CHARACTERIZING THE GENETIC DIVERSITY OF FINGER MILLET IN UGANDA

BY

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DECLARATION

I declare that the work in this thesis on Characterizing the Genetic Diversity of Finger Millet in Uganda is my own and has not been submitted for the award of a degree to any other university.

Signed

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This thesis has submitted with full approval of my supervisors

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SUMMARY

Finger millet [*Eleusine corocana* (L) Gaertn.] is a staple cereal, widely cultivated in the eastern, northern, northwestern, and southwestern parts of Uganda and in many other semi-arid regions of Eastern and Southern Africa as well as South Asia. The production of finger millet in Uganda is constrained by biotic and abiotic stresses, impeding the realization of yield potential. Moreover, a comprehensive review of the published literature shows that finger millet has received little research attention with limited studies conducted on diversity among Ugandan germplasm hence remaining uncharacterized, and under-utilized for breeding purposes. The objectives of this study were to (1) characterise the phenotypic diversity among the selected finger millet accessions using agronomic traits; (2) investigate the presence of pre-and post flowering drought tolerance in selected finger millet accessions; (3) determine genetic diversity among the sampled finger millet using simple sequence repeats (SSR) molecular markers; (4) determine the cross compatibility of cultivated finger millet *Eleusine Corocana* L with its wild relatives *E. africana and E. Kigezienesis.*

The studies involved field evaluations conducted at the National Semi Arid Resources Research Institute (NaSARRI) Serere, Screen house experiment at Makerere University Agricultural Research Institute (MUARIK) Kabanyolo and laboratory work conducted at ICRISAT biotechnology laboratory in Nairobi, Kenya. The evaluations were conducted during first and second season rains of 2011 with a total of 400 finger millet accessions. Planting was done by hand with seeds spread in 4 rows to ensure germination using a lattice design of 16 X 25. The screen house experiments ran from March to July of 2012 with a total of 15 genotypes selected from the results of the field evaluations.10 drought tolerant, 2 varietal checks and 3 susceptible

lines. The experiment was setup using a split plot design in 45 cm x 30 cm buckets with two replications. Marker characterization involved 105 finger millet accessions classified as 51 land races, 49 exotic lines, along with 5 released varieties screened against 20 polymorphic SSR markers. The determination of the cross compatibility between finger millet and the wild relatives *Eleusine* gnus included 18 wild collection of *E africana* and *E kigezenesis* that were crossed with *E corocana* species as female parents and ran from August 2011 to July 2012 at MUARIK

Analysis of variance was used to determine the agronomic trait variations, means and diversity among the accessions; the response of genotypes to post and pre flowering drought stress whereas principal component analysis was used to access the contribution of each trait to the total variation captured. Drought tolerance was investigated by measuring chlorophyll count at 1 day, 21 days and 42 days after water stress while other agronomic traits were measured using the descriptors for finger millet. The DNA extracted from the accessions were screened against 20 polymorphic SSR markers. The SSR marker PCR products were size-separated by capillary electrophoresis on an ABI 3730*x*l DNA analyzer (Applied Biosystems, Inc.). The fragment sizes generated from the Gene Mapper for all the 20 markers were used in the basic statistics analysis using Power Marker version 3.25, including the polymorphic information content (PIC), allelic richness, and the occurrence of unique, rare, common, and most frequent alleles. Principal coordinate analysis was computed based on Nei's distance matrix with 1000 bootstrapping. Meanwhile successful crosses were determined by seed formation after 3-4 weeks which was followed by morphological characterization of the F1 using parent morphology and confirmed by SSR markers.

Significant variations were observed among the finger millet accessions for various agronomic traits with the ear and maturity traits having the greatest contribution to the total phenotypic variation captured which was consistent with reports of previous studies. The genotypes also responded to differential pre and post flowering drought stress with significant genotype to drought treatments interaction observed for chlorophyll content 42 day after stress application and the most drought tolerant genotypes were identified. Meanwhile unique pattern of diversity was revealed by the SSR markers with a total number of 23 loci identified 226 alleles generated by 19 (out of the 20) highly polymorphic markers; alleles generated per locus ranged from 3 to 20 with an average of 9.6 alleles per locus. The polymorphic information content (PIC) varied from 0.09 to 0.88 with an average of 0.53. 62 rare alleles (27.43%), 130 common alleles (57.52%) and 34 the most frequent alleles (15.04%) and 17 most diverse accessions were also identified. Furthermore the cross between finger millet *E corocana* subsp *corocana (2n =4x= 36*), the wild relatives *E corocana* subsp *africana and Eleusine kigezienesis (2n = 4x = 36)* all allotetrapliod was successful hence compatible which was consistent with previous studies but specific to genotypes with 50% of the F1s true fertile F1 hybrids

The results show that the finger millet accessions used in this study are genetically diverse and could be used to initiate a breeding program. Moreover, the gene flow within the *Eleusine* genus provides an opportunity for finger millet genome enhancement and improvement since varietal yield improvement is dependent on the availability of sufficient diversity. It was therefore, concluded that the diverse parents identified in this study could be used for developing finger millet populations' for mapping contributing traits and studying their inheritance. The already identified genetically diverse and similar accessions could also be used for developing mapping

populations for QTL mapping. The new sources for drought tolerance could be used for improving finger millet accessions with farmer preferred traits but there is need to evaluate them for various agronomic traits for suitability. The gene flow within the *Eleusine* genus could also be exploited for improving important traits such as disease resistance, drought tolerance, tillering, earliness and yields to improve the cultivated finger millet.

DEDICATION

To my father Andema Rofino, in gratitude for your continued fatherly guidance and prayers amidst physical disability

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CHAPTER ONE

INTRODUCTION

1.1 Importance of finger millet to Uganda's economy

Finger millet [*Eleusine corocana* (L) Gaertn.] is a staple cereal, widely grown in the semi-arid areas of Eastern and Southern Africa as well as South Asia (Kiran and Thakur *et al*., 2012). The crop is believed to have been first cultivated in Uganda about 5000 years ago where domestication extending from western Uganda to Ethiopian highlands. Today finger millet is important minor millet in the tropics grown in more than 25 countries of Africa and Asia and accounts for 12% of the global millet area (ICRISAT, 2008).

Major finger millet producers are Uganda, India, Nepal, China, Kenya, and Ethiopia. For instance in Uganda, finger millet is cultivated in the eastern, northern, northwestern, and southwestern regions. Annual production is estimated at 841, 000 metric tons on 460, 000 hectares of land, making it the second most important cereal after maize (Wanyera, 2007). Currently, finger millet has been recognized as a highly nutritious food for the weak and immuno-compromised people in Uganda (Takan *et al*., 2012). The grains are a rich source of protein (5.6 to 12.7 %), proportionately higher in brown seeded than in white seeded genotypes. The grain contains high levels of essential amino acids with 44.7% lysine, threonine, and valine above the 33.9% of FAO reference content of similar essential amino acids in rice, wheat and sorghum and maize (Singh and Raghuvanshi, 2012). Besides, finger millet grain has sulphur containing amino acids that are crucial for human health, and rich source of minerals like, calcium, iron, zinc, and manganese (Bhatt *et al*., 2003; Singh and Srivastava, 2006).

However, the production finger millet in Uganda is conducted by small scale farmers with average land holdings of 1-4 ha, under low input system (Patrick, 2011). The low input and

management system compromise the farmer yields. In Uganda, average farmer's yield is between 0.5 T/Ha to 1 T/Ha though yields of 1.6 T/Ha have been reported under experimental conditions. Albeit improved varieties have a potential of 3 T/Ha, the actual yield potential of finger millet is more than 6 T/ha under optimal irrigated conditions. Given the importance of finger millet to smallholder farmers' in the semi-arid areas of Africa, Asia and of Uganda in particular, more effort is required to support finger millet breeding, and close the gap between farmer yield and actual yield potential.

1.2 Constraints to production

Finger millet production is constrained by a number of diseases, insects, and a biotic stresses impeding the realization of high yield potential. The most important disease limiting production is blast caused by *Pyricularia grisea* (Cooke) Sacc. [teleomorph: *Magnaporthe grisea* (Hebert) Barr.], which can causes up to 90% loss in yield (Mgonja *et al.,* 2007; Nagaraja *et al.,* 2007). Blast disease affects the neck and panicles (Takan *et al*., 2012;). Other constrains include drought, striga, and weeds especially the finger millet wild relative (*Eleusine indica*), insect pests mainly shoot fly (*Atherigona spp*) and stem borers (*Busseola spp, Chilo spp* and *Sesamia spp*). Finger millet also suffers,narrow genetic base, limited funding for research, lack of agronomic recommendations, and changing farming systems that increase likelihoods of genetic erosion of finger millet (Kisandu *et al*., 2005). Therefore, blast disease, drought, striga and lodging are some of the constraints that need immediate research attention (NRC, 1996; CGIAR, 2005; Odouri, 2008). However, beginning a successful and sustainable breeding program on finger millet will require that the variation among the germplasm needs to be characterised, genetic base enhanced through wild hybridisations and the information on the traits of economic importance generated to support breeding efforts.

1.3 Justification of the study

Finger millet, though hardy, is severely affected by both biotic and abiotic stresses. Currently farmers grow low yielding varieties that are very susceptible to many stresses. With little information is available germplasm, improvement of finger millet is limited.. Extensive genetic diversity has been observed especially among the landraces for a number of agronomic traits such as panicle compactness and shape, tillering ability, plant height, plant pigmentation, panicle length and width, , days to harvest maturity, days to 50 % flowering, ear exertion, ear size, discontinuity of spikelet, grain colour and blast disease resistance (Odouri, 2005; Wanyera, 2007). However this diversity has not yet been tapped for crop improvement in addition to remaining un-described, being poorly understood and under-utilized (Oduori, 2005; Upadhyaya *et al*., 2008).

Furthermore, the wild ancestral relatives of finger millet *E. africana and E. kigezienesis* are genetically diverse and thrive in the wild as aggressive weeds resistant to stress. The natural hybrids between the *E. corocana* x *E. africana* are also reported to be weedy, fertile aggressive colonizers (Dida *et al*., 2007; Andersson and De Vicente, 2010; Liu *et al*., 2011). Thus efforts to mine the existing genetic diversity among wild relatives to improve resilience of cultivated finger millets, is worth exploiting, albeit limited efforts have been made in the past. Of particular interest are crosses involving other wild relatives such as between, *E. corocana* and *E. africana* or *E. kigezienesis* (Andersson and De Vicente, 2010). This study therefore seeks to characterise a wide collection of finger millet germplasm so as to generate information on the available genetic variation, and investigate the cross compatibility of finger millet *(E. corocana*) with E. *africana* and *E. kigezienesis* to support use of wild accessions for improving yields and other farmer-preferred traits in this nutritious cultivated cereal.

1.4 Objectives of the study

1.4.1 General objective

To contribute to the development of finger millet varieties with farmer preferred traits by generating information that will underpin breeding efforts.

1.4.2 Specific objectives

1. Characterise the phenotypic diversity among the selected finger millet accessions using agronomic traits.

2. Investigate the presence of pre-and post-flowering drought tolerance in selected finger millet accessions.

3. Determine genetic diversity among the sampled finger millet accessions using simple sequence repeats (SSR) molecular markers.

4. Determine the cross compatibility of cultivated finger millet *Eleusine Corocana* L with its wild relatives *E. africana and E. Kigezienesis.*

1.4.3 Hypothesis

1. Accessions of finger millet assembled from different regions and farming systems of Uganda are genetically diverse

2. Accessions of finger millet respond differently to drought stress at pre and post flowering growth phases

3. Members of the genus *Eleusine* are cross-fertile, and can therefore be used as parents in wide hybridization and breeding programmes

CHAPTER TWO

LITERATURE REVIEW

2.1 The origin and distribution of finger millet

Finger millet [*Eleusine corocana* (L.) Gaertn.] belongs to the family *Poaceae* (*Gramineae*). It's a staple food crop in the semi arid areas of the world and an important food security crop in Uganda (Wanyera, 2007). The crop is believed to have originated in Uganda about 5000 years ago with domestication extending from western Uganda to the Ethiopian highlands (ICRISAT, 2008). The domestication were subsequently moved to the highlands of Africa around 1000 B.C and introduced into India through the sea trade that existed (Dida *et al.*, 2008; Liu and Peterson *et al.,* 2011). *Eleusine* species occupy diverse habitats, ranging from open, dry places to forests under-covers. Finger millet is extensively grown in the semi-arid regions of Africa and India through the selection of genotypes adapted to the different agro ecological conditions (Werth *et al.,* 1994; Das *et al.,* 2007*).*

Besides, the crop is cultivated in diverse eco-geographical areas where it displays high variability in vegetative, floral, and seed morphology. Hilu and de Wet (1976) and Upadhyaya *et al.* (2007) identified three eco- geographical races: African highland race cultivated in the East African highlands, a lowland race grown in the lowlands of Africa and south Indian and an Indian race with its centre of diversity in northeast India. The African highland race is the most primitive, diverse and the precursor of the lowland race, which was subsequently introduced to southern India that developed into a secondary center of diversity, resulting in the Indian race. Natural selection may have played a significant role in finger millet evolution (Hilu and de Wet, 1995a). Archaeological evidence also indicates that finger millet was a staple crop of Southern Africa before the introduction of maize. Today, finger millet is cultivated in the Eastern and Southern

Africa and is a major cereal in the northern, eastern, and western regions of Uganda, displaying a lot of trait variability however not collected, characterised, and underutilized for breeding programmes (NRC, 1996;Wanyera,2007; AGPC, 2008).

