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Genetic characterization of jute mallow (corchorus spp.): a traditional African vegetable

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GENETIC CHARACTERIZATION OF JUTE MALLOW (*CORCHORUS SPP.*): A TRADITIONAL AFRICAN VEGETABLE

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A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Life Science of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

Jute mallow (*Corchorus spp.*) is a very nutritious traditional leafy vegetable. Its leaves contain an average of 15% dry matter,4.8 g of protein, 259 mg of calcium, 4.5 mg of iron, 4.7 mg of vitamin A, 92 µg of folate, 1.5 mg of nicotinamide and 105 mg of ascorbic acid per 100g of leaves. In spite of its nutritional importance, cultivation of Jute mallow is limited due to lack of scientific information with regard to available cultivars and their adaptation to specific environments. The aim of this study was to analyze the genetic diversity and evaluate the accessions which are well adapted to specific environment for leaf and seeds production. Ninety accessions were evaluated in the field trial experiments for two growing seasons. ISSR and SSR markers were employed for molecular characterization as well as leaf and seed yield evaluation. The results showed significant ($p<0.01$) variability in accessions in all morphological traits. Traits such as plant height ($r=0.448$), petiole length ($r=0.237$), primary branches ($r=0.319$) and number of leaves per plant ($r=0.333$) were significantly ($p<0.01$) correlated with biomass yield. First five Principal components (PCs) with Eigen values ≥1 explained 72.9% of the total variability in the accessions. Cluster analysis grouped the accessions into five major clusters mainly based on their origin. Number of alleles per locus ranged from 2-4 with an average of 2.63 alleles per locus. Polymorphic information content (PIC) ranged from 0.278 in primer SSR17 to 0.78 in primer SSR24. Average Nei's gene diversity (*h*) and Shannon's information index (*I*) were 0.348 and 0.557, respectively. In ISSR markers a total of 85 bands were amplified and average polymorphic bands per primer was 2.75. PIC values ranged from 0.39 to 0.76 with average of 0.53. The highest Nei's pair wise genetic distance (0.431) was observed in East African accessions. UPGMA cluster analysis grouped the accessions into five main clusters at genetic similarity coefficient of 0.53. In terms of leaf and seed yield, significant ($p<0.01$) differences among the accessions in all traits was observed. Leaf fresh weight ranged from 18.3g/plant to 121.3g/plant in accessions TOT 6747 and TOT 8532, respectively. Seed yield ranged from 1.0g/plant to 35.5g/plant in accessions TOT 7980 including eight others and TOT 7866, respectively. Evaluation of genetic diversity and the agronomic traits for leaf and seed yield in this germplasm has revealed useful information for breeders in their efforts to improve the yield as well as selection of accessions with good agronomic traits. It is recommended that detailed study to document ethnomenclacture, ethnobotanical uses, progress towards domestication and challenges faced by farmers be conducted.

DECLARATION

I, **Munguatosha Samwel Ngomuo** do hereby declare to the senate of Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being submitted concurrently for degree award in any other institution.

Munguatosha Samwel Ngomuo

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The above declaration is confirmed

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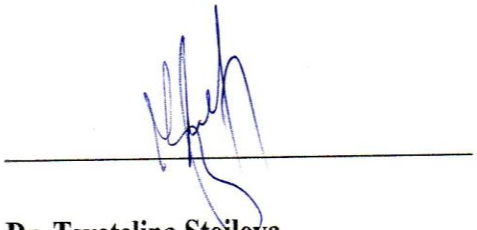

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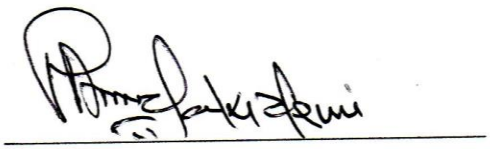

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CERTIFICATION

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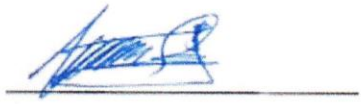

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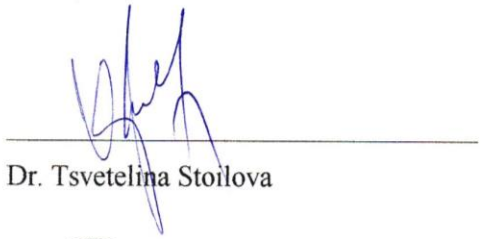

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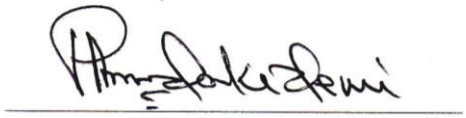

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Last but not the least; I would like to thank my family: my parents Samwel and Ngiwanza, for investing in my education at first place and supporting me morally and spiritually throughout my life.

DEDICATION

This work is dedicated to the LOVE and INFINITY GLORY of God

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LIST OF ABBREVIATIONS AND SYMBOLS

AFLP	Amplified fragment length polymorphism
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
AVRDC	Asian Vegetable Research and Development Center
CGIAR	Consultative Group in International Agriculture and Research
CTAB	Cetyltrimethylammonium bromide
DAP	Diamonium phosphate
dNPTs	deoxyribonucleoside triphosphate
ESA	Eastern and Southern Africa
FAO	Food and Agriculture Organization
GD	Genetic distance
ISSR	Inter simple sequence repeat
kD	Kilo Daltons
LA	Leaf Area
MC	Moisture content
NIHORT	National Institute of Horticultural Research and Training
NM-AIST	Nelson Mandela African Institution of Science and Technology
NPGRC	National Plant Genetic Resources Center
NPK	Nitrogen Phosphorus and Potassium

PAGE	Polyacrylamide Gel Electrophoresis
PCA	Principal Component Analysis
PCoA	Principal Coordinate Analysis
PGR	Plant Genetic Resources
PIC	Polymorphic Information Content
PVC	Polyvinyl Chloride
QTL	Quantitative TraitLoci
RAPD	Random Amplified Polymorphic DNA
SDS	Sodium dodecyl sulphate
SRAP	Sequence related amplified polymorphism
SSR	Simple sequence repeat
STMS	Sequence tagged microsatellite sites
UPGMA	Unweighted pair group method with arithmetic mean

CHAPTER ONE

Introduction

1.1 Back ground information

Vegetables are one of the most important components in diet for many households in Africa. Vegetable farming is practiced in small scale including at back and front yard gardens as well as at large scale for commercial purposes. These vegetables are of different types (Maina and Mwangi, 2008).

In tropical Africa there are various types of vegetable species (Grubben and Denton, 2004). Among the 275 vegetable species grown in the continent, 207 are native to Africa, 45 were brought in the past but became acclimatized, and 23 vegetables have been introduced (Grubben and Denton, 2004). African traditional vegetables have significant roles in our diet, food availability, food diversity, economic growth and sustainability in land use and care. They are a source of income as they can be marketed or traded locally, regionally and exported (Weinberger and Msuya, 2004). Several traditional vegetables are not used as food only but are consumed for their protective as well as for their healing properties (Keding *et al.,* 2007). Common traditional African vegetables in city markets and rural areas include African nightshade, African eggplant, amaranth, celosia, cowpea leaf, okra, spider plant, jute mallow, and roselle (Grubben and Denton, 2004; Keding *et al.,* 2007). These vegetables have fed Africans for centuries.

Corchorus spp. (Jute mallow) is among African traditional vegetables which is very nutritious and has been in use in many households for a long time. Its ecological adaptation to various climates and its resistance to pest and diseases is important incentive to many farmers. The origin of this vegetable is Africa and it has a wide spread of plant types present in the continent (Kundu, 1951; Benor *et al.,* 2011). The vegetable is extensively grown for the sliminess of the leaves in local dishes. It is one of the leading leafy vegetables in West Africa (Grubben *et al.,* 1977). It prefers warm, sunny conditions and a range of soils including sandy loams. It does not grow well in water logged soils, very cold weather or shade. It can be grown as a monocropor mixed with nightshade, amaranth or any other crop.

In many African countries particularly Tanzania, commercial farming of the crop is practiced to a very limited extent compared with other vegetables. However, for a long time, it has been semi-domesticated, and sometime grows as a volunteer crop in farmers' fields and on fertile soils close and around most homesteads. Although there is some limited farming of leafy vegetables in some countries in East Africa such as Kenya, Uganda and Tanzania, Jute mallow is not popular among them (Mnzava and Ngwerume, 2004).

1.2 Problem statement and justification

One way of adaptation to climate change is to reach into the genetic resource of the so-called minor crops. These crops are thought to be more resilient to climate change while being more nutritious than the domesticated global vegetables. It is vital to characterize these crops in order to gather information that will help in their widespread usage.

The status of Jute mallow in some parts of Tanzania as a wild plant and weed renders it unappealing for consideration, because it is often seen as a poor man's crop. Cultivation of wild edible plants is necessary in widening the nutrition and food base in developing countries. However, these vegetables are viewed by researchers as minor crops and hence get little attention in most research and crop development programs.

To put in place improved varieties in response to the needs of farmers, it is necessary to characterize the local ecotypes commonly used by small holder farmers. These local ecotypes are usually more of populations than pure varieties (Chweya and Eyzaguirre, 1999). Very little or no work has been centered on the real evaluation of the potential of different cultivated local and exotic vegetables including Jute mallow.

Research on the genetic diversity of Jute mallow (*Corchorus spp.*) helps in exploitation of the desirable nutritional properties of the crop for better nutrition and good health, essentially within the rural communities in Africa (Oguntona and Akinyele, 1995). Significant analysis of germplasm diversity is important for proper understanding and exploitation of genetic variability among accessions and their characters. This will help in coming up with accessions with high yield and those which are tolerant to various abiotic and biotic stresses. Traditional vegetables are highly nutritious yet are not getting satisfactory scientific consideration and detailed research

despite their importance in food and nutritional security in Africa. It is important to remember that the inclusion or conservation of edible wild and non-cultivated plant resources could be very useful for healthy people particularly for vulnerable groups in our society such as children under the age of five, pregnant and lactating mothers. Plant breeders can only achieve success in any crop improvement program with the knowledge of the extent of genetic variability that exists among accessions of the species that they are working with. This information will benefit seed production companies and researchers in their crop improvement and development efforts. Breeding of high yielding varieties which are well adapted to specific environment requires information related to genetic diversity of the respective crop. This usually facilitates selection of the accessions to include in breeding programs. The information from this study will also help in the conservation of the species, its management and further development of plant genetic resources.

In view of the above, this research focused on genetic characterization and evaluation of Jute mallow accessions available in the germplasm of World Vegetable Center (AVRDC) in order to address the challenges of developing and making use of this crop.

1.3 Objectives

1.3.1 General Objective

To study the genetic diversity of Jute mallow and evaluate morphological and quantitative traits of the accessions conserved in the gene bank.

1.3.2 Specific objectives

i. To analyze the morphological characteristics of traditional African vegetable; Jute mallow accessions.
ii. To study the genetic diversity among Jute mallow accessions by using ISSR and SSR molecular markers.
iii. To evaluate the leaf and seed yield of Jute mallow accessions under field conditions for two growing seasons.

1.4 Research Hypothesis

i. There are morphological variations among Jute mallow accessions
ii. Genetic diversity exists among Jute mallow accessions at molecular level
iii. There is variation in leaf and seed yield of Jute mallow accessions under field conditions

1.5 Significance of the Study

Due to the effects of climate change which has led to changes in crop adaptation to the adverse environment, it is important to widen the nutritional base by using alternative crops to supply nutrients. Jute mallow is underutilized and neglected crop. This study aimed at identifying sources of variation within the jute mallow germplasm that will help in breeding of improved Jute mallow varieties. Leaf and seed yield evaluation will help in selection of accessions which can be used directly by farmers due to good adaptation to local environment. This will improve the productivity of this crop and ensures household food and nutritional security.

