diverse genotypes are used in the hybridization or crop improvement programme. The DNA fingerprinting analysis provides a good method for the discrimination of germplasms at the interspecific level (Jia *et al*., 2000; Conner and Wood, 2001). .

5.3 Comparison of genetic diversity of finger millet genotypes based on morphological and SSR markers.

The results suggest that some genotypes had similar grouping patterns for SSR and and morphological dendrograms. These results suggest that finger millet diversity for both the genetic and phenotypic variability is high and the use of these markers separately or in combined analyses will be more reliable to detect genetic differences among finger millet genotypes (Kumari and Pande, 2010). Compared to phenotypic traits, molecular markers have the advantage of not being influenced by the environment, specific, reliable and wider range of genome sampling but have the disadvantage of accessing the genome as a whole and not only the regions responsible for the expression of traits of interest.

The Mantel matrix correspondence test was used to compare matrices generated from morphological and SSR and combined data using cophenetic correlation coefficients (r). Cophenetic correlation coefficients were used to objectively compare matrices generated from SSR and morphological data, using the Mantel test. The cophenetic correlation coefficient of the combined dendrogram (r=0.7)

showed good agreement between the graphical representation of the distances and the original matrices, which enables more accurate visual inferences to be drawn.

Consensus tree analysis (CIc) was used to compare the different dendrograms. Dendrograms are considered identical when the consensus fork index CIc equals one. Dendrograms obtained from SSR and morphological data were compared using consensus tree analysis and it was 60% identical (CIc= 0.60). The consensus fork index value of 0.6 indicated high resolution of the dendrograms obtained from morphological and SSR data. The correlation coefficient was significant (r=0.998) between SSR and combined SSR and morphological analysis.

Morphological characterization has been traditionally used despite their limitation (Upadhyaya *et al.,* 2007). Its usefulness is still valid for farmers, breeder and curators, as well as for variety registration and release. However, genetic markers have received extensive attention in the last decade as a tool to improve knowledge about the genetics of various traits, and to enhance breeding efficiency (Soller and Beckmann, 1983). DNA-based molecular markers can facilitate the precise identification of genotypes without the confounding effect of the environment, thus increasing heritability. They also contribute to the efficient reduction of large breeding populations at the seedling population. The genetic diversity analysis using association of SSR and morphological data, is more powerful because both analysis are incorporated and a large proportion of variation detected by molecular markers are not subject to the environment. Conventional breeding methods in combination with molecular markers have been advantageously reported in development of elite productive varieties in cotton (Abdukarimov *et al.,* 2003).

CHAPTER SIX:

CONCLUSION AND RECOMMENDATION

6.1: Conclusion

The results from this study indicate that there is sufficient genetic diversity among finger millet genotypes studied which could be exploited for breeding and selection programmes for improved genotypes for increased finger millet production.

1) Cluster analysis showed that genotype Lira was clustered alone from the other genotypes in the morphological dendrogram.
2) Both morphological descriptors and SSR markers were able to group finger millet genotypes into distinct groups. For reliability and efficiency of genetic diversity studies, use of morphological markers, should be backed with DNA markers.

6.2: Recommendation

Planning of future germplasm collection in many geographical areas should be carried out instead of collecting extensively within individual region. Priorities of germplasm collection should focus on areas with relatively large variation. Exploiting the genetic diversity existing in the available germplasm could be beneficial to breeders in finger millet improvement through genome-based utilization of unexploited gene pools because, so far, a very small fraction of the total available collections of finger millets have been used in the national breeding programs in Kenya. Performance of this crop should be improved through the use of appropriate breeding techniques to overcome the challenges faced in finger millet production.

REFERENCES

Abdukarimov, A.S., Djataev, S., Abdukarimov, I. 2003. Cotton research in Uzbekistan: Elite varieties and future cotton breeding. Proceedings of World Cotton Research Conference. Cape Town, South Africa. pp 5-15.

Adnew, T., (2002). Genetic diversity and association among quantitative traits in tef germplasm. MSc. thesis. Alemaya University, Ethiopia.