2.3 Genetic variability in finger millet

2.3.1 Variability among cultivated finger millet

Extensive amounts of finger millet germplasm exist (Bennetzen *et al.,* 2003) with ICRISAT holding 5,000 accessions, University of Agricultural Sciences in Bangalore (4,500) accessions, National Dry land Farming Research Station of Kenya (1,500), Gene bank of Kenya (1,000), Plant Genetic Resource Centre in Ethiopia (1,000) and University of Georgia in US (1, 500) and Uganda (5,000). These large amounts of germplasm provide plant breeders with the necessary genetic material, to develop farmer-desired varieties besides being natural conservation facility for desirable genes due to genetic variability (Fakrudin *et al*., 2004: Das *et al*., 2007). High variability exists for agronomic traits including days to 50% flowering and maturity, plant height, number of productive tillers, main ear length, finger number per ear and grain yield as well as neck and head blast (Bedis *et al*. 2006; Bezaweletaw *et al.*,2006; Dagnachew et al., 2012). High genotypic and phenotypic coefficients of variation have also been reported for the number of productive tillers per plant, number of fingers per ear, ear weight, total dry matter, harvest index, and high heritabilities coupled with high expected genetic gain for productive tillers (John, 2006; Dagnachew et al., 2012). Moreover studies revealed, high broad sense heritabilities for days to 50% flowering, days to physiological maturity, plant height, number of tillers, number of fingers per ear, finger length, 1,000 grain weight and grain yield per plant (Sumathi *et al.,* 2007). Although recent studies revealed high morphological variability among finger millet accessions, breeding for resilient and high yielding varieties is only feasible through

accurate characterization and capture of the genetic variability among the accessions for effective utilization (Ali *et al.* 2007; Rajan and Deshpande *et al*., 2010)

2.3.2 Response to drought

Finger millet is a resilient crop well adopted for harsh conditions of the semi-arid tropics (Muhammad and Azam, 2007;Vadez *et al.,* 2012). Moreover, frequent occurrence of abiotic stresses such as water deficit stress has been identified as the key constraint leading to the low productivity under rain fed ecosystems. A forecast on climate change effects predict water deficit in years to come (Wassmann *et al.,* 2009). Severe water deficit in the wet season at the reproductive stage not only have an adverse effect on finger millet yields but also reduces the area sown under other crops like sorghum, maize, wheat, pulses, and oilseeds in the subsequent dry season because of the unavailability of sufficient moisture in the soil (Chaves and Oliveira, 2004;). Drought stress induces biochemical changes, such as decreased ribulose-1,5-bisphosphatase carboxyase/oxygenase activity, reduced photochemical efficiency, enhanced accumulation of stress metabolites and increased antioxidant enzymes in higher plants. Physiological changes include reduced relative water content, pigment degradation, decreased stomatal conductance, reduced internal CO_2 concentration, net photosynthetic rate (Pn) reduction and growth inhibition prior to plant death (Vadez *et al* ., 2012).

Investigations reveal that some crop parameters especially yield related traits are critical for improving water deficit tolerance through breeding besides being the most important trait for water-deficit tolerance screening (Ashraf, 2010). Knowledge of crop water usage at key stages of growth is critical for drought adaptation in the field conditions (Zaman-Allah *et al.,* 2011). Identifying suitable screening tools and quantifiable traits would facilitate investigation of genotype responses to water deficient stress especially during critical growth stages

2.3.3 Genetic diversity in wild finger millet

Finger millet and its wild relatives belong to the genus *Eleusine* Gaertn (*Poaceae,* subfamily *Chloridoideae*). The genus includes 10 annual and perennial species that are native to East African (Phillips, 1972; Dida, 1998; Andersson and De Vicente, 2010) that is *E. corocana, E. indica, E. intermedia, E. jaegeri, E. floccifloia ,E. kigezienesis, E. multiflora* and *E. semisterilis* except one New World species (*E .tristachya)* native to Argentina and Uruguay (Andersson and De Vicente, 2010). The primary centre of diversity for the genus *Eleusine* is East Africa where 8 of the approximately 10 species occur. India is considered to be a secondary centre of diversity. The genus includes diploids and tetraploids with basic chromosomes number of 8, 9, and10. This has been extended by widespread introduction of the crop (*E. corocana)* throughout the tropics; the common weed often associated with cultivation *E. indica* (Dida *et al.,* 2007; Liu and Peterson *et al.,* 2011). A lot of diversity has been reported in the genus *E. corocana, E. africana, E. indica* and *E. kigezienesis* (Dida, 2007). A study of the genome has reported a close relationship between *E. corocana, E. africana, E. indica* and *E. kigezienesis* with allotetraploid attributes (Liu *et al.,* 2011).

2.4 Gene flow within the genus Eleusine

Within the genus *Eleusine,* the cultivated finger millet *E. corocana* sub spp *corocana* and the wild *E. corocana* sub spp *africana* both allotetraploid are cross compatible (de Wet, 1991; Dida, *et al.,* 2008; Andersson and De Vicente, 2010). In finger millet, gene flow may occur either through seeds or pollen, though successful hybridization does not necessarily lead to gene introgression but depends on the nature of the transferred genes (Andersson and De Vicente, 2010) *Eleusine kigezienesis* (*2n= 4x = 36,* some forms 38) another allotetrapliod species closely related to finger millet and its wild progenitor are cross compatible though both produce male

sterile F1 hybrids if crossed with a male fertile *E corocana* plants. This indicates that *E. kigezienesis* carry AABB genome (Dida, *et al.,* 2008; Andersson and De Vicente, 2010). Nevertheless, other wild relatives are genetically isolated from cultivated finger millet due to differences in their ploidy level with no natural hybrids between finger millet and these wild relatives (Hiremath and Salimath, 1992).

Although finger millet shows a lot of morphological diversity, it is prone to stress like drought, blast disease because of the narrow genetic base occasioned by population bottlenecks during domestication yet the wild relatives thrive under the same conditions (Dida *et al*., 2008; Andersson and De Vicente, 2010; Liu and Peterson *et al.,* 2011). The cross compatibility within *Eleusine* allotetrapliods provides a unique opportunities for new sources of novel genes for blast disease resistance, drought tolerance, earliness and yields for improving the cultivated finger millet through possible new genetic recombination's enhanced genome

2.5 Tools used to study genetic diversity

2.5 1 Use of Agronomic traits

In finger millet and most related wild species, morphological descriptors that include attributes, characteristics, or measurable traits observed in an accession have been greatly used in diversity studies (Bioversity International, 2007). The descriptors in finger millet germplasm characterization include qualitative traits; plant pigments, growth habit, inflorescent compactness, grain colour, lodging, plant aspect. Quantitative traits; days to 50% flowering, plant height, basal tillers per plant, culm branches per plant, flag leaf length, flag leaf width, flag leaf sheath length, peduncle length, inflorescent length, inflorescent width, longest finger length, longest finger width and panicle per plant (IBGPR,1985; Gopal and Upadhyaya *et al.,* 2007). Though morphological descriptors are highly influenced by environment, their accurate

estimations show the expression of adaptive genes that enhance evolutionary potential, local adaptation to changing environmental conditions if well maintained (Heather and Joanna, 2011). A considerable number of morphological based diversity studies have been conducted on different crops such as tef (Kefyalew *et al*., 2000), chickpea (Workeye, 2002), pearl millet (Rai *et al.*, 2009), proso millet (Gopal *et al.*, 2007) and wild ginger (Noladhi *et al*., 2011). Recent characterization studies conducted on finger millet show high morphological diversity among finger millet accessions of both African and Indian origins (Gopal and Upadhyaya *et al.*, 2009). Therefore, use of morphological descriptors is an effective approach in estimating phenotypic variability among a germplasm collection

2.5.2 Neutral genetic markers

In recent years, progress has been made in approaches to studying genetic diversity. The most current being use of genetic markers for assessing genetic diversity at single locus or sequence of DNA presumed neutral (neither influenced by selection nor affecting fitness), often abundant in a genome. This has been exploited in the development of molecular markers (Heather and Joanna, 2011). Recent advances in molecular marker techniques and technologies have led to the characterization of selectively neutral genomic regions (Heather and Joanna, 2011). The most commonly used molecular markers include random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), microsatellites and single nucleotide polymorphisms (SNPs). Although molecular markers are not influenced by environmental conditions, their wider use has been limited because of the high level of expertise required, cost of consumable and lack of technical capacity especially in developing countries.

2.5.3 Types of molecular markers used in diversity studies

Of recent, advancement in molecular technologies have resulted in development of molecular marker categories which are being extensively used in crop improvement programmes to detect polymorphisms in nuclear DNA. In genetic diversity studies, the most frequently used markers categories for finger millet are

2.5.3.1 First generation genetic markers

2.5.3.1.1 Random amplified polymorphic DNA (RAPD) markers

RAPD markers are DNA fragments amplified by PCR using short synthetic primers (generally 10 bp) of random sequence (Dida *et al.*, 2008; Kumari and Pande *at al.*, 2010). These oligo-nucleotides serve as both forward and reverse primers, and each pair of primers usually amplify fragments from 1-10 genomic sites. Amplified fragments (usually within the 0.5 - 5 kb size range) are separated on agarose or polyacrylamide-gels stained with ethidium bromide. Polymorphisms are detected by the presence or absence of bands of particular size considered primarily due to variation in the primer annealing sites. They can also be generated by genotype differences in the length of the sequence amplified between the primer annealing sites. The main advantage of RAPD markers is that they are quick and easy to assay. Since PCR is involved, only low quantities of template DNA are required (usually 5-50 ng per reaction), random primers are commercially available and also no sequence data for primer construction are needed. Moreover, RAPD markers have a very high genomic abundance and are randomly distributed throughout the genome. The main drawback of RAPD markers is their low reproducibility (Dida *et al.*, 2007; Kumari and Pande *at al.*, 2010). RAPD analyses generally require purified DNA of high molecular weight, and precautions are needed to avoid contamination of DNA samples because the short random primers that are used can amplify DNA fragments in a variety of

organisms. Altogether, the inherent problems of reproducibility make RAPDs unsuitable markers for estimating diversity for comparing of results among research teams working in a similar species and subject. As with most other multi - locus techniques, RAPD markers are not locus-specific, band profiles cannot be interpreted in terms of loci and alleles (dominance of markers), and similar sized fragments may not be homologous.

2.5.3.1.2 Restriction fragment length polymorphisms (RFLP) markers

RFLPs are based on the principal of endonuclease digestion southern blotting and hybridization (Dida *et al.,* 2007). Restriction enzymes cut genomic DNA at specific nucleotide sequences, the restriction sites. The digested DNA fragments are separated on agarose gels and transferred to nylon or nitrocellulose membranes. Restriction fragment length polymorphisms results from base pair changes in endonuclease target sites or from DNA rearrangement between sites recombinations and mutations. The major advantage of RFLP marker is being co-dominant. RFLPs have been used to construct genetic maps of several plant species like peas (Ellis *et al* 1992); sunflower (Jan *et al.,* 1998); diversity studies (Garcia *et al.,* 2004; Dida *et al.,* 2007). The major disadvantage of RFLPs as a genetic marker for diversity studies is that the cost of analysis is very high, laborious and difficult to automate, requires a relatively large amount of high quality DNA for good analysis.

2.5.3.1.3 Amplified fragment length polymorphism (AFLP) marker

AFLP combines the use of restriction endonuclease digestion and PCR- based finger printing (Vos *et al*., 1995). It involves selective amplification of template DNA fragments generated using two restrictive enzymes; a rare cutter and a frequent cutter. The rare cutter largely determines the number of fragments that will be amplified and the digestion with the frequent cutter generates amplifiable products of optimal size (less than 1Kb). The cut fragments are

ligated to adapters that provide target sites for primer annealing and amplification using selective primers. Amplification products are then separated on denatured polyacrylamide gels and visualization of products are separated through autoradiography. AFLPs are dominant markers (Vos *et al*., 1995). Variations at many loci can be arrayed simultaneously to detect single nucleotide variations of unknown genomic regions in which a given mutation may be frequently present in undetermined functional genes. However, a disadvantage is that they show a dominant mode of inheritance, this reduces their power in population genetic analyses of within-breed diversity and inbreeding. Nevertheless, AFLP profiles are highly informative in assessing diversity (De Marchi *et al*., 2006; Dida *et al.,* 2007).