CHAPTER TWO

LITERATURE REVIEW

The genetic diversity of leaf vegetable Jute mallow (*Corchorus spp*.): A Review[1]

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Abstract

Corchorus spp. is among African traditional vegetables which is very nutritious and has been in use in many households for many years. Jute mallow contains high level of carotenoids (150µg/100g) a precusor of vitamin A, vitamin C, Iron (190µg/g), 1.3%Calcium and 25.0% protein. The leaves are also used in treatment of several diseases and have a wide antibacterial properties. The crop is semidomesticated and sometimes grows as a volunteer crop in farmers fields. The status of Jute mallow in some parts of Tanzania and other East African countries as a wild plant renders it unappealing for consideration in crop development programs. There is limited scientific information on Jute mallow diversity for use as a leaf vegetable in crop improvement and is a potential area for research. The variations exist among different accessions and cultivars based on leaf shapes and color. Characterization of Jute mallow by using molecular markers, proteins and isozymes is also reported. Variation is reported to be low within species and high among species. Most of released varieties have a narrow genetic base. Conservation of this species in Africa is scarcely reported.

Key words: *Corchorus spp*., Genetic diversity, Jute mallow, Leaf yield, Morphological characterization.

[1] Paper Published in Indian Journal of Agricultural Research (*Vol. 51, Issue 5, pp. 405 - 412*).

2.1 Introduction

In tropical Africa, there are various types of traditional vegetable species (Grubben and Denton, 2004). African traditional vegetables have significant roles in our diet, food availability, food diversity, economic growth and sustainability. They are a source of income as they can be marketed or traded locally, regionally and exported (Weinberger and Msuya, 2004). Several traditional vegetables are not used as food only but are consumed for their protective and healing properties as well (Keding *et al.*, 2007).

Ecological adaptation of Jute mallow to various agro-climates and its resistance to pest and diseases is important incentive to many farmers. There is wide variation among the species or local types in terms of height, stem color, pubescence, leaf and fruit shape and leaf production (Westphal-Stevels, 1985). Variation in morphological and physiological characters among *Corchorus* species has been documented by several researchers (Schippers, 2000; Kar *et al.*, 2009; Benor *et al.*, 2012; Mandal *et al.*, 2013; Soliman *et al.*, 2014). Molecular markers techniques have also been used to detect variation in *Corchorus* species both within and among genotypes (Basu *et al.*, 2004; Benor *et al.*, 2011; Banerjee *et al.*, 2012; Biswas, 2013; Ghosh *et al.*, 2014). Other markers that have been used to study genetic diversity of Jute mallow include proteins and isozymes. Collection and conservation of germplasm accessions in different national and international gene banks is also reported by several authors.

This review paper aims at exploring the current state of Jute mallow research as a leafy vegetable consumed in many rural areas in East Africa. In view of the above, a platform for identification of promising accessions for yield, quality and desirable horticultural traits for use in breeding programs and improvement of Jute mallow can be established.

2.2 Origin and Geographical Distribution

The origin of *Corchorus spp*. has been investigated extensively. White Jute is said to have originated from Indo-Burma while the dark Jute is said to have originated from Africa due to variation in morphological traits (Xiong, 2008). Now it is well established that the two cultivated species (*C. olitorius* and *C. capsularis*) in Asia originated from Africa from a study conducted using nuclear and chloroplast simple sequence repeats (SSRs) (Kundu *et al.*, 2013). This is also

supported by a wide spread of plant types present in the continent and occurrence of several related species (Kundu, 1951; Benor *et al.*, 2012). In addition, secondary centers of origin are considered to be Bangladesh, North India and Myanmar (Basu *et al.*, 2004; Grubben and Denton, 2004).

The species are known to occur in diverse ecological habitats and are widely distributed across Africa (Mir *et al.*, 2008). The plant is found in open acacia bushland, grassland, cultivated lands and near pans and dams in several countries such as Kenya, Tanzania, Botswana, Zambia and Zimbabwe (Nguni and Mwila, 2007). In Africa, most of the species are found in the eastern and southern parts of the continent (Benor *et al.*, 2010). Eight wild species of *Corchorus* and the two cultivated species occur in India in diverse ecological conditions and habitats (Mir *et al.*, 2008). *Corchorus* is also cultivated in Caribbean, Brazil, Cyprus, Greece, Crete, Bangladesh, China, Japan and in the middle East (Grubben and Denton, 2004; Thompson *et al.*, 2010). The variation in *Corchorus spp*. is said to be due to adaptation to various climates and habitats or occurrence over large geographical areas resulting in spatially isolated populations (Knight *et al.*, 2005; Benor *et al.*, 2011). Zhang *et al.* (2015) noted different centers of origin for the two cultivated species of Jute mallow (*C. olitorius* and *C. capsularis*) are responsible for their differences, i.e. strong sexual incompatibility barrier.

2.3 Taxonomy and Botanical Description

Jute mallow belongs to the family *Malvaceae*. At least 50-60 species are well known and 170 different names are used to describe them under the genus *Corchorus* in Index Kewensis (Edmonds, 1990; Palve and Sinha, 2005; Mir *et al.*, 2008).

The cultivated species (*C. olitorius* and *C. capsularis*) are diploid (2n=14) and they undergo regular meiosis. As for the identified wild species, the chromosome numbers of around 21 species have been reported. Majority of these wild species are diploid though tetraploid species (2n=28) are also known (Edmonds, 1990).

Most of the species are annual herbs reaching a height of up to 2.4m. Jute mallow can be unbranched or with very few primary and secondary branches. It has alternate simple, lanceolate leaves. The leaves margins range from finely serrate to coarsely serrate or lobed margin. The

plant has small yellow flowers with five petals which are hermaphrodite (Norman, 1972). The stem is long, slender and color varies from full green to dark red in case of *C. capsularis* and green or colored light red or deep red in case of *C. olitorius*. For *C. capsularis*, it has a round pods while *C. olitorius* has elongated pod. It has a well branched tap root system.

2.4 Use and Nutritional Importance

Corchorus olitorius and *C. capsularis* are mainly cultivated in Asian and Latin American countries as a major source of natural fiber (Roy *et al.*, 2006). In Africa, *C. olitorius* is grown as vegetable crop (Krebs, 2001; Grubben and Denton, 2004; Kinabo *et al.*, 2006; Dansi *et al.*, 2008; Benor *et al.*, 2012). Little is reported for these species as fiber crop in Africa (Benor *et al.*, 2010). Nevertheless, plants with short and branched stems with small leaves are widely used as leafy vegetable in Africa, Asia and Europe (Velempini *et al.*, 2003).

Jute mallow like other traditional leafy vegetables represents a cheap but quality nutrition for large segments of the population in urban and rural areas (Freiberger *et al.*, 1998; Kinabo *et al.*, 2006; van Rensburg Willem *et al.*, 2007; Lewu and Mavengahama, 2010). Its leaves contain an average of 15% dry matter, 4.8 g protein, 259 mg calcium, 4.5 mg iron, 4.7 mg vitamin A, 92 µg folate, 1.5 mg nicotinamide and 105 mg ascorbic acid per 100 g leaves (Grubben and Denton, 2004; Odhav *et al.*, 2007; Dansi *et al.*, 2008). The production of mucilage from the leaves is a property appreciated to make sauces that can be used together with starchy foods. A relish and potherb can also be produced by boiling the leaves like spinach (Benor *et al.*, 2010).

Apart from food value, *Corchorus* species are medicinal plants that are widely used for treatment of various ailments. The commonly used species include *C. olitorius*, *C. capsularis* and *C. aestuans*. These are used to treat general diseases and are also remedies for heart disease, enemas, parturition and febrifuges (Burkill 1995). Other diseases include chronic cystitis, gonorrhea, dysuria, and toothache (Hillocks, 1998). The seeds are used to treat fever and have wide antibacterial properties (Pal *et al.*, 2006). *Corchorus fascularis* is used as soap and soap substitute. The mucilage from *C. olitorius* is also used as source of fatty acids, oils and waxes. *Corchorus capsularis* is a source of glycosides, saponins and steroids (Benor *et al.*, 2010).

2.5 Genetic Diversity of Jute mallow

2.5.1 Morphological diversity

Genetic variation within individual species (*C. olitorius and C. capsularis*) is limited due to self-pollination (Hossain *et al.,* 2002). *Corchorus* species are thus characterized by a high level of inter-specific variability, but a low degree of intra-specific variability (Kar *et al.,* 2009). Studies on morphological characteristics of Jute mallow have been reported by several researchers. Nath and Denton (1980) found large morphological and physiological variations in leaves of *C. olitorius* grown by farmers in Nigeria. Variation in leaf shapes particularly was used to separate different local cultivars of *C. olitorius* (Denton, 1997). Considerable variation in other morphological traits was also observed by Akoroda (1985) in several local morpho-types.

Variation in number of vegetative characters among 3 Egyptian cultivars of *C. olitorius* was also reported by (Soliman *et al.*, 2014). Denton and Nwangburuka (2012) conducted a study on six morphological characters (plant height at maturity, number of leaves per plant, fresh leaf weight, stem weight, total plant weight and harvest index) by using single linkage cluster and principal component analyses. In their study, clustering scores among the principal component analyses suggested a strong relationship among individuals in the cluster.

Another study involving two sets of Jute mallow genotypes, 40 from *C. incifolus* and another 40 from *C. olitorius* was conducted by Ogunkanmi *et al.* (2010) In this study, they reported a continuous distribution of mean value for the parameters they measured except for number of leaves in *C. olitorius* which indicated superiority of a leafy vegetable over other genotypes (Ogunkanmi *et al.,* 2010).

Begum and Kumar (2011) characterized 25 released or notified varieties and 7 common knowledge varieties of both species using 17 morphological characters for Distinctiveness Uniformity and Stability (DUS) testing. Out of 17 morphological traits, 8 were monomorphic, 7 dimorphic and 2 polymorphic in *C. capsularis*.

In another study, Palve and Sinha (2005) used 6 accessions of *C. capsularis* and 7 accessions of *C. olitorius* to study variation and interrelationships among fiber yield attributes. Highly

significant differences were observed among accessions for plant height, number of days to first flowering and fiber strength (Palve and Sinha, 2005). Benor *et al*. (2012) used 101 accessions of *C. olitorius* species to study genetic diversity and relationships inferred from molecular and morphological data. In their morphometric analysis, it was found that qualitative traits especially those related to leaf morphology, branching habit and stipule color were the taxonomically very informative traits.

In efforts to widen a base for assessment of Jute mallow traits, Mandal *et al.* (2013) conducted a study on pollen grains. In their study, pollen morphological parameters revealed differences between the two species and a correlation matrix revealed no significant relationship between the two species. These are associated with reproductive outcomes and heredity and they are important in detailing morphological variations which define taxonomic relationships among plant taxa at different levels. Morphological markers are important in establishing the variation between plant species and they can be used as initial step towards breeding for Jute mallow traits crop improvement.

2.5.2 Morphological assessment with respect to vegetative characters of *Corchorus spp.*

Crop improvement programmes on Jute mallow for selection of varieties with finer and high quality fiber have received considerable attention over years in Asian countries such as China, Bangladesh and India. However, improvement programs on Jute mallow as a leafy vegetable has been very limited (Nyadanu *et al.*, 2016).

Because of variations in numerous local types in traits such as plant height, stem color, pubescence, fruit and leaf shape and leaf production, Westphal-Stevels (1985) proposed a classification of Cameroon materials and other West African countries into three cultivar groups that is cv group *olitorius, incisifolius* and *Geant de Bertoua* the main distinguishing feature being leaf shape. Morphological studies on several accessions have been done in Nigeria to separate segregating populations into different leaf types (National Horticultural Research Institute, 1986).

A study to determine heritability, genetic advance and association of quantitative vegetative characters with leaf yield of *C.olitorius* was conducted by Nwangburuka and Denton (2012) on

15 accessions, in which significant difference was found among the genotypes for all characters studied except in stem weight per plant, suggesting a prospect for meaningful selection for improvement of Jute mallow.

In Benin, 40 cultivars of *Corchorus spp*. were evaluated for their genetic diversity with reference to all phenotypic traits. Results of this study led to grouping the target plant materials into six classes based on all quantitative traits under the study. Similar research was conducted by Armand *et al*. (2013) on okra. Nine varieties of okra were used to identify those with best agro-morphological traits.

Choudhary *et al.* (2013) conducted a study on 17 genotypes belonging to six Jute mallow species to assess leaf area, foliage yield and some nutritional parameters. In their study, they were able to link foliage yield and nutritional content. The leaf area and foliage yield were found to have positive phenotypic correlation with potassium mineral content.