Attere A.F., (1993). Distribution and collection of finger millet (Eleusine) in East and Southern Africa. Pages 375-380 *in* Advances in small millets (Riley KW, Gupta SC, Seetharam A and Mushonga JN, eds.). New Delhi, India: Oxford and IBH Publishing.

Bedis, M. R., Patil H.S.., Jangle G.D. and Patil V.S., (2006). Correlation and path analysis in fingermillet (*Eleusine coracana* Gaertn.). Crop Res., 31(2): 261-266.

Bendale V. W., Bhave S. G. and Pethe U. B., **(**2002). Genetic variability, correlation and path analysis in finger millet (*Eleusine coracana* Gaertn.). Journal of Soils and Crops 12: 187-191.

Bezaweletaw, K., P. Sripichit, W. Wongyai and V. Hongtrakul, (2006). Genetic variation, heritability and path-analysis in Ethiopian finger millet (*Eleusine coracana* (L.)Gaertn) landraces. Kasetsart J. Nat. Sci., 40: 322-334.

Bisht M.S. and Mukai Y., (2001). Genomic *in situ hybridization* identi- Wes genome donor of Finger millet (*Eleusine coracana*). *Theor Appl Genet* 102:825–832.

Botstein D., White R. L., Skolnick M., Davis R. W., (1980). Construction of a genetic map in man using restriction fragment length polymorphism. American Journal of Human Genetics 32: 314-330.

Chen L. and Yamaguchi S., (2005). RAPD markers for discriminating tea germplasms at the inter-specific level in China. *Plant Breed.* 124: 404-409.

Conner P.J. and Wood B.W., (2001). Identification of pecan cultivars and their genetic relatedness as determined by randomly amplified polymorphic DNA analysis. *J. Am. Soc. Hort. Sci.* 126: 474-480.

Consultative Group on International Agricultural Research (CGIAR), (2001). CGIAR Research: Areas of Research–Millet. [online] Available: http://www.cgiar.org/research/res_millet.html [2004,Apr.]

De Wet, J.M.J., Prasada Rao K.E., Brink D.G. and Mengesha M.H., (1984). Systemic evolution of *Eleusine coracana* (Graminea). Amer. J. Bot. 7: 550-557.

Dida M. M., Wanyera N., Dunn M. L. H., Bennetzen J. L. and Devos K.M. (2008). Population structure and diversity in finger millet *Eleusine coracana* germplasm. *Trop. Plant Biol*. 1, 131–141.

Dida MM, Srinivasachary, Sujatha, Amakrishnan, Jeffrey, Bennetzen L, Mike D, Katrien M, Devos, (2007). The genetic map of finger millet, *Eleusine coracana*. Theoretical and Applied Genetics, 114: 2-32.

Doyle J.J. and Doyle J.L., (1990). Isolation of plant DNA from fresh tissue. *Focus* 12:13-15.

Duarte M. C., Santos J. B., Melo L. C., (1999). Comparison of similarity coefficient based on RAPD markers in the common bean. *Genetics and Molecular Biology* 22: 427-432.

Edwards A., Civitello A., Hammond H. A. and Caskey C. T., (1991). DNAtyping and genetic mapping with trimeric and tetrameric tandem repeats. *American Journal of Human Genetics,* 49: 746-56.

Ekwamu, A., (1991). Influence of head blast infection on seed germination and yield components of finger millet (*Eleusine coracana* L. Gaertn). Trop. Pest Manage., 37: 122-123.

FAO, (2009). Cassava diseases in Central, Eastern and Southern Africa (CaSESA), Strategic programme framework 2010-2015. *http://www.fao.org*.

Ganapathy S., Nirmalakumari A. and Muthiah A.R., (2011). Genetic Variability and Interrealtionship Analyses for Economic Traits in Finger Millet Germplasm. World Journal of Agricultural Sciences 7 (2): 185-188.