2.5.3.2. Modern genetic markers

2.5.3.2.1 Simple sequence repeats (SSR) marker

Currently, microsatellites are the most popular markers in crop genetic characterization studies (Dida et al., 2007). Simple sequence repeats (SSR) are regions of DNA that consist of short tandem repeated units (2-6 bp in length) found within the coding or non coding regions of all eukaryotic organisms (Quellar *et al*., 1993). If nucleotide sequences in the flanking regions of the microsatellite are known, specific primers (generally 20–25 bp) can be designed to amplify the microsatellite by Polymerase Chain Reaction (PCR). Different alleles can be detected at a locus by PCR using conversed DNA sequences flanking the SSR as primers. SSR markers have been used initially to detect polymorphism between parent cultivars (Beshir *et al*., 2010). Although costly to develop relative to some other classes of genetic markers, once developed, analysis by SSR markers is both easy and inexpensive. The preference of simple sequence repeat (SSR) markers are because of many desirable attributes including hyper variability, multi-allelic nature, co-dominant inheritance, reproducibility, relative abundance, extensive genome coverage,

chromosome-specific location, amenability to automation and high-throughput genotyping (Kalia *et al.,* 2011, Vetriventhan *et al.,* 2012). SSR markers have been used to study genetic diversity in several crops (Ali *et al.,* 2007; Agrama *et al.,* 2007; Ste˛pien´ *et al.,* 2007; ;Vetriventhan *et al.,*2012).

2.5.3.2.2 Single nucleotide polymorphisms (SNPs) markers

Single nucleotide polymorphisms (SNPs) markers are used as an alternative to microsatellites in genetic diversity studies. Several technologies are available to detect and type SNP markers (Syvanen, 2001). Being biallelic markers, SNPs have rather low information content, and larger numbers have to be used to reach the level of information obtained from a standard panel of 30 microsatellite loci. However, ever-evolving molecular technologies are increasing automation and decreasing the cost of SNP typing. This is likely, in the near future, to permit the parallel analysis of a large number of markers at a lower cost. SNPs seem to be appealing markers to apply in the future for genetic diversity studies because they can easily be used in assessing either functional or neutral variation (Deulvot *et al.,* 2010; Galeano *et al.,* 2012). However, the preliminary phase of SNP discovery or SNP selection from databases is critical. SNPs can be generated through various experimental protocols, such as sequencing, single-stranded conformational polymorphism (SSCP) or denaturing high-performance liquid chromatography (DHPLC), or *in silico*, aligning and comparing multiple sequences of the same region from public genome and expressed sequence (EST) databases. When data has not been obtained randomly, standard estimators of population genetic parameters cannot be applied. A frequent example is when SNPs initially identified in a small panel are then typed in a larger sample of chromosomes. By preferentially sampling SNPs at intermediate frequencies, such a protocol will bias the distribution of allelic frequencies compared to the expectation for a random sample.

SNPs do hold promise for future applications in population genetic analyses; however, statistical methods that can explicitly take into account each method of SNP discovery have to be developed (Nielsen and Signorovitch, 2003).

2.6. Sectional conclusion

Finger millet though a food security crop, high nutritious and indigenous to Uganda has attracted limited research attention and funding besides the existing diverse germplasm. Characterizing the germplasm for diversity would generate information for breeders for effective utilization to support breeding efforts. Of recent molecular tools have been used to fasten breeding especially simple sequence repeat (SSR) markers because of their high discriminatory power in diversity studies (Vetriventhan *et al.,* 2012). However little information is available on the use of SSR markers in assessing the genetic diversity in finger millet especially among the Ugandan land races. Albeit finger millet is reported cross compatible with the other allotetrapliods in the genus *Eleusine* little is reported about the use of the transferred genes for finger millet improvement in breeding programs. The focus of this study is to generate information on diversity of the finger millet accessions in Uganda both at morphological and molecular levels and investigate the potential of the transferred genes from the inter species crosses for germplasm enhancement and crop improvement for blast disease resistance, drought tolerance and earliness

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of study sites

The studies were conducted at the National Semi Arid Resources Research Institute (NaSARRI) Serere and Makerere University Agricultural Research Institute (MUARIK) Kabanyolo. Serere is located at an elevation of 1038 m above sea level, $1^{o}39'$N and $33^{o}27'$E. The area has sandy soils with low organic matter content. It receives a bimodal type of rainfall with an annual mean of 1427 mm and large variation between years. NaSARRI experiences an annual mean temperature is 24 ^{0}C, with a minimum of 17.9 ^{0}C and maximum 34.4 ^{0}C. The relative humidity ranges from 72% to 84%. Kabanyolo is located in central Uganda about 19 km north of Kampala (0° 28' N. 32° 37' E; altitude 1200 m). The upland soils of Kabanyolo are classified as ferralitic, naturally rich in potash and low in nitrogen and phosphorous, with a pH between 5.2 - 6.0, and 2-3 % organic matter content in the surface horizon. Kabanyolo has a moist tropical climate with an annual mean temperature of 21.95°C, minimum 15.9°C and maximum 28.5°C. The area receives bimodal rainfall with a mean of 1150 mm, peaking in April and November (MUARIK, 2011)

3.2. Description of the germplasm used and experimental layout

The germplasm used in the study was a core collection of 400 genotypes selected based on origin and to represent 80 % of total diversity in the germplasm collection at NaSARRI, grouped as 322 Ugandan accessions, 72 ICRISAT collections and 6 Ugandan released varieties. There was little information about the traits of economic importance during the selection of these materials for the study. Two field evaluations were conducted during the first and second rains of 2011 at NaSARRI. The first trial ran from April to August, 2011A and involved 100 genotypes that were planted in an α- lattice design of 10 blocks x 10 entries, with 2 rows per accession in two

replications. In order to increase the number of local material for evaluation, additional 300 accessions were included in the second season that ran from September to December 2011B. The experiment was set up using the same α- lattice design of 16 blocks x 25 entries, with a spacing of 30 cm x 15 cm. In order to ensure good germination, seed was spread in 2 row plots by hand during field planting. Seedlings were thinned to the recommended spacing three weeks after germination and weeded twice at 4 and 8 weeks after planting. The accessions were grown under natural environmental conditions without any fertilizer to mimic farmer conditions.

3.3.1 Study 1: Assessing phenotypic diversity among finger millet accessions in Uganda

3.3.1 Introduction

The existence of genetic diversity among crop species provides useful information for selecting the most diverse accessions that could be used as parents for making crosses to study segregating generations and develop mapping populations. Accurate estimation of phenotypic diversity that exists in plant genetic resources is essential for proper utilization in crop improvement. This section describes the approaches that were used to estimate and enhance diversity among finger millet accessions in Uganda.

3.3.1.1 Phenotypic characterization of selected finger millet accessions

The accessions were characterized using morphological traits based on the International Board for Plant Genetic Resources descriptors for finger millet (IBPGR, 1985). The experimental unit consisted of five plants that were selected randomly from the four rows of test plants. Data collection included the number of primary tillers, secondary tillers, plant height, finger length and width, number of fingers, length of the longest finger, grain yield, grain colour, shape of the head, colour of the nodes, days to 50% flowering, days to physiological maturity, days to harvest maturity and blast disease severity scores (based on observation of 50% per row of the respective

genotypes). Disease assessments were performed using a 1-5 scale for neck blast, while a scale of 1-9 was used for leaf blast, stay green and leaf rolling (Kiran and Thakur., *et al* 2012). Other special procedures included the use of genotype E11 as a spreader row for blast disease planted after every 10 experimental plots.

3.3.1.2 Management of data and analysis

Data was collected in the field on plot basis for two seasons and subjected to analysis of variance (ANOVA), correlation and principal component (PCs) analysis using GenStat 14th edition. The ANOVA for the α–lattice design was done using the Linear Mixed Model option in the Restricted Maximum Likelihood (ReML) procedure GenStat. In cases where the lattice blocks were not effective as shown by negative variance components, analysis was done using the randomized complete block design. The analysis procedures used the linear mathematical models shown below, with the general notations of b= lattice blocks, r = number of replications and g = number of genotypes. Blocks and replications were treated as random effects while genotypes were treated as fixed effects.

The linear mathematical model for the analysis of variance for the balanced lattice was:

$$Y_{ij\kappa} = \mu + b_i + r_j + g_\kappa + (b/r)_{ij} + (g/b)_{i\kappa} + e_{ij\kappa}$$

Where $Y_{ij\kappa}$ = observed effects for i^{th} blocks, j^{th} replication and k^{th} genotypes

μ = grand means for the experiment

b_i = effect of the i^{th} blocks

r_j = effect of j^{th} replications

$g\kappa$ = effect of the k^{th} genotype

$(b/r)ij$ = effect of the i^{th} blocks within the j^{th} replication

$(g/b)i\kappa$ =effect of the k^{th} genotype within i^{th} blocks

$eij\kappa$ = lattice effective error or random error of the experiment

Whereas the linear mathematical model for the analysis of variance for randomized complete block design was:

$Yij\kappa = \mu + bi + rj + g\kappa + eij\kappa$

Where $Yij\kappa$ = observed effects for i^{th} blocks, j^{th} replication and k^{th} genotypes

μ = grand means for the experiment

bi = effect of the i^{th} blocks

rj = effect of j^{th} replications

$g\kappa$ = effect of the k^{th} genotype

$eij\kappa$ = random error of the experiment

3.3.2 Study 2: Investigation of pre - and post- flowering drought tolerance in finger millet

3.3.2.1 Description of materials used

This study consisted of 15 genotypes selected from the 400 accessions based on the results of field evaluations in the second season rains of 2011 conduced at NaSARRI. The second season was characterised by terminal drought stress during which drought tolerance parameters such as, stay green trait and leaf rolling were measured. Assessments were conducted under controlled conditions using the materials described below at Makerere University Agricultural Research Institute Kabanyolo (MUARIK) (Table 1).

Table 1 Names and descriptions of accessions used in the drought experiment

Accession number	Accession Name	Description
101	IE 4497	drought tolerant
102	KNE 388	drought tolerant
103	Nanjala	drought tolerant
104	NU-1	drought tolerant
105	Odiang/2	drought tolerant
106	P 763	drought tolerant
107	SEC 217	drought tolerant
108	SEC 220	drought tolerant
109	SFMC# 402	drought tolerant
110	U 16	drought tolerant
111	Pese 1	Checks
112	SEC 915	Checks
113	Okiring	Susceptible
114	Q 95	Susceptible
115	SEC 132	Susceptible

3.3.2.2 Experimental site and design

The study consisted of field experiments conducted at NaSARRI (Serere) and screen house experiments at MUARIK. The field evaluations were conducted during the second season rains

of 2011 at NaSARRI with a total of 400 finger millet accessions. Planting was done by hand with the excess seeds spread into 4 rows to ensure germination using a lattice design of 16 blocks x 25 entries. Seedlings were thinned to the recommended spacing of 30 cm x 15 cm three weeks after germination, weeded twice (at 4 and 8 weeks after planting). The screen house experiments ran from March to July of 2012 at MUARIK with a total of 15 genotypes selected from the results of the field evaluations; 10 drought tolerant, 2 varietal checks and 3 susceptible lines. The experiment was set up using a split plot design in 45 cm deep x 30 cm diameter buckets with two replications. The genotypes were thinned 3 weeks after planting leaving 6 plants per bucket. Main plots were watering regime, with genotypes as subplots.

3.3.2.3 Management of water

The experiment consisted of 3 watering treatments, used as main plots. These were pre-flowering stress, post-flowering stress, and control. Each experimental unit consisted of a bucket containing six plants of the same genotype, considered a subplot. The experimental unit in each treatment was watered with half a liter of water weekly for 3 weeks. The plants were then stressed by withholding water for 17 days to ensure homogeneity in the experimental units before administering 0.5litre of water every 9 days until 100 days. This amount of water was calculated with the intent of producing moderately severe stress, based on rainfall information obtained from NaSARRI and from crop water requirements corresponding to the stage of growth. Pre-flowering drought was initiated at 45 days after planting while the post-flowering stress treatment was applied in the same manner as the pre-flowering stress, starting at 75 days after planting. Controls received 0.5 litre of water weekly throughout the experiment.