In this regard, only few studies on genetics of leaf yield traits have been conducted in Jute mallow and only few accessions have been used (Palit *et al.*, 1996; Denton and Nwangburuka, 2012; Osawaru *et al.*, 2012). In order to improve leaf yield of Jute mallow, the knowledge required is not only on the diversity and genetic variability of the available germplasm but also on genetic architecture of leaf yield traits and its attributes. Descriptive data for each accession conserved by gene banks facilitate the efficient use of accessions in research and plant breeding for improvement of the respective crop (Kristkova *et al.*, 2008). In view of this, genetic evaluation of the current germplasm by focusing on leaf yield related traits is important in order to identify the lines which have high yield and well adapted to local environment.

2.5.3 Genetic diversity based on Biochemical markers

i. Protein

When proteins are used as genetic markers and studies conducted using proper laboratory procedures, their electrophoretic migration rates are generally highly heritable and ample polymorphism are discernable for many germplasm management purposes (Bretting and Widrlechner, 1995). The variation in banding pattern of different proteins are assumed to be

equal to the variation in genes coding for these proteins (Osawaru *et al.*, 2012). In a study conducted using proteins to determine the relationship among three *Corchorus* species, traits such as growth habit and stem features appeared to be uniform. It was also found that determinate and medium branching habit and erect stems among the accessions studied were important traits for leaves production which are needed by farmers (Osawaru *et al.*, 2012). Seed protein polymorphism derived from electrophoretic banding pattern using SDS-PAGE in two cultivated Jute species (*C. olitorius* and *C. capsularis*) and seven wild species was conducted by (Das and Maiti, 1998). The results of this study indicated distinct polymorphism in electrophoretic banding pattern and led to detection of polypeptide bands ranging from medium (25.0kD to 49.9kD) to low (<25.0kD). Seven polypeptide bands ranging from 9.73 to 88.79kD were also recognized among 14 accessions of Jute mallow screened by Isuosuo and Akaneme (2015). These bands were moderately dissimilar showing moderate heterogeneity. Generally few studies have been conducted on Jute mallow using protein as markers. However, this technique has been used effectively to study genetic diversity among and between genotypes in different plant species (Thanh and Hirata, 2002; Nethra *et al.*, 2007; Chandra, 2008; Meena and Shukla, 2013). More reliable and useful information in detailing the diversity among Jute mallow genotypes can be generated by using protein markers.

ii. Isozymes

Isozymes are defined as structurally different molecular forms of an enzyme with qualitatively the same catalytic function. They result from amino acid alterations which cause changes in net charge or the spatial structure (conformation) of the enzyme molecule and thus their electrophoretic mobility (Dziechciarkova *et al.*, 2004). In Jute mallow, Ali *et al*. (2012) used allozymes/isozymes banding pattern to measure the genetic diversity of six leaf mutants of tossa Jute. The result of cluster analysis from the constructed dendrograms showed only two groups of peroxidases and five groups of esterase. The two clusters of peroxidase revealed 20% similarity with Jacquard's similarity coefficient of 0.1 among the researched genotypes in both clusters.

In another study, Khatun and Alam (2010) confirmed the species status of *C. trilocularis* and *C. pseudo-olitorius* by using isozyme assay. The activities of isozyme systems such as esterase, acid phosphatase and peroxidase used in their study and their banding pattern were different in the two species, indicating their usefulness in establishing the identity of the two species. For instance in acid phosphatase, *C. pseudo-olitorius* showed two bands which were dark and thick, while in *C. trilocularis* two light bands were observed. Similar work has been done in *C. aestuans*, a wild *Corchorus* species. In esterase system, five bands were observed while in peroxidase only one light band was observed. In acid phosphatase, two bands were observed, one thick and one light band. One band in both esterase and peroxidase system was common for all species of wild Jute, while the rest were specific for *C. aestuans* (Khatun *et al.*, 2011). The specific bands can be used as markers for *C. aestuans*.

Isozyme assay of three species of *Corchorus* (*C. fascicularis*, *C. pseudo-capsularis* and *C. tridens*) was carried out by Khatun *et al.* (2011). The banding properties in esterase and acid phosphatase were different in three species. In peroxidase *C. fascicularis* showed a dark and thick band while *C. pseudo-capsularis* had light thick band. The bands in peroxidise can be used as markers for these two species. Generally, the activities of different isozyme systems such as esterase, acid phosphatase and peroxidase have proved to be powerful tools in studying genetic diversity of different plant species. In jute mallow, only a few studies have been reported on the use of isozymes as markers. Besides Isozymes, many studies have been reported on the use of DNA markers (genomic DNA and chloroplast DNA), the approach that has been used to study the genetic diversity in other crop species.

2.5.4 Genetic diversity using DNA markers

Genetic diversity studies using molecular markers have been reported by different authors. Haque *et al.* (2007) conducted a study in which 18 genotypes of two cultivated species (*C. olitorius* and *C. capsularis*) were evaluated using RAPD markers. In this study, the genotypes representing the two species formed two major clusters. Other studies includes those of Qi *et al.* (2003) and Hossain *et al.* (2002) where they used RAPD primers in evaluating genetic diversity of Jute mallow in Bangladesh and China. The use of Amplified Fragment Length Polymorphism (AFLP) in Jute mallow has been reported (Basu *et al.*, 2004). In their study, 305 polymorphic

bands were detected by AFLP markers by using 10 pairs of primers (*Eco*RI and *Mse*I*)* from 49 genotypes of amplified template DNA of the two Jute mallow species. These markers revealed a high level of variation between *C. olitorius* and *C. capsularis* suggesting the distant maternal relationship and different centers of origins. Benor *et al.* (2012) employed AFLP in evaluation of genetic diversity of 101 *C. olitorius* accessions. Their analysis indicated low genetic diversity within the population as well as low Neis' gene diversity index which ranged from 0.046 to 0.096. Generally, there is low genetic diversity at species level.

Another study was conducted by Ghosh *et al.* (2014) using 63 genotypes of *C. olitorius* and *C. capsularis*. Both Simple Sequence Repeats (SSR) and AFLP markers were used in this study in which the combination of data from both primers divided the 63 genotypes into two different clusters. The similarity between the two species was low (0.003) indicating the divergence which exists in DNA sequences of these two species.

Mir *et al.* (2008) used 81 (45 *C. olitorius* and 36 *C. capsularis*) genotypes of commercially cultivated Jute mallow to study fiber related traits. This study revealed a quantitative nature of fiber yield related traits with more likelihood of dominance component in genetic variance. It was further noted that a subset of 45 set of SSRs derived from *C. olitorius* were more transferable to *C. capsularis* when they were used to study DNA polymorphism in *C. capsularis*. Average number of alleles for individual SSRs and average polymorphic information content (PIC) was low in both species. Similar study was also conducted by Huq *et al.* (2009) using 16 genotypes of elite Jute varieties from the same two species by use of SSR markers. In this study, 27 SSR primer pairs yielded a total of 171 different alleles, where the average alleles per locus were 6.33 $\pm$ 2.04. They also attained high polymorphism (92.2%) despite the crop being self-pollinated and incompatible for inter-specific crosses. Clustering of their data by using UPGMA resulted into two groups belonging to both species. This pattern of clustering is similar to those obtained in other studies by using RAPD and AFLP markers.

In another study, 172 SSRs were used to assess genetic diversity and population structure in 292 genotypes of two cultivated Jute species including indigenous and exotic accessions. In their study, PIC values did not differ significantly in the two species and 596 alleles were detected. In

both distance based analysis and structure based analysis, the exotic and indigenous genotypes were in separate groups (Banerjee *et al.*, 2012).

Inter simple sequence repeats (ISSR) markers are also reported by several authors in molecular characterization of Jute mallow. Qi *et al.* (2003) used 27 accessions of *Corchorus* consisting of two species *C. urticifolius and C. trilocularis* and unknown wild species to investigate their genetic diversity by use of ISSR primers. In their study, 283 bands were amplified by 25 primers in which 92.85% of the amplified bands were polymorphic making an average of 10.48 bands per primer. After cluster analysis, the accessions were grouped into three clusters belonging to different species. In another study, Roy *et al.* (2006) used ISSR and other markers to study genetic diversity of 20 exotic germplasm and 20 commercial varieties of cultivated Jute from *C. capsularis* and *C. olitorius*. Again, the results showed 98.44% polymorphism across all the species and low level of polymorphism within the species.

Sequence Tagged Microsatellite Sites (STMS) was used by Roy *et al.* (2006) to study genetic diversity in Jute mallow. These markers employ a pair of primers with sequences similar to the single copy of a sequence flanking microsatellite repeats, which upon amplification detects the variation which exist among individuals in terms of number of repeat motifs at a specific locus. In their study, all six STMS markers showed polymorphism among the four species of *Corchorus* under study and this polymorphism ranged from 50 to 100 with PIC ranging from 0.1 to 0.5.

Sequence related amplified polymorphism (SRAP) is reported by Soliman *et al*. (2014) in which three genotypes of leafy vegetable Jute mallow were evaluated for genetic diversity and phylogenetic relationships. The results from different primer combinations indicated highest percentage of polymorphism (72.7%) using Me4-Em3 combination and the lowest polymorphism (14.3%) were recorded in Me4-Em4 combination.

In efforts to quantify genetic diversity and to characterize accessions in germplasm collections, microsatellite markers and other DNA markers are useful tools when considering their advantages over other markers. They are unlimited in number and are not affected by environmental factors, growth stage and agronomic practices (Mir *et al.*, 2009). In many studies

conducted, the diversity of the genotypes at species level is very low for many accessions of Jute mallow and hence it is important to focus on the variation that exists between different species and establish how this variation can be used to improve the crop.

2.6 Conservation

Jute mallow, like other traditional leafy vegetables has been neglected by researchers, policy makers and agriculturists thus there is limited indigenous knowledge about it and reduced its biodiversity (Agong *et al.*, 2013). There is therefore a need of both *in-situ* and *ex-situ* conservation measures to ensure that the available genetic resources are not lost. In Africa, attempts to conserve the germplasm of *Corchorus spp*. in national institutional gene banks has been done in Nigeria, Ethiopia, Kenya, Sudan and Zambia (Attere, 1997). In Nigeria, National Horticultural Research Institute (NIHORT) maintains a large germplasm of a collection of local landraces that have been characterized (Opabode and Adebooye, 2005). Thirteen species of *Corchorus* are found in National Herbarium of Addis Ababa University in Ethiopia. Among the species, *C. urticifolius* is very rare, indicating high risk of genetic erosion in Ethiopia (Benor *et al.*, 2011). In Tanzania, the National Plant Genetic Resources Center (NPGRC) contains only 9 accessions of *Corchorus* of unknown species (National Report of PGR, FAO 2009), despite the high number of species reported to be found in the country by survey conducted by Edmonds (1990) and Benor *et al.* (2010). Two more accessions were collected in collaboration with other international centers and conserved at facilities of CGIAR and the world vegetable centre (AVRDC). In 2013, the World Vegetable Center in Tanzania had 2659 vegetable accessions of 48 species (Agong *et al.*, 2013). Among these accessions, 35 were accessions of Jute mallow from one species. However, currently due to active collection of important indigenous vegetables, the germplasm collection of Jute mallow contains 104 accessions of Jute mallow from different countries in the world comprised of several species. World Vegetable gene bank in Africa is an important source of vegetable germplasm for breeding, research, and any other uses for public as well as private sectors.

2.7 Conclusion

In order to improve Jute mallow as a leafy vegetable, the current germplasm should be exploited to come up with varieties with desirable horticultural traits. Based on the knowledge generated by studying the genetic diversity of present germplasm, it is also possible to lay down the strategies for its conservation. Several studies have been conducted using molecular markers to study the genetic diversity of Jute mallow. However, it is the enrichment and refinement of these markers as well as their validation that will contribute significantly to the genomic research and breeding of Jute mallow.

The presence of a vast array of germplasm in Africa and East Asia should be considered as a potential source of breeding materials for breeders. It is well documented that the currently cultivated species of Jute mallow have a very narrow genetic base. The option of reaching out to the wild species with superior traits; such as drought tolerance, tolerance to low temperatures and resistance to pests and diseases remains very important. Improved genotypes of Jute mallow will broaden the nutritional base of leaf vegetables and increase its utilization.