Gibbs-Russell, G.E., Watson, L., Koekemoer, M., Smook, L. Barker, N. P., Anderson, H.M., Dallwitz, M. J. (1989). Grasses of southern Africa. Memoirs of the Botanical Survey of South Africa, No. 58, National Botanical Institute, Pretoria.

Hilu K. W. and Dewet J. M. J., (1979). Racial evolution in *Eleusine coracana* (L.). American Journal of Botany, 63: 1311-1318.

Hilu KW, Johnson JL (1992) Ribosomal DNA variation in Finger millet and wild species of *Eleusine* (Poaceae). Theor Appl Genet 83:895–902.

Hiremath S.C., Salimath S.S. (1992). The A genome donor of *Eleusine coracana* Gaertn (Gramineae). Theor Appl Genet 84:747–754.

IBPGR (International Board for Plant Genetic Resources), (1985). Descriptors for Finger Millet. IBPGR Secretariat, Rome, pp: 20. 18.

Jaccoud D., Peng K., Feinstein D. and Kilian, A., (2001). Diversity Arrays: a solid-state technology for sequence information independent genotyping. *Nucleic Acids Research*: 29: e25.

Jacob H. J., Lindpainter K., Lincoln S. E., Kusumi Bunker R. K., Mao Y- P., Ganten D., Dzau V.J. and Lander E.S., (1991) Genetic mapping of a gene causing hypersensitive rat. *Cell,* 67:213-224.

Jaetzold R. and Schmidt H., (2006). Farm management Handbook of Kenya. Ministry of Agriculture (MOA) in Cooperation with German Agency of Technical Cooperation (GTZ). Vol. 11/C1. Printed in Nairobi.

Jia J.H., Wang P., Jia D.M., Qu X.P., Wang Q., Li C.Y., Weng M.L. and Wang B., (2000). The application of RAPD markers in diversity detection and variety identification of Porphyra. Acta. Botanica Sinica, 42: 403-407.

Kumari K. and Pande A, (2010). Study of genetic diversity in finger millet (*Eleusine coracana* L. Gaertn) using RAPD markers. African Journal of Biotechnology Vol. 9(29), pp. 4542-4549.

Litt M. and Lutty J. A., (1989). A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *American Journal of Human Genetics,* 44:397-401.

Lule, D., Bekele E. and Ayana A., (2008). Variability, heritability and genetic advance in quantitative traits of tef: Implication for genetic conservation and improvement strategies. Ethio. J.Biol.Sci., 7:159-177.

Maniatis T., Fritsch E. F., and Sambrook J., (1989). Molcular cloning. A laboratory manual 2nd Edition Clod Spring laboratory, New York.

Mantel, (1967). The detection of disease clustering and generalized regression approach. *Cancer Research* 27: 209-220.

Misra R. C., Das S. and Patnaik, M.C. (2009). AMMI Model Analysis of Stability and Adaptability of Late Duration Finger Millet (*Eleusine coracana*) Genotypes. World Applied Sciences Journal, 6: 1650-1654.

Mitaru, B.N. Karugia J.T. and Munene C., (1993). Finger millet production and utilization in Kenya. In: Riley, K.W., Gupta, S.C., Seetharam, A. and Mushonga, J.N. (ed.). Advances in small millets. pp. 247-254. New Delhi: Oxford and IBH.

Mnyenyembe P. H. and Gupta S. C., (1998). Variability for grain yield and related traits in finger millet germplasm accessions from Malawi. Short communication. African Crop Science Journal, Vol. 6. No. 3, pp. 317-322.

Mulatu, T., Debelo A., Gutema Z. and Degu E., 1995. Finger millet: A potential crop in Ethiopia. In: proceedings of work shop organized to re-establish sorghum and millet in Eastern and Central Africa. Kampala, Uganda 6-9 Nov.

Mullis K., Faloona F., Schard S., Saiki R., Horn and Erlich H., (1986). Specific enzymatic amplification of DNA in vitro: the polymerase enzyme chain reaction. Cold Spring Harbor Symposium. *Quantitative Biology* 51: 263-273.

Murray M.G. and Thompson W.F., (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8: 4321–4325.