3.3.2.4 Data management

Chlorophyll content readings were taken on days 1, 21 and 42 after stress application. The plant height, number of fingers per head, finger length, finger width and days to 50% flowering were subjected to analysis of variance using GenStat 14th edition statistical software. The linear mathematical model for the analysis of variance for the split plot design was:

$Y_{ijk} = \mu + b_i + r_j + br_{ij} + g_\kappa + bg_{i\kappa} + e_{ij\kappa}$

Where $Y_{ij\kappa}$ = observed effects for i^{th} watering regimes, j^{th} replication and k^{th} genotypes

μ = grand mean for the experiment

b_i = effect of the i^{th} watering regimes

r_j = effect of j^{th} replications

br_{ij}=main-plot of i^{th} watering regimes and j^{th} replications

g_κ = effect of the k^{th} genotype

bgi_κ =interaction between i^{th} watering regimes and k^{th} genotype

$e_{ij\kappa}$ = sub-plot error

3.4 Study 3: Assessing genetic diversity of finger millet accessions with Simple Sequence Repeats (SSR) Markers

3.4.1 Genetic materials

Molecular characterization was done in the ICRISAT biotechnology laboratory in Nairobi, Kenya. The 105 finger millet accessions were used in this study and selections were made based on origin and preliminary data . They included 51 land races, 49 exotic lines, and 5 released varieties.

3.4.2 DNA extraction and quantification

DNA was isolated from the accessions using a high-throughput 96-well mini-DNA extraction protocol (*Mace et al.,* 2003). Leaf tissues from 3 weeks-old seedlings were harvested and dried using silica gel from which two dry leaf tissue samples (~30- 50 mg) were sampled from each genotype and placed in 12 x 8 well strip tubes with strip caps (Marsh Biomarket, USA) in a 96 deep-well plate. Two 4-mm stainless steel grinding balls (Spex CertiPrep, USA) were added. To each sample was then added 450µL of preheated (65 ^{0}C) extraction buffer (100 mM Tris-HCL [pH 8], 1.4 M NaCl, 20 mM EDTA, CTAB [3 %w/v], β-mercaptoethanol [0.03-3% v/v] secured with 8 strip caps (Marsh Biomarket, USA) and the samples were ground using a GenoGrinder 2000 (Spex CertiPrep, USA), at 1500 strokes/min for 2-3 times at 2 minutes interval and then steadily for 20-30 minutes following manufacturer's instructions. This was then incubated for 30 minutes in a 65 ^{0}C water bath with occasional mixing.

450 µL of chloroform- isoamylalcohol (24:1) was added to each sample and inverted twice to mix before centrifuging at 3500 g for 10 minutes at 20^{0}C (Beckman Coulter, Allegra^{TM} 25R centrifuge) and a fixed volume (400 µL) of aqueous layer was transferred to fresh strip tubes (Marsh Biomarket, USA). 0.7 volume (~280µL) of isopropanol (stored at -20 ^{0}C) was added to

each sample and inverted twice to mix and centrifuged at 3500*g* for 15 minutes at 4^0C (Beckman Coulter, Allegra^{TM} 25R centrifuge). Supernatant was decanted from each sample and the pellet air dried for 30 minutes and added 200µL low salt TE (1 mM Tris, 0.1mM EDTA [pH 8], and 3 µL RNAse (10mg/mL), and incubated at 37^0C for 30 minutes. 200 µL of chloroform-isoamylalcohol (24:1) was added to each sample, inverted twice to mix before centrifuging at 3500*g* for 10 minutes at 24^0C (Beckman Coulter, Allegra^{TM} 25R centrifuge). A fixed layer of the aqueous layer was transferred to 96 well plates (Marsh Biomarket, USA).

315 µL of ethanol-acetate solution (30mL EtOH, 1.5mL 3 M NaOAc [pH 5.2] was added to each sample and placed in -20^0C for 1 hour. This was then centrifuged at 3500*g* for 10 minutes at 4^0C and supernatant decanted from each sample and pellets washed with 70% EtOH. This was followed by another centrifuge at 3500 g for 5 minutes at 10^0C and supernatant was decanted from each sample and pellets air dried for 1 hour and re-suspended in 100 µL TE (10mM Tris-HCL [pH 7.5], and 1 mM EDTA [pH 8]).

3.5.3 Determination of DNA quantity and quality

6 µL of genomic DNA extracted above was loaded on 0.8% agarose gel and stained with 5 µL gel red and run in 100mL of TBE-buffer in a horizontal gel system in agarose gel electrophoresis at 100 volts for 1 hour with λ DNA of known concentration (25ng, 50ng, 75ng) used as control (Bio-Rad, model 96, Bio-Rad laboratories, Inc. life CA, USA). This was then visualized under 100% UV light in a gel documentation system (Bio-Rad, model 1000, Bio-Rad laboratories, Inc. life CA, USA) and photographed. The DNA samples that produced sharp, single visible bands were considered to represent samples of good quantity and quality. The concentration of DNA was then measured by a Nanodrop ND-1000 spectrophotometer

(NanoDrop technologies, Delaware,USA), diluted to 10 ng/ µL and stored at -20^{0}C for use in Polymerase Chain Reactions (PCR).

3.5.4 Molecular markers used in the study

Simple sequence repeats (SSR) markers were used in this study. Twenty SSR markers were selected from thirty SSR markers, basing on their polymorphism when screened against finger millet genotypes at ICRISAT –Nairobi during SSR optimization for finger millet.

Table 2 SSR markers used in the analysis of genetic diversity

No	Marker	Mapped	Forward primer	Reverse primer
1	UGEP024	3B	GCCTTTTGATTGTTCAACTCG	CGTGATCCCTCTCCTCTCTG
2	UGEP053	2A	TGCCACAACTGTCAACAAAAG	CCTCGATGGCCATTATCAAG
3	UGEP084	No	GGAACTTCCGTCAGTCCTT	TGGGGAAGGTGTTGAAT
4	UGEP024	No	TTGCTCTGAGGTTGTGTTGC	TCAAGCATAGTGCCCTCCTC
5	UGEP098	No	GTCTTCCATTTGCAGCAACC	ACGAGTACTGACGTGATTG
6	UGEP095	No	AGGGGACGCTTGGTTATTTG	GCCTCTACCTGTCTCCGTTG
7	UGEP064	No	GTCACGTCGATTGGAGTGTG	TCTCACGTGCATTTAGTCATT
8	UGEP033	No	TAGCCCGTTTGCTTGTTTTG	AAGGCCCTAGAACGTCAAGC
9	UGEP067	No	CTCCTGATGCAAGCAAGGAC	AGGTGCCGTAGTTTGTGCTC
10	UGEP106	9B	AATTCCATTCTCTCGCATCG	TGCTGTGCTCCTCTGTTGAC
11	UGEP110	7AB	AAATTCGCATCCTTGCTGAC	TGACAAGAGCACACCGACT
12	UGEP057	No	CCATGGGTTCATCAAACACC	ACATGAGCTCGCGTATTGC
13	UGEP096	No	TAATGGGCCTAATGGCAATG	CAAAATCCGAGCCAAGATTC
14	UGEP066	No	CAGATCTGGGTAGGGCTGTC	GATGGTGGTTCATGCCAAC
15	UGEP046	No	CAAGTCAAAACATTCAGATGG	CCACTCCATTGTAGCGAAAC
16	UGEP079	No	CCACTTTGCCGCTTGATTAG	TGACATGAGAAGTGCCTTGC
17	UGEP020	No	GGGGAAGGCAATGATATGTG	TTGGGGAGTGCCAACAATAC
18	UGEP012	8B	ATCCCCACCTACGAGATGC	TCAAAGTGATGCGTCAGGTC
19	UGEP073	No	GGTCAAAGAGCTGGCTATCG	ACCAGAACCGAATCATGAGG
20	UGEP005	No	TGTACACAACACCACACTGAT	TTGTTTGGACGTTGGATGTG

3.5.5 Simple Sequence Repeats (SSR) amplification and Capillary electrophoresis

Twenty SSR markers were amplified in a thermocycler (GeneAmp PCR systems 9700, Applied Biosystem, Cary California USA), Polymerase Chain Reaction (PCR) was performed on each Simple Sequence Repeat (SSR) primer pair on the genomic DNA of the accessions. Each PCR

reaction was carried out in 10µl reaction volumes containing 1x PCR buffer, 2mM $MgCl_2$, 0.16mM dNTPs, 0.16 pmol of fluorescent labeled universal M-13 forward primer, 0.04pmol forward primer, 0.2pmol reverse primer, 0.2U of Amplitaq (Applied Biosystems, USA), and 30 ng finger millet template DNA. Amplification consisted of an initial denaturation at 94^0 C for 5 minutes followed by 35 cycle's touchdown annealing starting at 59^0 C for 30s, and extension at 72^0 C for 2 minutes. The program was finished with a final extension at 72^0 C for 20 minutes and cooled to 4^0 C. Amplification products were separated in 2% agarose gel and visualized by Gel red.

A set of 5 PCR multiplex (co-loading sets) were constructed based on the allelic size range estimates and type of the forward primer label of the markers. Each set consisted of four SSR markers with different labels and allele size. For post-PCR multiplexing, 1 ml PCR product of each of 6-Fam, Vic, Ned and Pet-labelled products were pooled (according to the above-mentioned criteria) and mixed with 7ml of Hi-Di formamide (Applied Biosystems, USA), 0.2ml of the LIZ-500 size standard (Applied Biosystems, USA) and 2.8ml of distilled water. The pooled PCR amplicons were denatured for 5 min at 958C and cooled immediately on ice and size-separated by capillary electrophoresis on an ABI 3730xl DNA analyzer (Applied Biosystems, Inc.). Raw data produced from the ABI 3730 x l DNA analyzer were analyzed using Genemapper software version 4.0 (Applied Biosystems, USA) and fragment size was scored in base pairs based on the relative migration of the internal size standard, LIZ 500.

3.5.6 Data analysis

The fragment sizes generated from Gene Mapper for all 20 markers were used in the basic statistics analysis using PowerMarker version 3.25 (Liu and Muse, 2005), these included the polymorphic information content (PIC), allelic richness as determined by the total number of the

detected alleles and the number of alleles per locus, gene diversity and the occurrence of unique, rare, common and most frequent alleles and heterozygosity (%). Unique alleles were considered as those present in one accession or in one group of accessions but absent in other accessions or group of accessions. Rare alleles were considered as those whose frequency is ≤1% in the investigated materials. Common alleles were those occurring between 1 and 20% in the investigated materials while those occurring < 20% were classified as most frequent alleles (Upadhyaya *et al.*, 2008b). The correlations among the number of repeat unit, the number of alleles per locus, gene diversity, heterozygosity and PIC were estimated. A neighbour-joining tree was constructed based on the simple matching dissimilarity matrix of 19 polymorphic markers genotyped across the finger millet accessions as implemented in DARwin 5.0.156 program (Perrier and Jacquemoud-Collet, 2006) with a bootstrapping value of 1000.The most diverse pairs of accessions were identified based on the dissimilarity matrix. The principal coordinate analysis (PCoA) was performed based on Nei's (1973) distance matrix.

3.6 Study 4: Determining the cross- compatibility of finger millet E. corocana with E africana and E kigezienesis

3.6.1 Description of materials

Twenty *Eleusine* spp collections were made from the highlands of Kigezi and Bwindi National park in western Uganda where there is a lot of species diversity for the genus *Eleusine*, including 18 wild collections. The *Eleusine corocana* species used in this experiment included Adoke, Gulu E, DR 23, Seremi 1, Seremi 3 and Pese 1, Serere 14 obtained from NaSARRI and were used as female parents

Table 3 Morphological characterization of the parents

Accession name	Altitude (masl)	DF	PHT	PG	P T	PL	LFL	LFW	NF	ES
E.kigeziensis	1844m	48	128	G	12	15	13	0.5	12	LO
E.africana	1263m	53	110	P	15	12	10	0.2	8	LO
Pese 1	1038m	65	100	G	5	9	6	2.8	7	TC
Seremi 1	1038	69	98	P	4	10	7	3.1	7	TC
Seremi 3	1038	70	96	P	5	8	7	2.7	6	SO

DF- days to 50% flowering(days), PHT- Plant height(cm),PG - Plant pigment PT- Productive tillers, PL- Panicle length(cm), LFL- longest finger length(cm), LFW – longest finger width(cm) NF- Finger numbers ES – Ear shape

3.4.2 Emasculation and cross pollination

Emasculation and crossing of finger millet is tedious and difficult because of the highly self-pollinating nature of the genus coupled with extremely small florets. In this study, a simpler method of obtaining crosses was used. All mature florets were cut off from a panicle branch. The branch was then covered using a white transparent polythene bag in the evening. Hot-water

emasculation of florets was done by dipping the flower heads in hot water at 52^0 C for 2 minutes to kill the pollen. Pollination was done by bagging both the emasculated and the pollinator heads together in one polythene bag very early the following morning. The bagged flowers were left alone for two to three weeks to monitor seed formation (Dida, 2007*;* Makhdoom and Mubashir *et al*., 2012). Plants grown from seed produced by this process were inspected, and only those showing traits from both parents were considered true F_1's.