CHAPTER THREE

Characterization of morphological diversity of Jute mallow (*Corchorus spp*)[2]

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Abstract

Jute mallow is a traditional leafy vegetable which is important part of daily diet for the majority of people in rural areas in sub Saharan Africa. This study employed quantitative and qualitative phenotypic traits to assess the morphological diversity of 90 accessions using univariate and multivariate analyses. Field experiments were conducted for two seasons to identify accessions suitable for leaf yield. The accessions were significantly variable in all traits. The highest variability among accessions was found in harvest index, biomass yield and 1000 seeds weight. The traits that significantly correlated with biomass yield include plant height (r=0.448), petiole length (r=0.237), primary branches (r=0.319) and number of leaves per plant (r=0.333). Principal component analysis showed that the first five PCs with Eigen values ≥1 explained 72.9% of the total variability in the accessions. Pods per plant, primary branches, secondary branches, and number of leaves per plant accounted for highest variability in PC1. Cluster analysis grouped the accessions into five major clusters mainly based on their origin. Thus, the collection displayed high variation in morphological traits particularly those related to leaf yield. These accessions are therefore useful in breeding for the improvement of the crop and germplasm management.

Key words: *Corchorus spp.*, Jute mallow, Leaf yield, Morphological characterization.

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3.1 Introduction

Jute mallow (*Corchorus spp.*) is a very nutritious African traditional vegetable and has been in use in many households (Westphal-Stevels, 1985; Krebs, 2001; Kinabo *et al.*, 2006; Nyadanu and Lowor, 2015). The genus *Corchorus* is comprised of annual or short lived perennial herbs and shrubs with many agriculturally useful species (Benor *et al.*, 2010). *Corchorus olitorius* has its centre of origin as Africa and is known for its high genetic diversity and wide geographical distribution (Benor *et al.*, 2011; Adebo *et al.*, 2015; Kundu, 1951; Benor *et al.*, 2012). The vegetable is extensively grown for the sliminess of the leaves used in local dishes. It is one of the leading leafy vegetables in West Africa (Grubben *et al.*, 1977).

Crop improvement programmes on Jute mallow for selection of varieties with finer and high quality fiber have received considerable attention over the years in Asian countries such as China, Bangladesh and India (Palit *et al.*, 1996; Das and Maiti, 1998; Palve and Sinha, 2005). However, improvement programs on the Jute mallow as a vegetable particularly *C. olitorius* and other wild relatives which are used as a vegetable in Africa is limited (Denton and Nwangburuka, 2012; Nyadanu and Lowor, 2015).

Characterization of genetic diversity among accessions of different germplasms using phenotypic traits is an initial step towards crop improvement (Julia *et al.*, 2016; Loumerem and Alercia, 2016; Peratoner *et al.*, 2016). The variation in these traits can be used to classify materials into different groups. In Jute mallow, because of variations in numerous local types in traits such as plant height, stem color, pubescence, fruit and leaf shape and leaf production Westphal-Stevels, (1985) proposed a classification of Cameroon materials and other West African countries into three cultivar groups that is *olitorius*, *incisifolius* and *Geant de Bertoua*. The main distinguishing feature for these 3 groups being leaf shape.

Morphological studies on several accessions have been done in Nigeria to separate segregating populations into different leaf types (National Horticultural Research Institute, 1986). Nwangburuka and Denton (2012) reported significant differences among 15 genotypes in terms of morphological characters such as plant height at maturity, number of leaves per plant, fresh leaf weight, stem weight, total plant weight and harvest index. Osawaru *et al.* (2012) used

morphological characters and presence of soluble proteins to determine the relationship among three *Corchorus* species. Traits such as growth habit and stem features appeared to be uniform except for stem color. They found that determinate growth habit, medium plant branching and erect stems among the accessions studied as important traits for production of leaves, which are edible part and needed by farmers. Nyadanu *et al.* (2016) reported high variability in morphological traits in local cultivars of Ghana. The cultivars were grouped based on their geographical origin and traits such as number of leaves per plant, leaf length and number of branches per plant were most informative traits.

Few studies on genetics of leaf yield and its components have been conducted on Jute mallow; however, only few accessions have been used (Palit *et al.*, 1996; Denton and Nwangburuka, 2012; Osawaru *et al.*, 2012). Benor *et al.* (2012) assessed patterns of morphological variation in Jute mallow of Ethiopian origin and compared them with those from other parts of the world. In their study, samples were collected from Ethiopia and compared with the samples from Asia and Africa. They found high genetic diversity among the African accessions and high similarity between North Africa accessions and Asian accessions. Accessions from West and Southern Africa and some samples from East Africa were not included in this study. These accessions which may be highly diverse remain unexploited for breeding and improvement of Jute mallow.

The exploitation of existing diversity of characters of each individual accession forms a major principle in plant improvement. Thus in order to improve leaf yield of Jute mallow, the knowledge required is not only of the diversity and genetic variability of the available germplasm but also of genetic architecture of leaf yield and its components.

In view of the above, our study is focused on vegetative, inflorescence, fruit and seed traits and their contribution towards divergence in these accessions as an important step in identifying those which have high yield potential and good adaptation to specific environment.

3.2 Materials and Methods

3.2.1 Study location

The experimental field was located at Madiira farm at an altitude of 1262m above sea level, latitude 03.38'S and longitude 36.87^{o}E. The soils are well drained. The monthly total rainfall and monthly average temperature during the growing season are presented in Fig. 1.

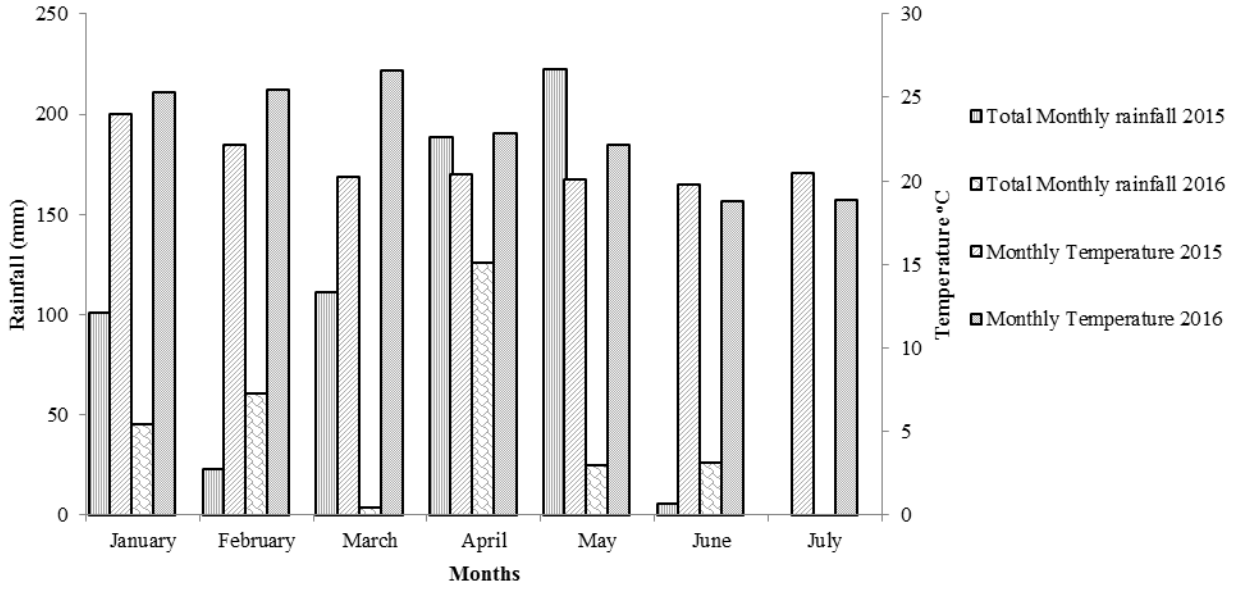


Figure 1: Meteorological data on monthly average temperature and total monthly rainfall during the two growing seasons.

3.2.2 Experimental Design and Layout

The experiments had two phases, first by sowing the accessions in the screen house then followed by transplanting of seedling to the field. In the screen house the seeds were sown in plastic trays (with 66 holes; diameter of 4cm and depth of 4cm) by using sterilized soil. After 28 days, the seedlings were transplanted to the field. Each plot size was 3m x 0.6m with three replications. Each replication contained 90 plots. The plots were arranged in a randomized

complete block design (RCBD). Each accession was planted in two rows, and the space between plants was 0.25m with 12 plants per row.

Fertilizer application was done one week after transplanting where Diammonium phosphate (DAP) and Urea fertilizers were applied at a rate of 143.8kgP/ha and 55.2kgN/ha, respectively. The experiments were conducted during the rain season. However, the plants were only supplemented with irrigation when necessary. Manual weeding was carried when it was necessary to maintain weed free plots.

3.2.3 The experimental materials

Comprised of 90 accessions of Jute mallow (*Corchorus spp.*) provided by the seed repository of the World Vegetable Center, Eastern and Southern Africa (Table 1). The experiments were carried out for 2 growing seasons in 2015 and 2016 (from February to June). Jute mallow collection represent one of the different types of traditional vegetables collected from farmers' field and preserved *ex-situ* for breeding, other research activities and farmers' purposes.

Table 1. The list of Jute mallow accessions (*Corchorus spp*.) used in this study

SN	Accession Name	Country of Origin	SN	Accession Name	Country of Origin
1	HS	Tanzania	42	UG	Uganda
2	BAFIA	Cameroon	43	IP-4	Kenya
3	EX-CHAMALAWI	Malawi	44	GKK 10	Malawi
4	IP 5	Kenya	45	CAMEROON MULA	Cameroon
5	IP10	Kenya	46	SUD-2	Sudan
6	UG-JM-1	Uganda	47	TOT 4316	Bangladesh
7	SUD-1	Sudan	48	TOT 4097	Tanzania
8	TOT 4879	USA	49	LOCAL BIG LEAVES	Mali
9	ML-JM-1	Malawi	50	GKK 25	Malawi
10	TOT 5877	Japan	51	ML-JM-12	Malawi
11	ES	Tanzania	52	CAMEROON EX. CO	Cameroon
12	ML-JM-14	Malawi	53	TOT4669	Bangladesh
13	SUD-4	Sudan	54	TOT 4051	Vietnam
14	CAMEROON	Cameroon	55	TOT 4312	Bangladesh
15	EX-ZIMBABWE	Zimbabwe	56	TOT 4352	Bangladesh
16	TOT 4064	Vietnam	57	ML-JM-7	Malawi
17	TOT 0124	Malaysia	58	TOT 4519	Bangladesh
18	IP-2	Kenya	59	TOT 6280	Vietnam
19	AZIGA	Cameroon	60	TOT 4800	Vietnam
20	ML-JM-5	Malawi	61	TOT 8975	Taiwan
21	TOT 6426	Kenya	62	TOT 4708	Bangladesh
22	TOT 5876	Japan	63	TOT 4670	Bangladesh
23	UG-JM-2	Uganda	64	TOT 7974	Bangladesh
24	TOT 6278	Vietnam	65	TOT 4589	Bangladesh
25	SUD-3	Sudan	66	TOT 4713	Bangladesh
26	UG-JM-13	Uganda	67	TOT 3499	Vietnam
27	TOT 4157	Vietnam	68	TOT 4067	Vietnam
28	MIX	Tanzania	69	TOT 4631	Bangladesh
29	TOT 4885	Japan	70	TOT 4500	Bangladesh
30	TOT 5999	Taiwan	71	TOT 8532	Unknown
31	TOT 4429	Bangladesh	72	TOT 6667	Philippines
32	ALV MN 059	Unknown	73	ML-JM-9	Malawi
33	KIPUMBULIKO	Unknown	74	TOT 6730	Unknown
34	ML-JM-2	Malawi	75	TOT 6425	Uganda
35	TOT 4712	Bangladesh	76	TOT 7980	Bangladesh
36	IP 1	Kenya	77	TOT 4413	Bangladesh
37	TOT 4721	Bangladesh	78	TZA 3070	Tanzania
38	TOT 7977	Bangladesh	79	TOT 9736	Unknown
39	ML-JM-4	Malawi	80	TOT 4624	Bangladesh
40	TOT 4541	Bangladesh	81	TOT 6749	Unknown
41	ML-JM-13	Malawi	82	TOT 4701	Bangladesh
83	TOT 4623	Bangladesh	87	TOT 6430	Cameroon
84	TOT 7865	Unknown	88	IP 13	Kenya
85	TOT 7866	Unknown	89	TOT 6427	Kenya
86	TZA 3002	Tanzania	90	TOT 3584	Unknown

3.2.4 Morphological study

Thirty three quantitative and qualitative morphological traits were used to estimate the level of variation among the accessions from seedling stage to seed characterization (Table 2). During vegetative growth, measurements of leaves and stem were recorded following Jute mallow descriptor list of World Vegetable center. Plant height, flower diameter, pedicel length together with plant canopy was measured at flowering. Fifty percent flowering was recorded for each accession. During maturity stage, data on first mature pod, fruit length, fruit shape and color were recorded. The number of seeds per pod and weight of 1000 seeds were also measured (Table 2).