Mushonga, J.N., F.R. Muza and, H.H. Dhliwayo, 1993. Development, Current and Future Research Strategies on Finger Millet in Zimbabwe. In: Riley, K.W., Gupta, S.C., Seetharam, A. and Mushonga, J.N. (ed.). Advances in small millets. pp. 11-19. New Delhi: Oxford and IBH.

Muza F.R., Lee D.J., Andrews D.J. and Gupta S.C., (1995). Mitochondrial DNA variation in finger millet (*Eleusine coracana*). Euphytica 81:199–205.

National Research Council, (1996). Finger millet in Lost crops of Africa: volume I: grains, National Academy of Sciences: 39-57.

Naveed, M., Nadeem M. and Islam N., (2007). AMMI analysis of some upland cotton genotypes for yield stability in different milieus. World J. Agril. Sci., 3: 39-44.

Obilana, A.B., E. O. Manyasa, J. G. Kibuka and S. Ajanga. (2002). Finger millet blast (fmb) samples collection in Kenya: Passport data, analyses of disease incidence and report of activities. ICRISAT, Nairobi, Kenya.

Odelle, S.E., (1993). Improvement of finger millet in Uganda. In: Riley, K.W., Gupta, S.C., Seetharam, A. and Mushonga, J.N. (ed.). Advances in small millets. pp. 75-83. New Delhi: Oxford and IBH.

Oduori C. O. A. (2000). Finger Millet. Kenya Agricultural Research Institute. [online] Available: http://www.kari.org/Farmers%20Guide/Information%20brochures/Finger%20Millet.Htm

Oduori, C.O., (1993). Small Millets Production and Research in Kenya. In: Riley, K.W., Gupta, S.C., Seetharam, A. and Mushonga, J.N. (ed.). Advances in small millets. pp. 67-73. New Delhi: Oxford and IBH.

Panwar P., Nath M., Kumar V. Y., and Kumar A., (2010). Comparative evaluation of genetic diversity using RAPD, SSR and cytochrome P450 gene based markers with respect to calcium ncontent in finger millet (*Eleusine coracana* L. Gaertn.). Research article. Indian Academy of Sciences.

Rachie K.O. and Peters L.O., 1978. *Eleusines*: a review of world literature. Patancheru 502 324, Andhra Pradesh, India: ICRISAT.

Rao S. A., Mengesha M. H. and Reddy, C. R., (1986). *New sources of dwarfing genes in pearl millet (Pennisetum americanum). TAG Theoretical* and Applied Genetics, 73 (2). pp. 170-174.

Reddy G. V., Upadhayaya H. D., Gowda C. C. L., and Singh S., (2009). Characterization of Eastern African finger millet germplasm for qualitative and quantitative characters at ICRISAT. International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Journal of SAT Agricultural Research 7. Patancheru, India.

Rohlf, (1982). Consensus indices for comparing classifications. *Mathematical Bioscience* 59: 131-144.

Rohlf, (2000). NTSYS-pc: Numerical taxonomy and multivariate analysis system, version 2.1. Exeter Software, New York, 98p.

Salimath S.S., de Oliveira A.C., Godwin I.D. and Benetzen J.L., (1995). Assessment of genome origin and genetic diversity in the genus Eleusine with DNA markers. *Genome* 38:757- 763.

Sneath P. H. A., Sokal R. R., (1973). Numerical Taxonomy. Freeman, San Francisco, USA.

Sokal R. R., Michener C. D., (1958). A statistical method for evaluating relationships. *University of Kansas Science Bulletin* 38: 1409-1448.

Soller M. and Beckmann J. S. 1983 Genetic polymorphism in varietal identification and genetic improvement. *Theor. Appl. Genet*. 67, 25–33.

Southern E. M., (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. Journal of Molecular Biology 98: 503-517.

Tadesse M. and Y. Kebede, (1993). Finger Millet Importance and Improvement in Ethiopia. In: Riley, K.W., Gupta, S.C., Seetharam, A. and Mushonga, J.N. (ed.). Advances in small millets. pp. 51-59. New Delhi: Oxford and IBH.