CHAPTER FOUR
RESULTS

4.1 Evaluations of finger millet accessions for phenotypic diversity

The field evaluations for agronomic performance were conducted during the 2011A and 2011B rain seasons to characterise accessions to identify new sources of novel genes among the germplasm for resistance to blast, drought, striga, and high yields. The evaluations indicated significant genotype differences among the selected accessions for the measured traits during the study and details for each trait are indicated below.

4.1.1.1 Plant pigmentation

Two distinct nodal colours (green and purple) were observed among the finger millet accessions and were used to differentiate the genotypes during the 2011A. Phenotypic characterization based on node colour among 100 accessions revealed that 76% of the accessions were classified as green pigmented, while 24% purple pigmented as in (Table 4).

4.1.1.2 Inflorescence compactness and shape

Inflorescence compactness is an important character in determining yield and useful in sub race classification. Four types of inflorescence shapes were recorded during the 2011A rain season that included incurved, short open, long open and tip curved. Majority of the accessions were tip curved (93%) followed by incurved (4%), long open (2%) and short open (1%).Most of the top curved were from Uganda as in Table 4.

Table 4 Inflorescence compactness and shape, and pigmentation of finger millet among accessions studied at NaSARRI during 2011A rain season

		Inflorescent compactness(shapes)				Pigmentation	
Origin	Accessions	INC	SO	LO	TC	Green	Purple
Uganda	76	2	0	2	72	58	18
Introductions	24	2	1	0	21	18	6
Totals	100	4	1	2	93	76	24

***Inflorescent compactness (head shapes) recorded. INC- Incurved heads; SO- Short open heads; LO- long open head; TC- Top curved heads*

4.1.1.3 Performance of finger millet accessions for quantitative traits

The two rainy seasons of 2011 were highly variable. The first rainy season (2011A) received lower but uniformly distributed rainfall compared to the second season that was highly variable, with no rains in December, a critical grain-filling stage (Figure1). Analysis of variance for Ugandan and exotic accessions revealed significant difference ($P \leq 0.05$) for plant height, days to 50% flowering, secondary tillers, panicle length, peduncle length, longest finger length while number of fingers, sheath length were significant among the exotic accessions (Table 5).

In addition, a combined analysis of variance for 2011B season evaluations under drought stress revealed significant variability for agronomic traits such as, days to 50% flowering, days to harvest maturity, dry weight, finger length, plant height, primary tillers, stay green, leaf rolling, and longest finger length except secondary tillers (Table 6). A similarly significant variability ($P \leq 0.05$) was revealed for exotic and Ugandan accessions separately analyzed though leaf rolling and stay green significantly varied ($P \leq 0.05$) among the exotics and not among the Ugandan accessions; with exception of primary tillers that were significantly different among the Ugandan accessions as in Table 7.

Table 5: Mean squares for distribution of 12 traits recorded on 96 finger millet accessions evaluated at NaSARRI during 2011A season.

		Mean squares											
Source of variation	Df	PHT	DF	PT	ST	PL	PW	Wt	Ped L	LFL	Sh L	LFW	NF
Ugandan accessions													
Replication	1	3.93	3.59	18.20	14.42	4.14	3.69	17391	18.68	2.90	1.40	0.12	1.33
Genotype	73	238.26***	46.24***	1.23Ns	6.95***	2.73***	0.51ns	1827***	9.50***	1.91*	1.48ns	0.08ns	1.21ns
Residual error	66	41.29	4.02	1.05	6.71	1.89	0.46	1433	12.19	1.70	1.92	0.06	1.46
Means		88.1	73.7	2.4	2.7	8.5	2.8	76.8	23.2	8.2	9.1	1.0	7.1
SEM		1.4	0.6	0.1	0.2	0.1	0.0	4.9	0.3	0.1	0.1	0.0	0.1
CV		12.6	6.8	28.7	69.0	12.6	10.8	51.1	9.6	11.9	10.4	19.2	10.3
Exotic accessions													
Replication	1	332.09	1.69	3.834	45.93	6.18	1.25	10003	41.91	2.05	1.38	0.04	0.08
Genotype	23	309.79***	67.25***	0.93ns	5.03***	4.54***	0.19ns	4553***	12.17***	3.76***	4.53***	0.04ns	0.69***
Residual error	22	40.19	4.36	1.54	4.89	3.52	0.11	3987.00	17.71	3.36	5.20	0.03	0.70
Means		86.3	74.1	2.5	2.5	8.6	2.9	95.7	22.8	8.1	9.6	1.0	7.0
SEM		2.06	0.82	0.15	0.29	0.24	0.12	12.41	0.39	0.22	0.21	0.03	0.12
CV		13.5	6.3	33.2	64.2	15.8	23.7	73.4	9.8	15.2	12.6	14.2	9.6

*Significant difference * P<0.05, ** P<0.01, *** P<0.001 , df- degree of freedom, SEM- standard error of means, CV- coefficient of variation, PHT- Plant height(cm), DF- days to 50% flowering (days), PT- primary tillers, ST, Secondary tillers, PL- Panicle length(cm),PW-Panicle width(cm),,Wt- Weight per stand(gm/Std), Ped L-Peduncle length (cm) LFL- longest finger length (cm), Sh L- sheath length (cm) LFW- Longest finger width (cm), NF- Finger numbers.*

Table 6: Mean squares for distribution of 10 quantitative traits recorded on 394 finger millet accessions evaluated at NaSARRI during 2011B season

		Mean squares									
Source of variation	d.f	DF	DHM	Dwt	FL	PHT	PT	SG	LR	ST	LFL
Replication	1	886.55***	424.84***	5523***	24.29***	152.96***	33.82***	4.50*	20.79***	0.75ns	16.79***
Rep/block	29	32.90***	61.89***	7227***	5.92***	50.21***	1.21ns	2.07**	2.84***	0.96ns	5.79***
Genotype	393	20.9***	112.27***	2229***	2.50***	143.7***	1.20*	1.3*	1.5***	0.72ns	1.95***
L E E	249	10.3	24.8	1612.0	1.3	25.6	0.7	1.0	0.7	0.5	1.1
R E	121	9.1	19.0	1474.0	1.4	17.8	0.7	0.7	0.7	0.5	1.1
Mean		68.96	107.3	66.52	7.86	47.76	1.207	1.96	3.54	0.91	7.32
SEM		0.16	0.36	1.68	0.054	0.43	0.041	0.03	0.04	0.032	0.05
CV		4.5	6.7	49.9	13.6	17.8	66.1	36.5	22.5	63.4	13.1

*Significant difference * P<0.05, ** P<0.01, *** P<0.001 df- degree of freedom, LEE- lattice effective error, RE- residual error, SEM- standard error of means, CV- coefficient of variation. DF- days to 50% flowering (days), DHM-days to harvest maturity(days) Dwt- dry weight (gm), FL-Finger length (cm), PHT- Plant height (cm), PT- primary tillers, ST, Secondary tillers, SG- stay green, LR- leaf rolling, LFL- longest finger length(cm).*

Table 7: Mean squares for distribution of 10 quantitative traits recorded on 394 finger millet accessions evaluated at NaSARRI during 2011B season

		Mean squares									
Source of variation	df	DF	DHM	Dwt	FL	PHT	PT	SG	LR	ST	LFL
Exotic accessions											
Replication	1	219.13***	69.56***	4564***	12***	58.16***	8.23**	7.41**	20.73***	2.48ns	18.33***
Rep/block	28	24.10***	52.91***	3134***	1.23ns	26.60***	0.66ns	1.44ns	2.03**	0.76ns	2.67***
Genotype	180	20.38***	115.15***	1609***	3.23***	144.54***	0.84ns	1.13*	1.45*	1.3ns	2.09***
LEE	93	10.6	28.0	1433.1	0.9	29.3	0.7	0.9	0.5	0.5	1.4
RE	76	10.9	22.4	1796.5	0.2	17.6	0.5	0.7	0.9	0.6	1.4
Mean		69.05	107.50	61.65	7.84	47.24	1.10	1.98	3.59	0.92	7.30
SEM		0.22	0.54	2.17	0.09	0.62	0.05	0.05	0.06	0.05	0.08
CV		4.327	6.707	46.92	14.71	17.63	57.28	36.47	20.88	63.75	14.51
Ugandan accessions											
Replication	1	4557.40***	366.61***	2959***	39.25***	94.80***	27.95***	1.17ns	6.02*	0.78ns	25.02***
Rep/block	27	18.51***	33.79***	4616***	4.33***	27.22***	1.09ns	1.43ns	1.62ns	0.26ns	3.55***
Genotype	212	21.76***	110.74***	2765***	2.39***	111.15***	1.63**	1.04ns	1.56**	0.67ns	1.83***
LEE	138	9.52	21.93	1932	1.31	26.26	0.74	1.01	0.81	0.56	0.98
RE	35	6.29	13.47	1171	1.28	23.13	1.06	0.71	0.48	0.04	0.71
Mean		68.89	107.20	70.60	7.89	48.20	1.30	1.95	3.50	0.91	7.34
SEM		0.22	0.50	2.47	0.07	0.59	0.06	0.05	0.06	0.04	0.06
CV		4.74	6.74	51.02	12.64	17.92	69.88	36.58	23.82	63.32	11.86

*Significant difference * P<0.05, ** P<0.01, *** P<0.001 df- degree of freedom, LEE- lattice effective error, RE- residual error, SEM- standard error of means, CV- coefficient of variation DF- days to 50% flowering(days),DHM-days to harvest maturity(days) Dwt- dry weight (gm) FL- Finger length(cm), PHT- Plant height(cm), PT- primary tillers, ST, Secondary tillers, SG- stay green,LR- leaf rolling, LFL- longest finger length(cm).*

4.1.1.4 Principal component analysis

Principal component (PC) analysis was performed for morphological traits of 400 finger millet genotypes. Eigen values between 3.47 and 1.0 were extracted from the accessions accounting for 71% of the total genotype trait variation captured among the genotypes for the first six principal components extracted from the mean of 11 normalized quantitative traits (Table 8). The PC1 accounted for 23% of the variability, whilst PC2, PC3, PC4, PC5 and PC6 accounted for 13%, 11%, 10% 8% and 7% of variability among the genotypes for traits under study.

The first PC was more related to head traits such as; number of fingers, finger length, finger width, longest finger length and primary tillers. In the second principal component, days to harvest maturity, days to 50% flowering, dry weight were more related traits.The third principal component was positive for leaf rolling but negative for staygreen. Genotypes were poor for ear shape, secondary tillers and plant height (Table 8).

Table 8: Principal component analysis for 14 quantitative traits for 400 finger millet accessions

Principal components	1	2	3	4	5	6
Eigen value	3.47	1.89	1.61	1.47	1.21	1
Percentage variance (%)	23	13	11	10	8	7
Cumulative variance (%)	23	36	46	56	64	71
DF	-0.047	0.585	-0.342	0.113	0.068	0.033
DHM	-0.01	0.623	-0.246	0.131	0.115	0.008
DW	0.282	-0.243	-0.107	0.076	0.108	0.04
ES	0.107	-0.132	0.008	-0.014	0.587	0.107
FL	0.388	0.174	0.116	-0.435	0.033	0.027
FN	0.405	-0.116	-0.35	0.259	-0.146	-0.05
FW	0.373	0.029	0.044	-0.142	-0.167	0.137
LFL	0.367	0.177	0.114	-0.462	0.062	0.06
NC	0.012	0.036	0.018	-0.071	-0.57	-0.473
PHT	0.182	-0.151	-0.203	0.112	0.246	-0.011
PT	0.202	0.192	0.295	0.057	-0.225	0.348
ST	-0.04	-0.024	0.139	0.314	-0.275	0.691
STG	0.2	0.18	0.416	0.396	0.166	-0.194
LR	-0.194	-0.098	-0.466	-0.363	-0.104	0.313

PC- Principal component DF-Days to 50% flowering, DHM- Days to harvest maturity, DW- Dry weight(gm),ES- Ear shape, FL- Finger length(cm),FN- Finger numbers, FW- Finger width(cm), LFL-Longest finger length(cm),NC- Node colour, PHT- Plant height(cm),PT-Primary tillers ST- Secondary tillers, STG- stay green, LR- leaf rolling.