Table 2. Qualitative and Quantitative characters used in morphological study

S/N	Character	Description
1.	Plant height (PH)	Height of the plant measured at ground surface at 50% flowering (cm)
2.	Leaf length (LL)	Leaf blade length excluding petiole length (cm)
3.	Leaf width (LW)	Mature leaf width measured at widest point (cm)
4.	Leaf length-Width ratio (LWR)	The ratio of leaf length to leaf width
5.	Petiole length (PL)	Length of leaf stalk (cm)
6.	Days to 50% flowering (50 FLR)	Number of days from sowing to 50% flowering
7.	Number of primary branches (PB)	Number of branches from main stem
8.	Number of secondary branches (SB)	Number of branches from the secondary stem
9.	Plant canopy (PC)	Plant width taken at widest point (cm)
10.	Flower diameter (FD)	The width of an open flower (mm)
11.	Pedicel length (PEDL)	The stalk of the flower (mm)
12.	Fruit length (FL)	Length of mature fruit excluding the pedicel
13.	Days to first mat. Pods (DMP)	Number of days from sowing to first mature pod
14.	Harvest index (HI)	Ratio of total harvestable leaves to total weight of the plant
15.	Number of leaves (NL)	Counted from individual plant during flowering
16.	Biomass yield (BY)	Total weight of the plant above the ground surface (gm)
17.	Number of pods/plant (NPP)	Counted from individual plant at maturity stage
18.	Weight of 1000 seeds (W1000S)	Measured in weighing balance after counting
19.	Seeds per pod (SP)	Counted from individual pod
20.	Cotyledon color (CC)	(3) Light green, (5) Green, (7) Purplish green
21.	Stem color (ST)	(3) Brown, (5) Green, (7) Red-Purple
22.	Leaf color (LC)	(1) Light green, (3) Green, (5) Dark green, (7) Purple, (9) Dark-purple
23.	Leaf lobe (LL)	(0)Absent, (1) Present
24.	Setae (S)	(1) Small, (2) Large
25.	Leaf shape (LS)	(1) Ovate, (3) Elliptical, (5) Cordate, (7) Palmate
26.	Leaf base (LB)	(1) Rounded, (3) Sagitate, (5) Acute
27.	Leaf apex (LA)	(1) Acuminate, (3) Caudate, (5) Acute, (7) Palmate
28	Leaf margin (LM)	(1) Coarsely serrate, (3) Cleft, (5) Double serrate, (7) Finely serrate, (9) Crenate
29.	Stem pattern (SP)	(1) Erect, (2) Semi erect
30.	Stipule color (SC)	(1) Green, (3) Green Stipule with dark red base, (5) Light purple
31.	Petiole color (PETC)	(1) Green, (3) Green with dark red base, (5) Purple
32.	Fruit shape (FS)	(3) Globule, (5) Long pod, (7) Round pod
33.	Fruit color (FRC)	(3) Pale Brown, (5) Brown, (7) Brown

3.3 Data analyses

For quantitative data, descriptive statistics that is mean, maximum, minimum and standard error were calculated followed by analysis of variance (ANOVA) to test the morphological variation among the 19 characters that were measured.

Model and Analysis of (CRDB) design was as follows;-

$Y_{hi} = \mu + \theta h + ri + \varepsilon hi$

Where:

Y_{hi}= is the random variable representing response of accession *i* observed in block *h*
μ = Overall mean
θh = Block Effect
ri = Treatment effect (Accession)
εhi = Random error for i^{th} accession in h^{th} block.

The selected quantitative characters were also plotted on box and whisker plots (Tukey, 1977) to reveal the dispersion of data set in different populations. Frequency distribution table was used to describe the qualitative traits. Simple correlations were used to compare the degree of association between leaf yield and other traits. Multivariate statistics were used to reveal the phenotypic diversity of the quantitative traits. The quantitative data were standardized before they were used as an input for PCA and cluster analysis. PCAs with Eigen values ≥ 1 were used to define the quantitative traits within the accessions. In order to group the accessions such that patterns of similarity and dissimilarity would be revealed, the traits data matrix was subjected to cluster analysis using complete linkage method. Descriptive statistics and ANOVA were computed with STATISTICA software version 12, edition 2013 (Statsoftinc., Tulsa, OK, USA). Multivariate analyses were performed with SAS software.

3.4 Results

3.4.1 Variation in qualitative characters

Fourteen qualitative characters of this study are summarized in a frequency distribution Table 3. At seedling stage, three types of cotyledon colors were observed in the accessions in which 67% were light green, 30.9% green, and 2.1% were purple green. During vegetative growth, green stem color was predominant among the accessions (52%) while brown and red-purple color was shared among the remaining accessions (24% each). Most accessions were characterized with light green and green leaf color. The presence of leaf lobe was only observed in 8.5% accessions. These accessions were also characterized with palmate leaf shape and cleft leaf margins. The remaining accessions had no leaf lobes. For leaf shape, ovate and cordate leaf shapes were observed in 38.3% and 35.1%, respectively. Leaf margins and stem pattern displayed little variation that is 62.8% accessions were coarsely serrate and 87.2% had erect stems. At maturity stage, among all the accessions, 62.8% possessed long pods while 9.5% had globule fruits. The remaining accessions had round pods. Three types of fruit color were also observed; pale brown (67%), brown (11.7%) and dark brown (21.3%). The accessions were also highly variable in terms of stipule color and petiole color.

Table 3. Frequency distribution table of qualitative morphological traits

S/N		Plant Traits	Percentage
1.	Cotyledon color	Light green	67
		Green	30.90
		Purplish green	2.10
2.	Stem color	Brown	24
		Green	52
		Red-Purple	24
3.	Leaf color	Light green	38.30
		Green	45.70
		Dark green	5.30
		Purple	8.50
		Dark Purple	1.10
4.	Leaf lobe	Absent	91.50
		Present	8.50
5.	Setae	Small	72.30
		Large	27.70
6.	Leaf shape	Ovate	38.30
		Elliptical	16
		Cordate	35.10
		Palmate	8.50
7.	Leaf base	Round	87.20
		Sagitate	6.40
		Acute	6.40
8.	Leaf apex	Acuminate	33
		Caudate	10.60
		Acute	46.80
		Palmate	7.40
9.	Leaf margin	Coarsely Serrate	62.80
		Cleft	9.60
		Double Serrate	13.80
		Finely Serrate	10.60
		Crenate	3.20
10.	Stem pattern	Erect	87.20
		Semi erect	12.80
11.	Stipule color	Green	46.80
		Green Stipule with dark red base	26.60
		Light purple	25.50
12.	Petiole color	Light green	43.60
		Dark green	28.70
		Light purple	21.30
		Purple	5.30
13.	Fruit shape	Globule	9.50
		Long pod	62.80
		Round	27.70
14.	Fruit color	Pale Brown	67
		Brown	11.70
		Dark Brown	21.30

3.4.2 Variation in quantitative characters

Significant ($p<0.001$) variation was revealed for vegetative, floral and seed traits. Harvest index, biomass yield and weight of 1000 seeds showed highest variation (Table 4). There was also a wide range of values in traits such as weight of 1000 seeds, number of pods per plant, seeds per pod and number of secondary and primary branches. Number of days to 50% flowering varied significantly ranging from 52 days to 110 days, this grouped the accessions into three categories which were early, mid and late flowering accessions. Days to first mature pod also corresponded to 50% flowering which ranged from 73 days to 135 days, thus there was late and earlier maturing accessions in terms of seed yield. Similarly, plant canopy exhibited a wide range resulting from the growth habit of the accessions; some had bushy canopy with many branches while others had small canopy with few primary and secondary branches. The wide range observed in fruit length from 0.5cm to 18.2cm is attributed to differences in fruit shapes; the accessions with round pods which belong to *C. capsularis* species had very small round fruits as compared to *C.olitorius* species. Other traits such as petiole length, leaf length and width, flower diameter and pedicel length though had a fairly narrow range, they differed significantly ($p<0.01$) among the accessions.

Box and whisker plots are displayed in Fig. 2 for selected quantitative traits. The accessions were divided into seven subpopulations based on their origin. Populations A, B, C and E were from Africa whereas population F and G were from Asia. All accessions were morphologically very diverse. In terms of plant height, the highest inter quartile ranges were observed in a group of accessions originating from South East Asia.

All accessions from Africa had almost the same average plant height irrespective of which part of the continent they originated. West and North African accessions had high interquartile ranges for both leaf length and leaf width.

Table 4. The results of descriptive statistics for quantitative characters

S/N	Trait	Mean ± S.E	Max	Min
1.	Plant height (cm)	78.08 ± 0.96**	137.50	52
2.	Leaf length (cm)	11.63 ± 0.15**	20.97	6.50
3.	Leaf width (cm)	5.41 ± 0.07**	10.05	2.73
4.	Leaf length-Width ratio	2.22 ± 0.08**	3.16	1.12
5.	Petiole length (cm)	4.85 ± 0.08**	10.22	1.99
6.	Days to 50% flowering	64.93 ± 0.54**	110	52
7.	Number of primary branches	12.16 ± 0.19**	21.60	1.00
8.	Number of secondary branches	15.22 ± 0.92**	79.80	0
9.	Plant canopy (cm)	71.88 ± 1.18**	125.10	10
10.	Flower diameter (mm)	11.22 ± 0.13**	15.20	5.52
11.	Pedicel length (mm)	2.28 ± 0.03**	4.22	1.14
12.	Fruit length	5.05 ± 0.13**	18.20	0.50
13.	Days to first mat. pods	107.6 ± 0.58**	135	72.50
14.	Harvest index	24.08 ± 0.65***	61.35	0.90
15.	Number of leaves	288.82±11.41**	1329	15
16.	Biomass yield (g)	332.40±7.62***	1001	43
17.	Number of pods/plant	111.96±6.48**	660	0
18.	Weight of 1000 seeds (g)	1.48 ± 0.04***	4.50	1
19.	Seeds per pod	136.42 ± 3.69**	275	8