Takan, J.P., S. Muthumeenakshi, S. Sreenivasaprasad, B. Akello, R. Bandyopadhyay, R. Coll, A. E. Brown, and N.J. Talbot, (2002). Characterisation of finger millet blast pathogen populations in East Africa and strategies for disease management.

Tautz D., (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acid Research 17: 6463-6471.

Upadhyaya H. D., Gowda C. L. L., and Reddy G. V., (2007). Morphological diversity in finger millet germplasm introduced from Southern and Eastern Africa International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India.

Upadhyaya, H.D., Gowda C.L.L., Pundir R.P.S., Reddy V.G. and Singh S., (2006). Development of core subset of finger millet germplasm using geographical origin and data on 14 quantitative traits. Gen. Res. Crop Evo., 53: 679-685.

Van Wyk, B.-E. and Gericke N., (2000). People's plants: a guide to useful plants of southern Africa. Briza, Arcadia.

Van Wyk, E. & Van Oudtshoorn, F., (1999). Guide to grasses of southern Africa. Briza Publications, Arcadia, South Africa.

Vos P. R., Hogers R., Bleeker M., Reijans M., Lee T., Hornes M., (1995). AFLP: a new technique for fingerprinting. *Nucleic Acids Res*. 21, 4407–4414.

Weber J.L. and May P.E., (1989). Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *American Journal of Human Genetics* 44: 388-396.

Welsh J. and McClelland M., (1990), Fingerprinting genomes using PCR with arbitrary primers, *Nucleic Acids Research* **18**: 6531-6535.

Werth C.R., Hilu K.W. and Langner C.A., (1994) Isozymes of *Eleusine* (Gramineae) and the origin of Wnger millet. *Am J Bot* 81:1186–1197.

Williams G.K.J., Kubellik R.A., Livak K.J., Rafalski J.A. and Tingey S.V. (1990). DNA polymorphism amplified by arbitrary primers is useful as genetic markers. Nucleic Acid Res. 18: 6531-6535.

Wyman A. R., White R., (1980). A highly polymorphic locus in human DNA. Proceedings of the National Academy of Sciences (USA) 77: 6754-6758.

Yang P., Wei H., Huang H.-L., Baum B.A., Hu Y.X., Kattawar G.W., Mishchenko M.I., Fu Q. (2005). Scattering and absorption property database for nonspherical ice particles in the near- through far-infrared spectral region. *Appl. Opt.*, 44, 5512-5523.

APPENDICES

Appendix I: Software Description

Numerical (NTSYS)

This is a computer package of programs that is used to find and display structure in multivariate data. The program was originally developed for use in biology in the context of the numerical taxonomy. The methods furnished in NTSYS are associated with the field of phenetics, but it also can be used in cladistics. It can be used to compute various measures of similarity or dissimilarity between all pairs of objects and then summarize this information either in terms of nested sets of similar objects (*cluster analysis*) or in terms of a spatial arrangement along one or more coordinate axes (*ordination analysis* or various types of *multidimensional scaling a*

Appendix II: Thermocycling Programme

The **thermal cycler** (also known as a **thermocycler**, **PCR machine** or **DNA amplifier**) is a laboratory apparatus most commonly used to amplify segments of DNA via the polymerase chain reaction (PCR). Thermal cyclers may also be used in laboratories to facilitate other temperature-sensitive reactions, including but not limited to restriction enzyme digestion or rapid diagnostics.[2] The device has a *thermal block* with holes where tubes holding the reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps.

Modern thermal cyclers are equipped with a heated lid that presses against the lids of the reaction tubes. This prevents condensation of water from the reaction mixtures on the insides of the lids. Traditionally, a layer of mineral oil was used for this purpose.

Some thermal cyclers are equipped with multiple blocks allowing several different PCR reactions to be carried out simultaneously. Some models also have a *gradient* function to allow for different temperatures in different parts of the block. This is particularly useful when testing suitable annealing temperatures for PCR primers

Source: Wikipedia, 2012