4.1.1.5 Correlations among phenotypic traits

In general, significant positive correlations among the phenotypic traits were observed. Days to 50% flowering was positive, and significantly correlated with days to harvest maturity (r^2 = 0.59P<0.05,). Days to harvest maturity was also positively and significantly correlated to primary tillers, stay green and negatively correlated to dry weight, Whereas dry weight was positively and significantly (P<0.05) correlated with number of fingers per ear, panicle width, longest finger length and plant height but negatively correlated with panicle length, primary tillers and leaf rolling. Panicle length positively and significantly correlated with longest finger length (r^2 = 0.71,P<0.05 number of fingers, panicle width, plant height and primary tillers while negatively correlated with secondary tillers and leaf rolling. Number of fingers positively and significantly (P<0.05) correlated with panicle width, plant height, primary tillers, stay green and negatively correlated with longest finger length and leaf rolling meanwhile stay green negatively and significantly correlated with leaf rolling (r^2 = 0.32,P<0.05,) (Table 9).

Table 9: Correlations among agronomic traits for finger millet accessions evaluated at NaSARRI during the 2011B rains

	DF	DHM	DWt	PL	NF	PW	LFL	PHT	PT	ST	STG	LR
DF	-											
DHM	0.766***											
DWt	-0.035	-0.063										
PL	-0.019	0.018	-0.017									
NF	0.027	0.016	0.262**	0.090								
PW	0.041	-0.075	0.142*	0.146*	0.239**							
LFL	0.012	0.009	0.041	0.845***	-0.057	0.066						
PHT	0.013	-0.042	0.120*	0.015*	0.197*	-0.009	-0.001					
PT	-0.145	0.171	-0.039	0.066	0.002	0.184	0.01	-0.016				
ST	0.062	-0.086	0.027	-0.089	0.005	0.015	-0.003	-0.056	0.146			
STG	0.024	0.048	-0.015	0.026	0.031	-0.006	0.012	0.071	0.093	0.09		
LR	0.126*	-0.062	-0.046*	-0.037	-0.019	-0.044	0.043	0.06	-0.075	0.034	-0.561***	-

*Significant difference at *P<0.5,**P<0.01,*** P<0.001 DF- Days to 50% flowering(days)g, DHM- Days to harvest maturity(days), DWt- Dry weight, LFL- Longest finger length (cm),NF- Number of fingers, PL- Panicle length (cm), PW-Panicle width(cm), PHT-Plant height(cm), PT-Primary tillers, ST-Secondary tillers, SHTL-Sheath length(cm), LFW -longest finger width(cm)*

4.1.2.1 Pre and post flowering drought tolerance in finger millet

Significant differences among genotypes for chlorophyll content, days to 50% flowering, finger length, finger width, Plant height, number of fingers were recorded. Significant variability for chlorophyll content, plant height, and finger length under different watering regimes ($P<0.05$) were found. There were non-significant interaction between the genotypes and watering regimes were found except for chlorophyll content at 50 days after drought stress was initiated (Table 10). The study showed lower means for the pre-flowering watering regime than for Post flowering and fully watered plants under the control. Significant differences were found for number of fingers per plant with a mean value of 3.5 compared with 3.4 and 3.3 in control and pre flowering watering regimes. Chlorophyll content, plant height, finger length were significantly affected by early water stress. The chlorophyll content measured in the control (10.3) was twice the post flowering water stress (5.4) though the overall mean was relatively high (6.8). Significant genotype differences in terms of chlorophyll content was also observed 28 days after water stress with the landrace Okiring initially susceptible under field conditions showing tolerance under screen house conditions (highest chlorophyll content after 45 days), followed by Pese 1, Seremi 3 and SEC 217 (Table 11).

Table 10: Mean squares for pre and post flowering drought tolerance for agronomic traits under screen house for 15 finger millet accessions

Source of variation	d.f.	CCT1	CCT 2	CCT3	DF(days)	FW(cm)	FL(cm)	NF	PHT(cm)
Replication	1	20.5	1.1	4.6	17	1.62	0.73	11.28	23.9
Watering regime	2	79.3ns	167.7ns	269.9**	6.2ns	0.35ns	12.8*	0.17ns	2201.5*
Residual	2	6.1	14.5	1.2	8.6	0.13	0.45	0.04	207.3
Genotype	14	3.2ns	5.6**	11.9***	217***	0.2*	3.4*	1.18***	301.5*
Watering regime. Genotype	28	2.5ns	2.6ns	4.8*	9.3ns	0.09ns	0.84ns	0.19ns	102.1ns
Residual	42	1.8	1.9	2.2	8.74	0.08	1.41	0.21	133.3
CV(%)		18.9	15.5	15	15	15.6	24.7	13.4	20
LSD ($P \leq 0.05$)		2.94	3.5	4.2	3.3	0.57	2.34	0.89	23.7

CCT- chlorophyll content, DF- days to 50% flowering(days), FW- Finger width (cm), FL- finger length(cm), NF- Number of fingers, PHT- plant height(cm)

Table 11: Means for agronomic traits for 15 finger millet accessions screened for pre and post flowering drought tolerance under screen house conditions

Genotypes	CCT 1	CCT2	CCT 3	DF	FL	FW	PHT	FN
SEC 915	7.1	4.9	4.0	55.0	3.5	1.6	47.0	2.4
NU-1	8.3	4.9	6.7	57.2	4.1	1.6	52.6	2.7
Pese 1	6.5	5.0	7.9	69.1	4.3	1.8	49.4	3.2
KNE 846	6.9	6.3	6.4	59.5	4.2	1.7	48.5	3.6
SEC 220	6.3	6.4	6.2	65.9	5.2	1.9	65.5	3.7
SEC 217	6.5	6.4	7.6	60.6	4.6	2.0	53.0	3.2
Seremi 3	6.0	6.5	7.8	70.7	5.2	2.0	62.6	3.6
Nanjala	6.1	6.5	7.3	70.9	5.0	1.8	56.1	3.8
P 763	7.7	6.6	6.9	59.4	4.9	2.1	59.3	3.4
U16	7.0	6.7	5.1	56.0	4.9	1.8	56.1	3.3
IE 4497	6.8	6.7	6.0	60.4	4.0	1.7	54.3	2.9
SEC 132	7.0	6.8	5.8	63.3	4.6	2.0	63.0	3.8
SFMC 402	7.6	7.5	7.6	68.4	5.0	2.0	63.2	4.0
Odiang/2	7.8	7.6	7.4	68.4	6.1	2.0	63.1	3.8
Okiring	8.3	8.3	10.0	73.5	6.3	1.9	71.6	3.6
CV(%)	18.9	15.5	15.0	15.0	24.7	15.6	20.0	13.4
LSD (P≤ 0.05)	1.6	1.6	1.73	3.45	1.4	0.3	13.5	0.5

CCT1,CCT2,CCT3 - chlorophyll content(1 day,21 days, 42 days after stress) , DF- days to 50% flowering(days), FW- Finger width(cm),FL- finger length(cm), NF- Number of fingers, PHT- plant height(cm)

4.1.2.2 Correlations among phenotypic traits

In general significant positive correlations among the phenotypic traits, chlorophyll content, were positively and significantly correlated with finger length (r^2 = 0.64 P<0.05), plant height, days to 50% flowering, finger width (cm) number of fingers, similarly positive and significant correlations were observed among other agronomic traits such as finger width, finger length, number of fingers and plant height. Days to 50% flowering had positive and significant correlations with finger length, number of fingers and plant height, positive but non significant correlations with finger width (Table 13).

Table 12: Correlations among agronomic traits measured during pre and post drought tolerance screening at MUARIK (March- July, 2011)

	CCT	DF	FW	FL	NF	PHT
CCT						
DF	0.639*					
FW	0.527*	0.478ns				
FL	0.799***	0.705**	0.669*			
NF	0.549*	0.688**	0.708**	0.708**		
PHT	0.723**	0.631*	0.642*	0.865***	0.684*	

CCT- chlorophyll content , DF- days to 50% flowering(days), FW- Finger width(cm),FL- finger length(cm),NF- Number of fingers, PHT- plant height(cm)

4.3 Genetic diversity based on simple sequence repeats (SSR)

A unique pattern of diversity was displayed among the 105 finger millet accessions with a total number of 23 loci identified 226 alleles generated by 19 highly polymorphic markers (Table 13). The number of alleles generated per locus ranged from 3 to 20 with an average of 9.6 alleles per locus. The polymorphic information content (PIC) varied from 0.09 to 0.88 with an average of 0.53. Similarly, high genetic diversity was observed among the land races with 23 loci identified and a total of 184 alleles generated by 19 polymorphic markers as in Table (14). The allele numbers generated per locus ranged from 1 to 15 with an average of 8.0 alleles per locus. The polymorphic information content (PIC) ranged from 0.07 to 0.87 with an average of 0.52. Of the 226 alleles, 62 were rare alleles, 130 common alleles and 34 the most frequent alleles. A total of 58 unique alleles were also detected (Table 15). However, highly polymorphic markers for the selected accessions (all) were UGEP067, UGEP053, UGEP066, UGEP024 and UGEP057 with PIC values above 0.80 and UGEP084, UGEP073, UGEP096-2 and UGEP096-1 had very low PIC values hence monomorphic. A high heterozygosity value for markers UGEP110, UGEP096, UGEP033, and UGEP020 was observed. However, UGEP005 had a unique characteristic pattern among the Ugandan germplasm, though with relatively high polymorphism, the availability of the locus among was generally low which could imply a non-allele situation for that locus for some accessions within Ugandan germplasm.

Table 13: Characteristics of 19 polymorphic SSR loci screened across 105 Finger millet genotypes

Marker	Range	No. of obs.	Allele Numbers	Major allele frequency	Heterozygosity	PIC
UGEP067	227-263	96	15	0.2083	0.1458	0.8762
UGEP053	225-257	102	13	0.2059	0.1176	0.8593
UGEP066	210-242	103	16	0.2136	0.1456	0.8436
UGEP024	171-219	102	20	0.2696	0.1667	0.8242
UGEP057	440-484	104	17	0.3462	0.1250	0.8136
UGEP064	215-253	98	16	0.3827	0.1633	0.7729
UGEP012	195-255	96	11	0.4479	0.1875	0.7353
UGEP027	259-293	101	15	0.4505	0.1188	0.7161
UGEP020_1	152-168	94	8	0.3316	0.0105	0.6693
UGEP079	173-191	104	7	0.4904	0.3654	0.5970
UGEP033_2	221-239	56	8	0.4800	0.0000	0.5713
UGEP 005	206-260	99	11	0.6071	0.2679	0.5711
UGEP098	194-296	101	10	0.5495	0.4158	0.4967
UGEP020_2	170-178	101	6	0.6961	0.0000	0.4611
UGEP110_2	195-217	104	4	0.6635	0.0000	0.4166
UGEP033_1	209-223	98	6	0.7273	0.0000	0.3829
UGEP095	214-238	100	7	0.8000	0.0500	0.3268
UGEP110_1	165-185	104	7	0.7981	0.0096	0.3263
UGEP106	172-206	105	12	0.8143	0.0571	0.3240
UGEP084	184-188	103	3	0.9029	0.0194	0.1679
UGEP073	228-248	103	5	0.9078	0.0097	0.1657
UGEP096_2	221-229	104	5	0.9231	0.0000	0.1429
UGEP096_1	203-209	103	4	0.9515	0.0097	0.0912
Mean		99	9.6	0.5725	0.1037	0.5283
Minimum			3	0.2059	0.0000	0.0912
Maximum			20	0.9515	0.4158	0.8762

Table 14: Genetic variability among finger millet genome based on 19 polymorphic SSR loci screened across 46 local land races from races from Uganda.