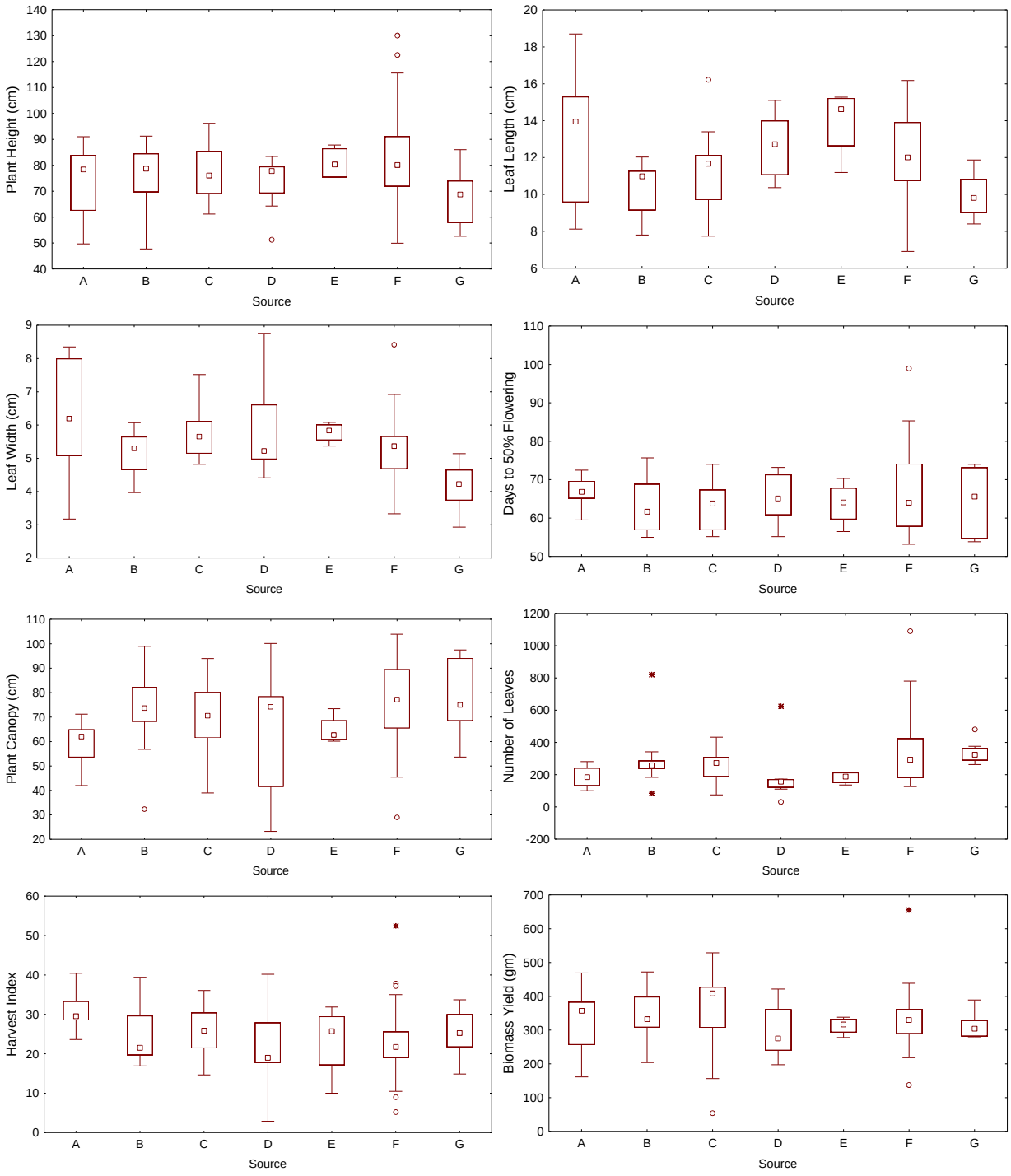
** Significant at $p<0.01$, ***Significant at $p<0.001$

South East Asian accessions had highest inter quartile ranges for number of leaves per plant as compared with the rest of other populations. Fifty percent flowering did not vary much across all populations. Harvest index and plant canopy traits indicated high variability among these

accessions which is reflected in leaf yield. The lowest interquartile ranges were observed in unknown accessions. The highest plant canopy was observed in accessions from East Africa.

3.4.3 Simple correlations

Biomass yield was statistically significantly (p=0.05) and positively correlated with plant height (r=0.448), petiole length (r=0.237), primary branches (r=0.319), days to first mature pods (r=0.306) and number of leaves per plant (r=0.333) (Table 5). Harvest index was positively and significantly correlated to leaf width (r=0.355), petiole length (r=0.310) and 50% flowering (r=0.270). It was also negatively correlated with secondary branches (r=0.350). Positive and significant correlation was also observed between number of leaves per plant and plant height (r=0.505), petiole length (r=0.261), fifty percent flowering (r=0.378), primary and secondary branches (r=0.577 and 0.559), pods per plant (r=0.582) and number of days to first mature pods (r=0.433). On the other hand, seed yield components such as number of pods per plant was positively and significantly correlated with plant height (r=0.357), fifty percent flowering (r=0.307), primary and secondary branches (r=0.620 and 0.680). Fruit length was positively correlated to plant canopy (r=0.378) and flower diameter (r=0.561). It was negatively correlated to fifty percent flowering and primary and secondary branches. Number of seeds per pod was positively correlated with leaf width (r=0.307), plant canopy (r=0.271) flower diameter (r=0.694) and fruit length (r=0.658).



Key**:** A- from West Africa, B- from East Africa, C- from South Africa, D-Unknown, E- from North Africa, F- South East Asia, G- East Asia

Figure 2: Box and whisker plots showing the variation in quantitative traits of the accessions from different parts

Table 5. Simple correlation matrix for the 19 quantitative traits of *Corchorus spp*.

	PH	LL	LW	LWR	PL	50 FLR	PB	SB	PC	FD	PED L	FL	NPP	DMP	NL	SP	HI	W 1000S	BY
PH																			
LL	0.10																		
LW	-0.01	0.50*																	
LWR	0.14	0.46*	-0.52*																
PL	0.35*	-0.07	0.38*	-0.42*															
50FLR	0.38*	-0.06	0.07	-0.09	0.29*														
PB	0.47*	-0.23*	-0.23*	0.11	0.25*	0.38*													
SB	0.28*	-0.32*	-0.57*	0.33*	-0.22*	0.13	0.48*												
PC	-0.03	-0.19	-0.24*	0.05	-0.15	-0.58*	0.02	0.18											
FD	-0.06	0.27*	0.48*	-0.26*	0.31*	-0.24*	-0.24*	-0.61*	0.10										
PEDL	0.29*	0.23*	0.40*	-0.14	0.30*	0.03	0.20	-0.06	-0.18	0.27*									
FL	-0.02	0.01	0.13	-0.18	-0.04	-0.42*	-0.23*	-0.44*	0.38*	0.56*	0.16								
NPP	0.38*	-0.19	-0.45*	0.34*	-0.01	0.31*	0.62*	0.68*	0.01	-0.66*	-0.12	-0.57*							
DMP	0.44*	-0.01	0.23*	-0.20	0.53*	0.65*	0.47*	0.15	-0.51*	-0.09	0.40*	-0.39*	0.32*						
NL	0.52*	-0.23*	-0.18	0.03	0.26*	0.38*	0.58*	0.56*	0.06	-0.44*	0.12	-0.32*	0.58*	0.43*					
SP	-0.27*	0.15	0.31*	-0.23*	0.09	-0.45*	-0.29*	-0.45*	0.27*	0.69*	0.16	0.66*	-0.66*	-0.31*	-0.42*				
HI	-0.22*	-0.05	0.36*	-0.36*	0.31*	0.27*	0.04	-0.35*	-0.11	0.21	-0.05	-0.15	-0.15	0.14	-0.05	0.06			
W1000S	-0.19	-0.18	-0.19	0.01	-0.03	-0.26*	-0.07	-0.03	0.29*	0.14	0.00	0.17	-0.12	-0.21	-0.09	0.13	0.04		
BY	0.45*	-0.11	0.11	-0.21*	0.24*	0.01	0.32*	0.14	0.16	0.07	0.17	0.14	0.10	0.31*	0.33*	-0.02	-0.16	-0.15	

***Significant at p<0.05**

Key: PH-Plant height, LL-Leaf length, LW-Leaf width, LWR-Leaf width ratio, PL-Petiole length, 50FLR- Fifty percent flowering, PB-Primary branches, SB-Secondary branches, PC-Plant canopy, FD-Flower diameter, PEDL-Pedicel length, FL-Fruit length, NPP-Number of pods per plant, DMP-Days to first mature pods, NL-Number of leaves per plant, SP-Number of seeds per pod, HI-Harvest index, W1000S-Weight of 1000 seeds, BY-Biomass yield.

3.4.4 Principal component analysis

Principal component analysis (PCA) of the 19 quantitative traits is summarized in Table 6. The first five PCAs had Eigen values greater than one (data not shown) and they explained 72.97% of the total variation for the morphological traits of the accessions under study. Number of primary branches, secondary branches, pods per plant and number of leaves per plant loaded highly in PC1 and they accounted for 27.33% of the total variation of the studied samples.

Table 6. Eigen values, proportion of variance and morphological traits that contributed to the first five PCs

Traits	PC 1	PC 2	PC 3	PC 4	PC 5
Plant Height	0.22	0.19	0.28	0.28	-0.02
Leaf Length	-0.11	0.09	-0.19	**0.60**	0.16
Leaf Width	-0.19	**0.38**	-0.09	0.09	-0.10
LW ratio	0.12	-0.28	-0.08	**0.48**	0.31
Petiole Length	0.03	**0.39**	0.13	-0.17	0.17
Fifty percent flowering	0.24	0.27	-0.24	-0.07	0.01
Primary Branches	**0.30**	0.11	0.24	-0.03	0.23
Secondary Branches	**0.33**	-0.19	0.15	0.01	0.00
Plant Canopy	-0.08	-0.25	**0.44**	-0.11	0.06
Flower diameter	-0.32	0.21	0.16	0.07	0.17
Pedicel length	-0.02	0.29	0.18	0.26	0.26
Fruit Length	-0.29	-0.00	**0.36**	0.06	-0.07
Pods per plant	**0.38**	-0.09	0.02	0.01	0.09
Days to first mature pods	0.23	**0.38**	-0.04	0.02	0.06
No of leaves	**0.33**	0.09	0.22	-0.06	0.03
Seeds per Pod	-0.34	0.05	0.22	0.02	0.05
Harvest Index	-0.07	0.22	-0.22	-0.39	0.25
Weight of 1000 seeds	-0.09	-0.14	0.15	-0.22	**0.66**
Biomass yield	0.09	0.17	**0.41**	0.05	-0.40
Eigen values	5.19	3.54	2.24	1.81	1.08
Proportion variance (%)	27.33	18.65	11.81	9.51	5.66
Cumulative variance (%)	27.33	45.98	57.79	67.30	72.97

PC2 accounted for 18.65% of the total morphological variation among the accessions. Leaf width, petiole length and days to first mature pods loaded more in PC2. PC3 accounted for 11.81% of the total variation. Traits such as plant canopy, fruit length and biomass yield loaded more in PC3. PC4 contributed 9.51% of the total morphological variation in these accessions with only leaf traits that is leaf length and leaf length-width ratio loading highly. PC5 accounted for 5.66% of total variation with seed traits that is weight of 1000 seeds loading highly. Generally, for the 19 morphological traits studied, PC1 and PC2 constituted 45.9% of the total morphological variation with most vegetative traits and seeds related traits. This indicated that these traits can be used to classify the materials under this study.

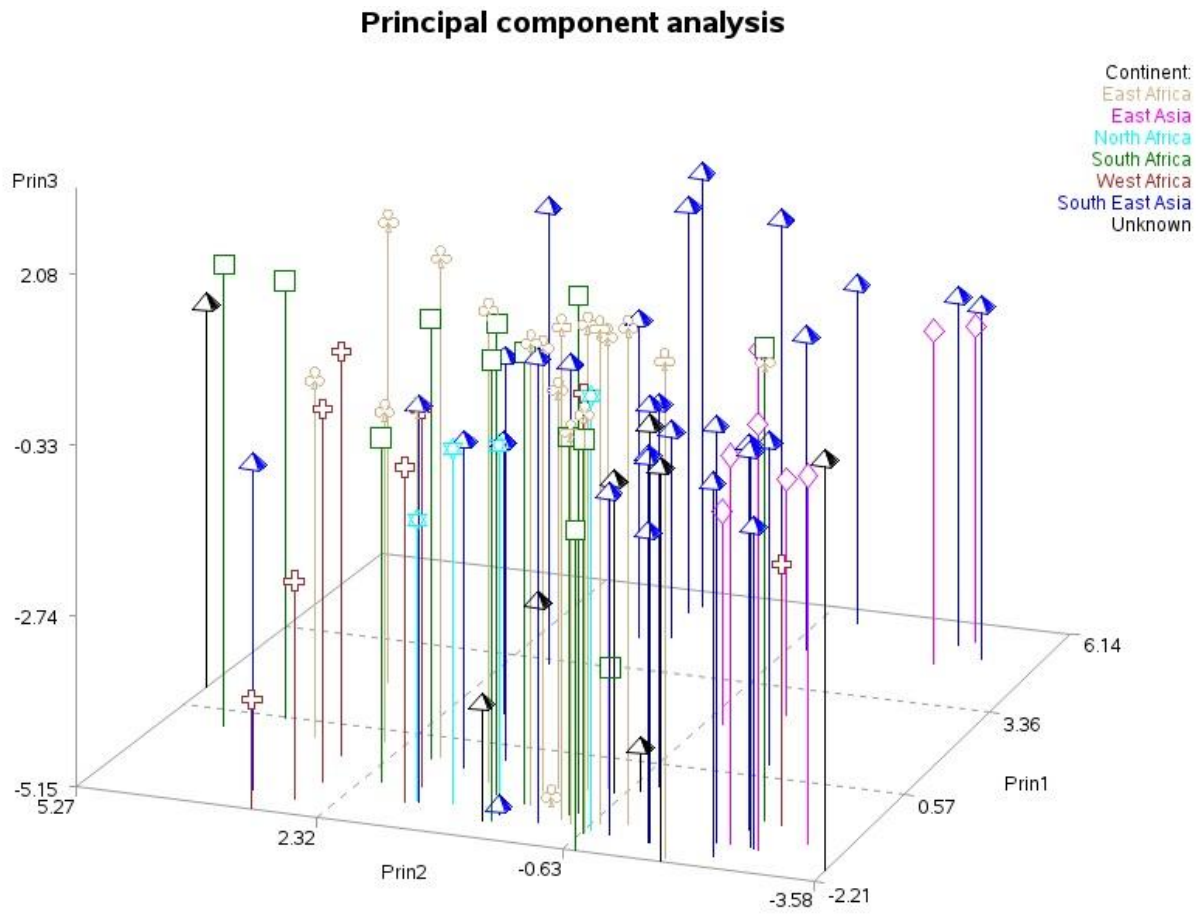


Figure 3: Scatter plot of *Corchorus spp*. accessions based on quantitative data. Different symbols and colors have been used to represent accessions from different parts.