Marker	Range	No. of obs.	Allele Numbers	Major allele frequency	Heterozygosity	PIC
UGEP067	227 – 259	52	14	0.231	0.154	0.875
UGEP053	233 – 257	53	11	0.198	0.113	0.858
UGEP057	450 – 478	56	15	0.277	0.125	0.837
UGEP024	181- 213	56	15	0.277	0.179	0.824
UGEP066	210 – 242	54	12	0.269	0.185	0.814
UGEP027	265 – 293	54	10	0.370	0.111	0.754
UGEP012	195-255	52	11	0.433	0.192	0.741
UGEP064	215 – 251	51	14	0.431	0.098	0.739
UGEP020-1	156 – 168	52	6	0.327	0.000	0.671
UGEP079	183 – 191	56	7	0.491	0.357	0.603
UGEP 005	2228- 258	37	9	0.622	0.297	0.544
UGEP020-2	170 – 178	55	5	0.636	0.000	0.511
UGEP033-2	231- 239	52	6	0.577	0.000	0.503
UGEP098	194 – 222	53	7	0.557	0.472	0.457
UGEP110_2	195 – 217	55	4	0.655	0.000	0.411
UGEP033-1	209 – 223	51	5	0.706	0.000	0.404
UGEP095	224 – 228	53	5	0.755	0.038	0.371
UGEP106	172 – 192	56	8	0.821	0.054	0.303
UGEP110_1	165 – 175	55	5	0.818	0.018	0.295
UGEP096-2	221 – 229	56	5	0.911	0.000	0.165
UGEP084	184 – 188	56	3	0.911	0.018	0.153
UGEP073	228-246	55	4	0.918	0.018	0.148
UGEP096-1	209- 209	54	3	0.963	0.000	0.071
Mean		53	8.00	0.572	0.106	0.524
Min		37	3	0.198	0.000	0.071
Max		56	15	0.963	0.472	0.875

Table 15: Summary statistics based on the two groups and the entire finger millet core collection using the 20 simple sequence repeat loci

Statistics	Entire collection	Groups	
		land races	Exotic
Sample size	105	56	49
Total Number of alleles	226	185	173
Rare alleles	62	32	11
Common alleles	130	102	133
Most frequent alleles	34	51	29
Unique alleles	58	49	22

4.3.2 Cluster analysis

Based on the 226 alleles, The weighted neibour-joining tree analysis was used for phenetic analysis and the process grouped the 105 accessions into 3 main clusters. Group A was sub divided into 3 clusters comprising 52 accesions. While, group B, had 2 sub clusters with 31 accessions and group C sub divided into 2 sub clusters with 22 accessions (Figure 2). The estimated genetic dissimilarity for each pair of the 105 finger millet genotypes ranged from 0.18 to 1.00. The greatest genetic diversity in this study was observed between KNE 88 and KAL, PN 113, Odiang/1, P 788, NU-1 and Adoke (Table.16). KNE is of Kenyan Origin. The weighted neibour-joining tree analysis for land races grouped them into 3 main clusters, Group I,II,II sub divided into 2 sub clusters each compring of 16, 21 and 14 genotypes respectively with ogubati andCherema/Oderema being the most dsitant from the rest of the groupings

Table 16: Dissimilarity coefficients of finger millet accessions as revealed by SSR markers

	Muhana white	KAL	PN 113	Odiang/1	P788	P 231	P 236	NU-1	SX 11	Oligo brown	Adoke
Ogubati	0.95	0.98	0.95	0.95	0.95	1	0.95	0.95	1	0.83	1
DWARF SEL	0.97	0.94	0.94	0.89	0.94	0.94	0.94	0.88	0.97	0.89	1
Cheram/Oderema	0.84	0.89	0.93	0.93	0.92	0.95	0.95	0.92	0.92	1	0.91
ACC/FMB/01	1	0.89	0.95	0.89	0.92	0.92	1	0.89	0.86	0.82	0.94
KNE 88	0.96	1	1	1	1	0.97	0.97	1	0.97	0.95	1
Q 1299	0.91	1	0.93	0.90	0.974	0.97	0.95	0.97	0.95	0.9	0.94

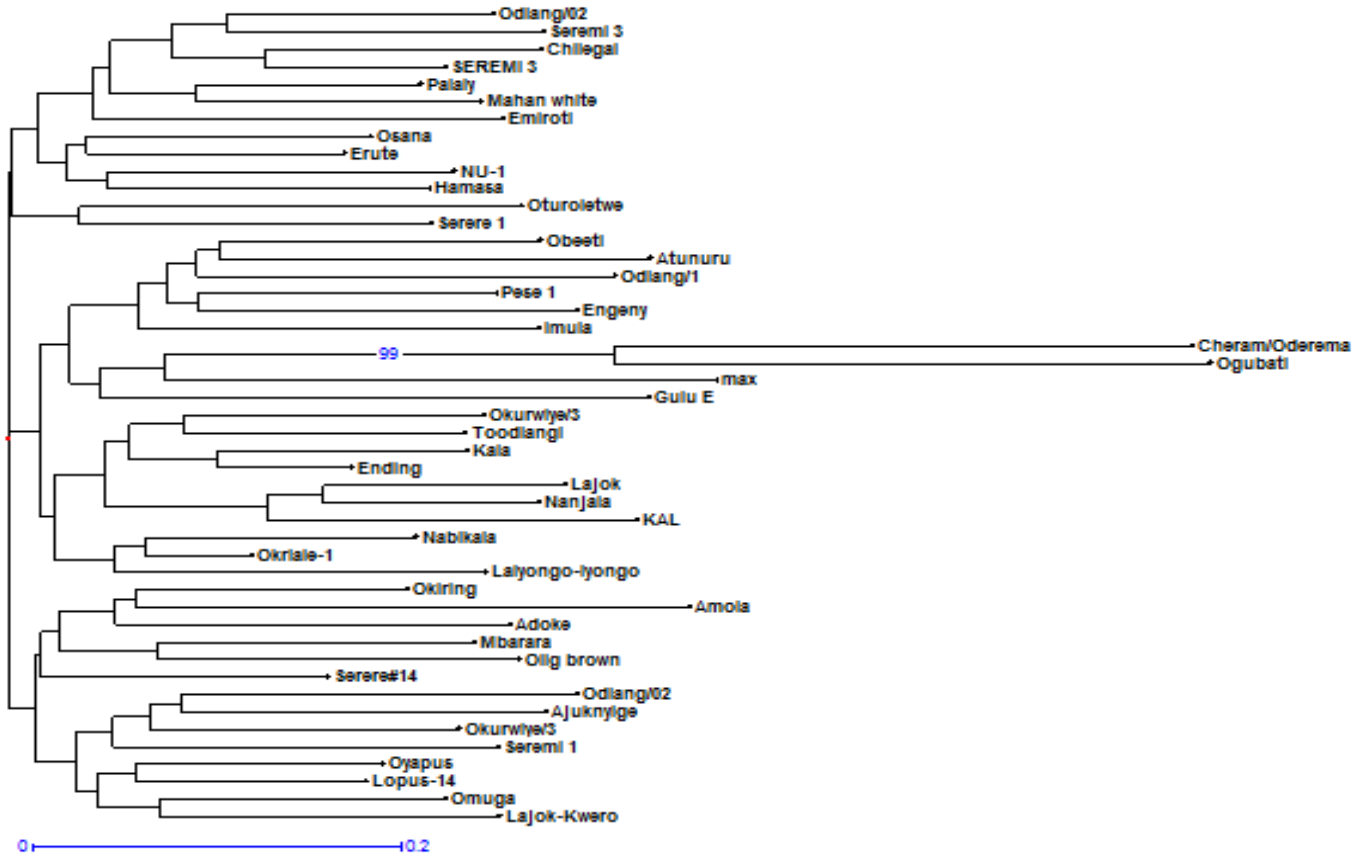

Figure 1: Dendrogram of finger millet land races generated using neighbor joining based on SSR data

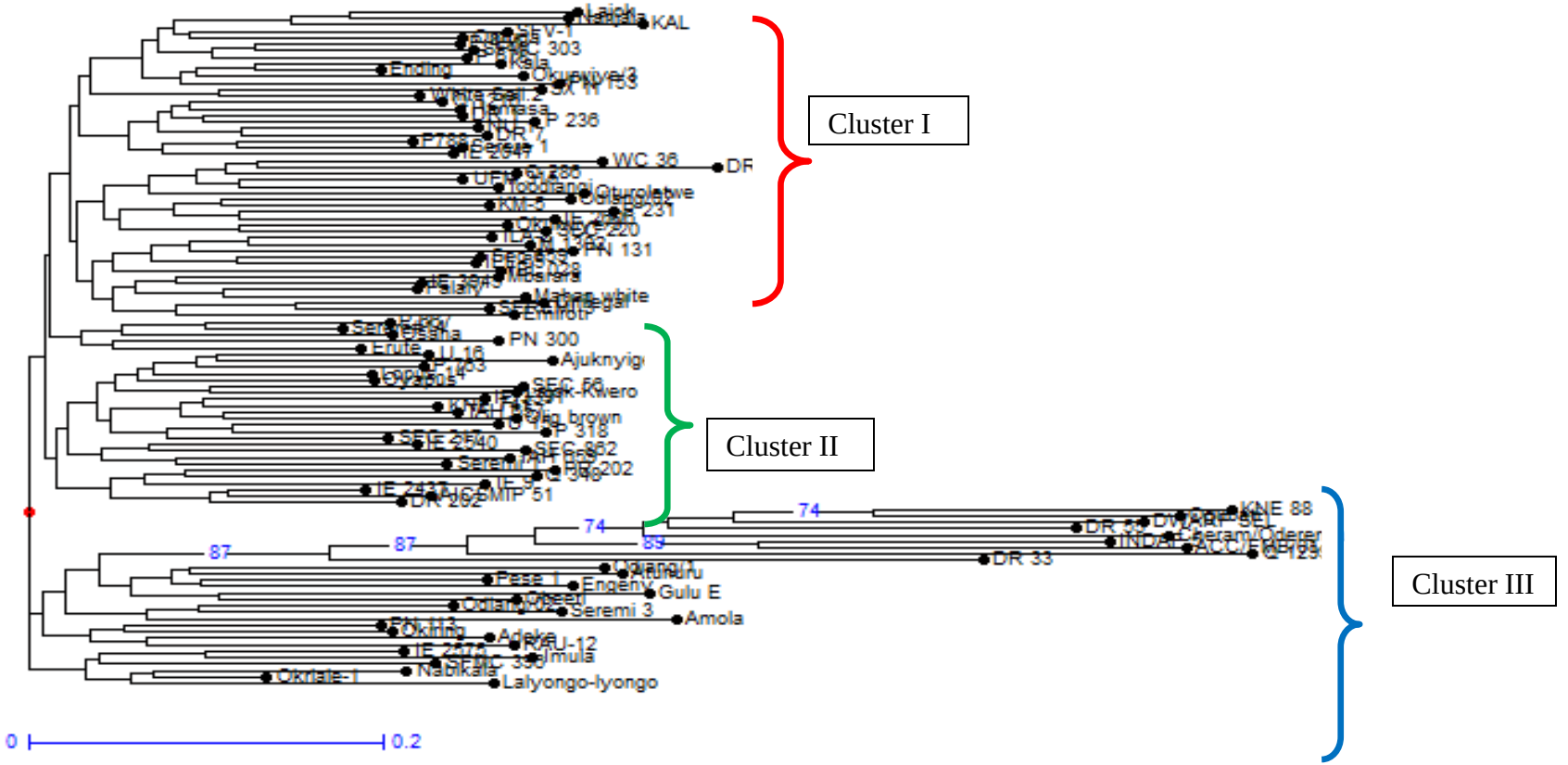

Figure 2: Dendrogram showing clusters of finger millet accessions revealed by genetic dissimilarity estimates based on SSRs

4.4 Cross compatibility within the Eleusine genus

A total of 60 crosses were attempted using the hot emasculation technique resulting in 3 crosses successfully forming seed (Table 17). Four hundred F1 plants were obtained from pre germination before transplanting to check for true hybrids using parent morphology and SSR markers. 50% of the 43 sampled to screening against SSR markers were true F1s (Plate.2). High plant vigour was observed among the F1 plants with some exhibiting cultivated finger millet traits only whereas, others were purely wild type in their phenotype (Plate 1)

Table 17: Number of attempted inter species crosses and seed formation after 3-4 weeks

Attempted Cross	Seed set (3 -4 weeks)
Pese 1X E.K(04)	Yes
DR 24 X E K(04)	No
Serere 14 x E K	No
Adoke x E K	No
Seremi 1x E K	No
Seremi 3x E K	No
Pese 1X E A(011)	No
Adoke x E A(010)	No
Gulu Ex E.A(010)	No
Seremi 1x E A(20)	Yes
Serere 14 x E A(20)	No
Seremi 3x E A(020)	Yes
FM(001)XE A(018)	No

E K - *Eleusine kigezienesis*; E A - *Eleusine africana;* FM- *finger mille*

F1plants purely cultivated type **F1plants purely wild type**

Plate 1 F1 hybrids of the interspecies crosses between the cultivated and wild finger millet

E.corocana F1 *E. kigezenesis* *E.corocana* F1 *E.africana*

Plate 2 True F1s of the inter species crosses between the cultivated and the wild finger millet as revealed by parent morphology

CHAPTER FIVE

GENERAL DISCUSSION

5.1 Genetic variability based on phenotypic assessment of accessions

5.1.1 Genotypic variations for agronomic traits among the selected finger millet accessions

There were significant differences among the selected finger millet accessions for most quantitative traits assayed such as plant height, secondary tillers, panicle length, panicle width, number of fingers per ear, dry weight, days to 50 % flowering, finger length, longest finger length across the two rain seasons of 2011. These findings are similar to earlier studies where significant differences were reported among various finger millet accessions (Kebere *et al.,* 2007; Ganapathy *et al.,* 2011). In this study, it was also clear that most of the Ugandan finger millet accessions were taller (range 20-79 cm, compared to exotic accessions: 19-77cm), late flowering (56 – 87 days, compared to exotic accessions; 53- 87 days) yet early maturing (88 – 133days, exotic; 90-139 days), longer fingers (2.8- 13.17, exotic;4-13); more productive tillers (0-10, compared to exotic accessions; 0-5.6), green plant types over pigmented (purple) with tip curved inflorescence compactness (93%) supporting earlier studies. This indicates that finger millet accessions used in this study are genetically diverse; moreover trait variability across the two different seasons showed genotype stability. This implies that the Ugandan finger millet germplasm are genetically diverse offering an opportunity for breeders to undertake further breeding activities through selection of the most diverse parents.

The principal component analysis revealed the contribution of each trait consequently individual genotype to the overall genetic variability observed among accessions. In this study, the first six principal components for the agronomic traits assayed had eigen values greater than one and

contributed 71 % of the total variation observed among finger millet accessions. High positive effects were registered for ear and maturity traits in PC1 and PC2 hence the major contributors to the significant genotype differences for agronomic traits among the finger millet accessions. This therefore indicates that the finger millet accessions varied highly for ear and maturity traits that could be used as basis for selecting parents (Ashfaq*et al*., 2012). Similarly grain yield per plant was reported to have been significantly influenced by finger length and width among finger millet genotypes from diverse regions of India (Bondale *et al.*, 2002). Moreover previous studies reported that high variability observed in finger millet characteristics including days to 50% flowering and maturity, plant height, number of productive tillers, finger per ear and grain yield could be utilized in the selection of diverse parents to undertake hybridization breeding (Bedis *et al.*, 2006; Bezaweletaw *et al*., 2006). This therefore indicates that the finger millet accessions used in this study are genetically diverse and each agronomic trait assayed contributed to a varying degree for the overall morphological variability among finger millet accessions. Also yield in finger millet is significantly influenced by ear and maturity traits as they contributed highly to the observed morphological difference among the finger millet accessions

Correlation analysis conducted revealed positive correlations for phenotypic traits with 65.1% of the total traits association. This positive correlation could be due to the presence of common genetic elements that controls the characters in the same direction Observed was significantly strong positive correlation between days to 50% flowering and days to harvest maturity as expected because the two are maturity traits; dry weight and number of fingers; Panicle length and length finger length. Similarly significant positive correlations were observed among Ethiopian land races for both ear and maturity traits (Dagnachew et al., 2012). This significant

positive correlation could have resulted from the effect of strong coupling linkage between genes or due to pleiotropic genes that control these traits in the same direction. However, environmental correlation caused by traits being influenced by the same environmental conditions could result in similar or dissimilar responses (Bezaweletaw *et al.,* 2006). Also observed in this study was significant negative correlation between days to 50% flowering, days to harvest maturity and dry weight similar to earlier findings (Bezaweletaw *et al.,* 2006; Dagnachew et al., 2012). This can probably be explained by the exhaustion of the available resources for production of profuse vegetative growth at the expense of assimilates that should be stored in the seeds (Dagnachew et al., 2012). With 65.1% of the traits showing positive and highly significantcorrelations especially for yield parameters, yield improvements by conventional breeding is achievable through simultaneous selection of traits.

5.1.2 Pre and post flowering drought tolerance in finger millet

This study revealed significant responses among finger millet genotypes for pre and post flowering drought tolerance for both phenotypic and physiological traits. These responses ranged from the ability to maintain relatively high chlorophyll content to drying of the whole plant under the different watering regimes. In general accessions that were able to hold high amounts of leaf water were presumed more drought tolerant similar to findings in other crops like wheat, corn and barley (O'Neill *et al*., 2006). Also observed was significant positive correlation among phenotypic traits, this could reveal environmental correlation causing traits influenced by the same environmental conditions result in similar responses (Bezaweletaw *et al.,* 2006).

Significant differences were observed in chlorophyll content, finger length and plant height under the pre and post flowering drought treatments indicative of genetic variability for

staygreen a critical element of drought tolerance. In recent studies, higher yield under drought tolerance was directly associated to the traits linked to soil/plant water status while the yield-biomass association with biochemical traits (Vadez *et al.,* 2012). This study also revealed that finger millet genotypes respond differently to drought stress depending on the physiological growth stage at which the stress is subjected. This therefore supports the use of phenotypic and physiological characteristics as a selection criterion for yield performance and improvement in finger millet under drought stress. Water shortage is one of the major limitations to crop productivity worldwide, and among the feasible solutions is to improve the drought tolerance of crop varieties through breeding.

Therefore the significant differences for agronomic traits among the collection indicates the level of morphological diversity of finger millet accessions in Uganda, moreover the genetic diversity with trait variability across the two seasons showing genotype stability. This implies that the 400 finger millet accessions used are genetically diverse; a rich genetic resource coupled with the generated information offers an opportunity to undertake further breeding activities through selection of the most diverse parents

5.2 Genetic variability based on SSR genetic markers

The success of a crop improvement programme depends on the knowledge and availability of genetic variability present in that crop for efficient conservation, management and effective utilization of plant genetic resources (Mondini *et al.,* 2009; Liu *et al.,* 2011; Vetriventhan *et al.,* 2012). Finger millet is as an important crop, having an allotetrapliod genome (AABB), C4 panicoid crop and short duration, it has a great potential for improvement.. The results of this study showed large molecular diversity in the finger millet collection. The SSR markers used in

this study were highly polymorphic and informative, and detected a total of 226 alleles with an average of 9.6 alleles per locus. The number of alleles per locus ranged from 3 to 20, however lower than that reported in barley (4–32 alleles; Matus and Hayes, 2002), rice (3–32 alleles; Borba *et al.,* 2010), chickpea (14–67 alleles; Upadhyaya *et al.,* 2008b) and maize (2–38 alleles; Wang *et al.,* 2008). An average of 9.6 alleles per locus was detected, which was more than those reported by previous studies in other finger millet diversity studies (Dida *et al.,* 2008) and other crops, e.g. 7.6 (Wang *et al.,* 2009) and 4.79 (Shehzad *et al.,* 2009) in sorghum, 8.23 in maize (Yang *et al.,* 2010) and 8.2 (Agrama *et al.,* 2007). In the finger millet collection, 62 were rare alleles (27.43%), 130 common alleles (57.52%) and 34 the most frequent alleles (15.04%). Rare alleles from cultivated and wild accessions could be used to select specific accessions for allele mining (Upadhyaya *et al.,* 2010). A total of 58 unique alleles were detected in the whole collection, which were specific to a particular accession. Unique alleles are important because they may be diagnostic of a particular type of genotype (Senior *et al.,* 1998) and useful for cultivar identification but they also indicative of centers of diversity. The presence of many rare and unique alleles could be due to the higher rate of mutation at SSR loci (Henderson and Peters, 1992; Senior *et al.,* 1998) and a highly diverse nature of the accessions present in Ugandan finger millet collection PIC in this study was higher (0.52) than that reported in rice (0.42; Jin *et al.,* 2010), Sorghum (0.49; Ali *et al.,* 2011) foxtail millet (0.381; Lin *et al.,* 2012) but lower than that reported in sweet sorghum (0.54; Wang *et al.,* 2009), chickpea (0.603, Pervaiz *et al.,* 2009, 0.854; Upadhyaya *et al.,* 2008b) and foxtail millet (0.7; Vetriventhan *et al.,*2012). Maximum allelic richness and the maximum number of rare and unique alleles can be explained by several factors such as diversity among the germplasm, number of accessions used, number of SSR loci

and SSR repeat type. A larger number of SSR loci and the use of dinucleotide repeat SSRs rather than trinucleotide or higher may lead to a higher number of alleles and higher genetic diversity (Yang *et al.*, 2010).. Moreover, the higher number of alleles may also be attributed to the material used in this study; this collection represents the diversity of the entire germplasm collection conserved at NaSARRI, Uganda which is an indication of the greater diversity present in the collection and its potential as a reservoir of novel alleles for crop improvement. Although finger millet is a self pollinating crop and DNA was extracted from single representative plant of each accession, some SSR loci showed relatively high heterozygosity (20%). This could be due to allotetrapliod nature of the genome (AABB), out crossing averaging about 1% (de Wet, 1995), heterozygous individuals or residual heterozygosity in germplasm (Blair *et al.*, 2009) for particular SSR loci.

In summary, the results of this study provide evidence for the presence of a high level of genetic diversity in the finger millet collection as well as within and between the two groups. The selected finger millet accessions also contained a good amount of diversity for important agronomic traits. Also identified were 17 most diverse accessions that high level of marker diversity (Muhana white, KAL, PN 113, Odiang/1, P788, P 231, P 236, NU-1, SX 11, Oligo brown,Adoke, Ogubati, DWARF SEL, Cheram/Oderema, ACC/FMB/01, KNE 88, Q 1299). These accession pairs could be used to initiate finger millet breeding as parents in the Cereals breeding programme for making crosses to identify and (or) to study superior segregants, for the development of mapping populations. These SSR markers were useful in differentiating closely related germplasm sources. Hence, these SSR markers could be used to supplement

morphological and agronomic data used for the protection of plant variety and/or germplasm identification.

5.3 Cross compatibility within the Eleusine genus

The results of this study revealed cross compartbility between *E corocana* subsp *corocana (2n =4x= 36*), and the wild relatives *E corocana* subsp *africana and Eleusine kigezienesis(2n = 4x = 36)* all allotetrapliod giving rise to fertile F1 hybrids.. However, some of the crosses did not produce F1 at all hence incompatible.. Molecular characterisation of the hybrids using SSR analysis confirmed these where indeed interspecies hybrids similar to earlier reports by (Dida *et al.,* 2008). This study also revealed that the wild relatives have a great potential for finger millet improvement through transfer of genes for tillering ability (primary tillers only. *E africana;* 15, *E kigezienesis*; 12), early flowering (E *a;* 53 days, *E k;* 48 days), taller with longer panicles that have many fingers. Though successful hybridization does not necessarily lead to gene introgression new sources of traits for earliness, tillering and yields have been identified among the F2 segregating population.

Therefore, the results of this study provide evidence for specific cross compatibility between the *Eleusine* genus not reported in earlier studies (de Wet 1991; Dida *et al.*, 2008; Andersson and De Vicente, 2010). While it's not certainly clear of the possible recombinations and gene interactions as a result of the gene mix, the potential benefits of full expression of transferred genes or accidental advantageous gene interactions expressed offers great opportunity for increasing the genetic base of finger millet, crop improvement of local adapted genotypes and land races for economically important traits such as diseases resistance, drought tolerance, earliness, tillering and increased yields.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study investigated genetic diversity of finger millet germplasm in Uganda and involved field evaluations to characterize phenotypic diversity among the selected finger millet accessions using morphological markers and genetic markers. Additionally cross- compatibility of finger millet *Eleusine corocana* with *Eleusine africana* and *E. Kigezienesis* was performed to access the potential use of the gene flow within the *Eleusine* genus for finger millet improvement.. The significant variation among genotypes for agronomic traits indicates the high level of morphological diversity of finger millet accessions in Uganda with potentially new sources for drought tolerance genes identified. Besides the results of SSR markers analysis revealed a high level of genetic diversity among the finger millet collection as well as within and between the two groups.

The specific cross compatibility within the *Eleusine* genus observed in this study provided more evidence for the gene flow within the genus. New source of variability have been successfully incorporated from the two wild allotetrapliod genomes to the cultivated finger millet genome; moreover the action of the new genes are yet to be determined. There is therefore great opportunity for finger millet improvement for economically important traits such as diseases resistance, drought tolerance, earliness, tillering and increased yields.

Therefore the finger millet accessions used this study are genetically diverse; rich genetic resource coupled with the generated information offers a virgin opportunity for undertake further

breeding activities through selection of the most diverse parents for finger millet yield improvements.

6.2 Recommendations

1. The variability among the finger millet accessions can be useful for selecting most diverse parents for use in developing finger populations' for mapping quantitative traits and studying their inheritance.

2. The already identified genetically diverse accessions can be used for developing mapping populations for QTL mapping with the use of the SSR molecular markers.

3. The new sources for drought tolerance can be used for improving finger millet accessions with farmer s preferred traits but there is need to evaluate them for various agronomic traits for suitability.

4. The gene flow within the *Eleusine* genus can be exploited for improving important traits such as blast diseases resistance, drought tolerance, tillering, earliness and yields to improve the cultivated finger millet

5. More field research is needed to mine the available genetic resource of useful genes that could be used to enhance breeding of finger millet for farmer-preferred traits.

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