Three dimensional principal component showing relationships among quantitative traits of studied accessions are presented in Fig. 3. Accessions from West Africa, East Africa and North Africa had higher values for PC2, traits related to leaves and days to first mature pods. Accessions from Asia had higher values for PC1, those related to branching habit, pods per plant and number of leaves per plant. These accessions were most dispersed and diverse as compared to other accessions.

3.4.5 Cluster Analysis

Dendrogram for complete linkage cluster analysis of quantitative and qualitative traits is presented in Fig. 4. The results indicated that the accessions were grouped into five major

clusters. Cluster 1 contained 7 accessions from Bangladesh and Vietnam. These are characterized by stems and leave which are purple in color as well as have round pods. Also these accessions had high numbers of branches (Table 7). Cluster 2 contained 7 accessions from Bangladesh and Vietnam with addition of two accessions from Malaysia and one from Taiwan. These were characterized by both green stems and purple stems and green leaves. Cluster 2 had highest plant height and belongs to *C. capsularis* species except the three accessions on top of this cluster which had long pods and more branches. Cluster 3 contained 12 accessions of which 7 came from Bangladesh and Japan. Four accessions are from unknown origin and 1 from Kenya. These were classified with semi erect stems and few pods and high plant canopy compared with other clusters. All had long pods and belong to species *C. olitorius*. Cluster 4 was the largest one with 33 accessions from almost all countries. These had both erect and semi erect stem pattern. Cluster 5 contained 29 accessions, 9 from East and Southern Africa, and 8 accessions from Asia, 4 from unknown, 2 from North Africa and 6 from West Africa. The accessions (8) in the upper cluster are characterized with palmate leaves with cleft margins. The other 10 accessions are characterized with big leaves. In this cluster, accessions such as Bafia and Aziga had globule pods as compared to other accessions which had long and round pods. These had erect stems and are late flowering. Generally, cluster 1 and 2 contained accessions from Asia while cluster 5 contained accessions from Africa. This grouping pattern indicates their distinct centers of origin and ecological adaptation. Clusters 3 and 4 contained mixed accessions from different countries.

Table 7. The mean traits values for clusters I-V

	CLUSTER				
Traits	**C1**	**C2**	**C3**	**C4**	**C5**
Plant Height (cm)	83.51	**98.50**	63.88	76.43	78.38
Leaf Length (cm)	10.38	11.36	10.60	11.24	**12.85**
Leaf Width (cm)	3.94	4.63	4.96	5.16	**6.43**
LW ratio	**2.70**	2.48	2.18	2.22	2.04
Petiole Length (cm)	4.70	4.36	3.45	4.74	**5.75**
50% Flowering (days)	72.79	**75.28**	62.21	**59.73**	67.20
Primary Branches	**16.81**	14.06	9.22	11.95	11.92
Secondary Branches	**37.18**	33.26	10.10	14.28	7.03
Plant Canopy (cm)	73.10	57.98	73.59	**79.77**	65.30
Flower Diameter (mm)	7.49	8.28	10.29	11.96	**12.63**
Pedicel Length (mm)	1.85	**2.48**	1.93	2.32	2.44
Fruit Length (cm)	2.13	4.01	5.32	**5.97**	4.95
Pods per plant	**351.38**	222.41	74.60	77.70	74.29
Day to first Mat. Pod	112.48	**116.48**	**99.44**	103.69	111.65
Number leaves	502.85	**505.12**	182.66	245.00	264.49
Seeds per Pod	29.88	50.69	134.95	**166.32**	155.23
Harvest Index	**28.47**	15.65	23.85	22.94	28.33
Weight of 1000 seeds (g)	1.55	1.06	1.38	**1.62**	1.49
Biomass yield (g)	344.29	**377.27**	250.29	347.56	332.24

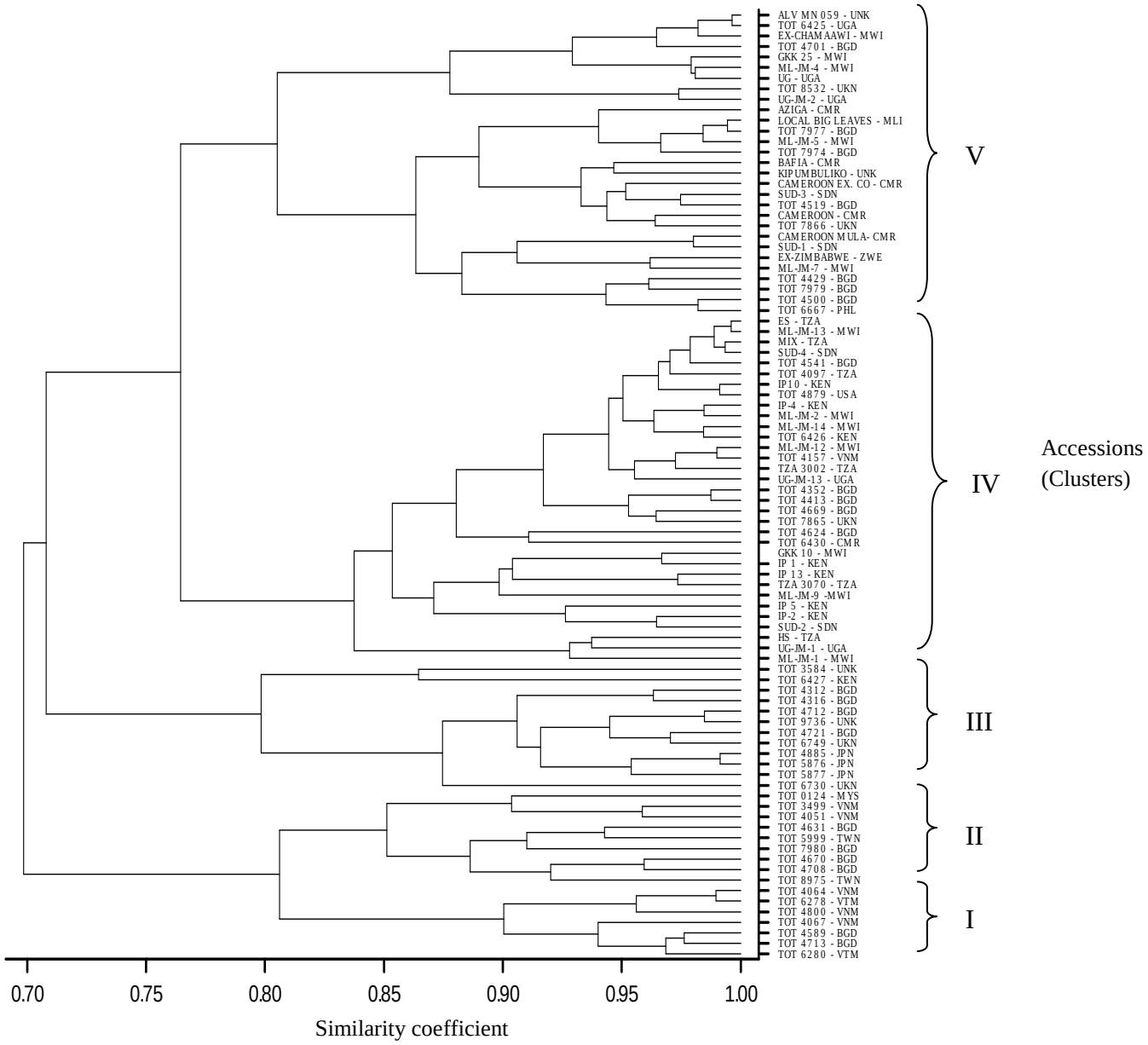


Figure 4: Dendrogram of 90 accessions of *Corchorus spp*. explained by complete linkage clustering of 19 quantitative traits and 14 qualitative traits

Key: The source of accessions is indicated by the country code of respective accession and UKN is unknown accession.

3.5 Discussion

Germplasm collections from underutilized vegetables such as Jute mallow can be an important step in breeding for improvement of this valuable crop (Nelson, 2011; Andini *et al.,* 2013). Accessions, particularly those from East and Southern Africa remain unexploited. The accessions under this study are morphologically very diverse according to the multivariate analysis of both quantitative and qualitative traits. The evaluation of vegetative, floral and seed traits showed high variation among the accessions. The accessions demonstrated high variation in harvest index, biomass yield and weight of 1000 seeds. These are important aspects to consider during selection of accessions with high leaf yield. Denton and Nwangburuka (2012) and Adebo *et al.* (2015) reported that the number of leaves per plant, plant height at maturity and harvest index as discriminating traits between *C. olitorius* accessions. In breeding for leafy vegetable, these materials can be used as parental stock. Variation in primary branches, secondary branches and pods per plant are important informative traits in differentiating accessions particularly those desirable for leaf production. Accessions from East Africa had thick plant canopy which is as a result of high branching and large leaves. These traits are attributes of leaf yield. Similar results are reported by Benor *et al.* (2012) and Shukla *et al.* (2006) in Jute mallow and amaranth, respectively. Accessions from East Asia had lowest interquartile ranges for leaf length, leaf width and number of leaves. This indicates these accessions are more desirable for fiber production than leaf production.

The analysis of simple correlations among the traits revealed that plants with high plant height, petiole length and large number of primary branches had high biomass yield as well as fruits. Positive correlations recorded among the accessions suggest that these traits can be used as selection criteria for accessions with high leaf yield. Plant height was also positively and significantly related to number of days to 50% flowering. Similar to our study Saha and Harza (2008) reported significant correlation between days to 50% flowering and plant height in lentil. These traits can be transferred to desired accessions through breeding due to high heritability. Positive correlation between harvest index and 50% flowering is a challenge for leaf harvesting of these accessions. The most limiting and significant factor to leaf production and yield is early and prolific flowering and berry production (Agong *et al.*, 2013).

Traits such as number of primary branches, pods per plant and number of leaves per plant account for the variation recorded in the accessions in PC1. Similar to our study, Nyadanu *et al.* (2016) reported that leaf length, number of branches per plant and leaves per plant were the traits that defined PC1 in their study. In contrast, leaf width, days to first mature pods and petiole length accounted for the variation observed in the accessions in PC2. Total cumulative variance was 45.98% indicating the high degree of diversity among the traits under study. Furthermore, the traits can be used as phenotypic markers in differentiating the accessions.

Cluster analysis grouped the accessions into five clusters. Cluster one and two contained accessions from Asia only. These accessions are from *C. capsularis* species. Few numbers of branches, high plant height and low number of leaves characterized these accessions Figure 2. This is attributed by unique selection pressure that aimed to improve fiber yield. Palit *et al*. (1996) reported greater homogeneity in accessions from Asia that was due to uniformity in selection pressure from commercial considerations. It is interesting that one accession from East Africa clustered with accessions from Asia. This pattern of clustering indicates the diversity of population within these geographical areas and on the other hand similarity of accessions from different geographical areas. These results agree with the report of Alemayehu *et al*. (2002) in *Brassica carinata*. This is due to some level of similarity in other quantitative traits though the accession belongs to *C. olitorius* species. Cluster four contained mixed accessions from all countries under study. Twenty five out of thirty three accessions were from Africa, the remaining accessions from Asia and USA indicate frequent exchange of germplasm across the countries (Edmonds, 1990). Similarly cluster five contained most accessions from Africa, a clustering pattern that indicate the distinct nature of the accessions. Most of these accessions were from East and Southern Africa. Some materials with uncertain origin appeared in cluster three, four and five. However, the origins of the accessions can be traced through their clustering pattern. Generally, the clustering of all accessions in our study indicate significant difference in diversity among their origins as most of Asian accessions are clustered at lower part of dendrogram that is cluster one, two and three. Accessions from Africa are mostly found in the upper part of the dendrogram that is cluster four and five.

3.6 Conclusion

Morphological diversity studies in leafy vegetable Jute mallow (*Corchorus spp*.) were carried out in 90 accessions. The results from ANOVA, simple correlations and multivariate analyses indicated high variation among the study materials. Significant and positive correlation between biomass yield and other leaf yield-related attributes indicate the potential of using these accessions for improvement of foliage yield. Traits such as plant height, primary branches and number of leaves per plant proved to be superior in contributing to biomass yield. Based on biomass yield and harvest index traits, accessions Aziga, TOT 8532, UG-JM-13, UG-JM-2, GKK 25, TOT 7980, ML-JM-1, UG-JM-1, Local big leaves and Ex-chamalawi, had highest yields and could be used as a potential parental lines for improvement of leaf yield in Jute mallow. On the other hand, principal component analysis showed that the variations observed in the accessions are mainly caused by traits such as number of leaves per plant, petiole length, leaf width, days to first mature pods and branching habit, indicating that their contribution was important in discriminating the accessions. Cluster analysis grouped the accessions based on their origin into five clusters that showed high diversity for most of the traits, demonstrating the homogeneity of accessions from specific location, except for cluster four. This clustering pattern can be exploited for identification of highly diverse accessions falling in different clusters for future genetic improvement of this crop. Direct selection can also be made for the accessions with high harvest index and biomass yield based on the recorded performance of these accessions during the field experiments. These results can further be confirmed by molecular studies as it has been confirmed by other studies using different materials. In our study these accessions showed another allele diversity that has remained unexploited for improvement of this vegetable. Therefore, plant breeders can use these germplasms for improvement of Jute mallow.

CHAPTER FOUR

Genetic Diversity of vegetable Jute mallow (*Corchorus spp*.) accessions using SSR markers[3]

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Abstract

Jute mallow is an important indigenous leafy vegetable used in many households in rural and urban areas. Little is known about the genetic diversity of the accessions from Africa. Moreover, all the accessions are not thoroughly characterized in terms of leaf yield traits and no information regarding their genetic diversity and phylogenetic relationship. The purpose of this study was to analyze the genetic diversity of Jute mallow accessions by using SSR markers. The study comprised of 83 accessions that are locally grown by farmers mainly from Africa and Asia. The number of alleles per locus ranged from 2-4 with an average of 2.63 alleles per locus. Polymorphic Information Content (PIC) values ranged from 0.278 in primer SSR 17 to 0.78 in primer SSR 24. Average Nei's gene diversity (*h*) and Shannon's information index (*I*) were 0.348 and 0.557, respectively. Highest pair wise genetic distance (0.666) was observed between accessions from North Africa and accessions from West Africa. Analysis of molecular variance showed that 41% of variation was distributed among individuals in population with estimated genetic differences (F_{ST}) of 0.713. An estimate of gene flow (Nm) among all populations was 1.382. Principal Component 1 (PC1) and PC2 axis explained 15.3 and 12.5% of the total variation, respectively. UPGMA dendrogram divided the accessions into five main clusters. The genetic diversity information obtained from this germplasm collection was for the first time in a leafy vegetable and is useful for breeding, research and conservation of this underutilized and neglected crop.

[3] Submitted to Journal of Plant Biochemistry and Biotechnology – Springer (Under review)

Key words: *Corchorus spp*., Indigenous vegetable, Jute mallow, Simple sequence repeat markers.

4.1 Introduction

Jute mallow (*Corchorus spp.*) is among important indigenous leafy vegetables. The genus consists of about 50-60 species widely known and distributed across the tropics, sub tropics and in temperate regions (Edmonds, 1990; Palve *et al*., 2003; Bayer and Kubitzki 2003). It is regarded as a vegetable of regional importance, and in Tanzania and Ghana it ranks among the top ten indigenous cultivated vegetables (Schippers, 2000; Nwangburuka and Denton, 2012; Kinabo *et al*., 2006). Jute mallow is also popular leafy vegetable in Nigeria, Cameroon, Sudan and Egypt. Other parts where Jute mallow is grown as vegetable include Middle East, Malaysia and Latin America (Westphal-Stevels, 1985). Jute mallow is also one of the most important fiber crops and it stands only second to cotton in India (Mir *et al*., 2008; Zhang *et al.,* 2015). The two cultivated species, *C. olitorius* and *C. capsularis* are used as a major source of natural fiber and the cultivation is believed to have started over 200 years ago (Basu *et al*., 2004; Benor *et al*., 2012).

In Africa, the rate of genetic exploitation of the current germplasm for breeding of improved cultivars for leaf yield has not received enough attention despite the efforts to conserve the accessions of *Corchorus spp.* in Institutional and National gene banks (Nwangburuka and Denton, 2012). Vegetative characters that are associated with green leaf yield such as number of branches, plant height and leaf size among others are reported to be highly variable as well as qualitative traits (National Horticultural Research Institute, 1986). These conventional methods of establishing diversity in Jute mallow that is morphological characterization have been done by several researchers using different germplasm resources (Nwangburuka and Denton, 2012; Osawaru and Ogwu, 2012; Palit *et al*., 1996). Field evaluation, despite being important step in initial screening of germplasm for breeding and conservation, it is time consuming and highly influenced by the environment. These limitations may be overcome by molecular markers because they are large in number, not affected by environment, more reproducible and more objective in analysis of genetic variation among genotypes. Thus they can be used to complement morphological characterization (Oh *et al*., 2012).

The genetic diversity within and among *Corchorus spp* has been investigated in several studies using different types of molecular markers such as RAPD (Ogunkanmi *et al.*, 2010; Hossain *et al*., 2002; Haque *et al*., 2007; Roy *et al*., 2006) and AFLP (Benor *et al*., 2012). Other markers include Sequence tagged microsatellite markers (STMS) and Sequence related amplified polymorphism (SRAP) (Roy *et al*., 2006; Soliman *et al*., 2014). Microsatellite markers particularly Simple sequence repeats (SSRs) occur frequently in many eukaryotic genomes and they can be very informative (Ntuli *et al*., 2015). They have been reported in Jute mallow in several studies (Akter *et al*., 2008; Mir *et al*., 2008; Mir *et al*., 2009; Basu *et al*., 2004; Ghosh *et al*., 2014). These markers have also been widely used in other crops because they are highly polymorphic, reproducible, multi-allelic and are co-dominant in nature (Becher *et al*., 2000; Formisano *et al*., 2012).

In many studies conducted using these molecular markers on Jute mallow, efforts have been directed towards improvement of fiber yield-related qualities such as fiber fineness and lignin content. Thus the molecular maps developed for QTL interval mapping are mainly useful for fiber yield improvement (Mir *et al*., 2009). The current germplasm contains accessions from Africa and South East Asia. In the studies conducted previously, majority of the samples originated from Asia where Jute mallow is mainly grown for fiber production. Thus little is known about the genetic diversity of the accessions from Africa. Moreover, all the accessions are not thoroughly characterized in terms of leaf yield traits and no information regarding their genetic diversity and phylogenetic relationship.

Understanding the genetic diversity and relationships among the current germplasm will be a useful and important tool in screening and identifying accessions to be used for future breeding of Jute mallow vegetable. Thus the aim of this study was therefore to determine the genetic diversity of *Corchorus spp* accessions present in the World Vegetable Centre by use of SSR markers.

4.2 Materials and methods

4.2.1 Plant materials

Molecular analysis was performed on 83 accessions of *Corchorus spp* from the seed repository of the World Vegetable Centre, Eastern and Southern Africa. The list of germplasm accessions used in this study is presented in Table 8.

Table 8. List of *Corchorus spp*. accessions used for the molecular characterization

SN	Accession Name	Country of Origin	SN	Accession Name	Country of Origin
1	ALV MN 059	Unknown	42	**TOT 4413**	Bangladesh
2	AZIGA	Cameroon	43	TOT 4429	Bangladesh
3	BAFIA	Cameroon	44	TOT 4500	Bangladesh
4	CAM EX CO	Cameroon	45	TOT 4519	Bangladesh
5	CAM MULA	Cameroon	46	TOT 4541	Bangladesh
6	ES	Tanzania	47	TOT 4623	Bangladesh
7	EX CHAMALAWI	Malawi	48	TOT 4624	Bangladesh
8	EX ZIMBABWE	Zimbabwe	49	TOT 4669	Bangladesh
9	GKK 10	Malawi	50	TOT 4701	Bangladesh
10	GKK 25	Malawi	51	SUD - 2	Sudan
11	HS	Tanzania	52	TOT 4712	Bangladesh
12	IP - 10	Kenya	53	TOT 4713	Bangladesh
13	IP - 13	Kenya	54	TOT 4721	Bangladesh
14	IP - 2	Kenya	55	TOT 4800	Vietnam
15	IP - 5	Kenya	56	TOT 4876	Japan
16	IP -4	Kenya	57	TOT 4885	Japan
17	MIX	Tanzania	58	TOT 5876	Japan
18	ML-JM-1	Malawi	59	TOT 5877	Japan
19	ML-JM-10	Malawi	60	TOT 5999	Taiwan
20	ML-JM-12	Malawi	61	TOT 6278	Vietnam
21	ML-JM-13	Malawi	62	TOT 6370	Unknown
22	ML-JM-14	Malawi	63	TOT 6425	Uganda
23	ML-JM-2	Malawi	64	TOT 6426	Kenya
24	ML-JM-3	Malawi	65	TOT 6427	Kenya
25	ML-JM-4	Malawi	66	TOT 6430	Cameroon
26	SUD 1	Sudan	67	TOT 6667	Philippines
27	SUD 3	Sudan	68	TOT 6669	Philippines
28	SUD 4	Sudan	69	TOT 6730	Unknown
29	T0T 4067	Vietnam	70	TOT 6749	Unknown
30	TOT 0124	Malaysia	71	TOT 7865	Unknown
31	TOT 3499	Vietnam	72	TOT 7866	Unknown
32	TOT 4064	Vietnam	73	TOT 7974	Bangladesh
33	TOT 4097	Tanzania	74	TOT 7977	Bangladesh
34	TOT 4140	Vietnam	75	TOT 7979	Bangladesh
35	KIPUMBULIKO	Unknown	76	TOT 7980	Bangladesh
36	TOT 4157	Vietnam	77	TOT 8532	Unknown
37	TOT 4235	Bangladesh	78	TOT 9736	Unknown
38	TOT 4312	Bangladesh	79	UG	Uganda
39	TOT 4316	Bangladesh	80	TZA 3002	Tanzania
40	TOT 4352	Bangladesh	81	TZA 3070	Tanzania
41	TZA 681	Tanzania	82	UG-JM-1	Uganda
83	UG-JM-13	Uganda			

4.2.2 DNA Isolation

DNA from each of the 83 accessions was extracted from the leaves of 28 days old seedlings. The seeds were sown and raised in a screen house of the World Vegetable Centre (ESA). DNA from the leaves was isolated using modified Cetyltrimethyl ammonium bromide (CTAB) method according to Doyle and Doyle (1990). The DNA was purified by RNase treatment followed by Sodium acetate and ethanol. The quality and concentration of DNA was checked on 0.8% agarose gel.

4.2.3 SSR Primers

Twenty five SSR genomic primers specific to *Corchrus spp*. were used to study genetic diversity among the 83 accessions (Table 9). Twenty different accessions were used for primer screening. Of the 25SSR primers, 19 primers amplified and produced clear polymorphic bands (Table 9). These primers were used to study the genetic diversity of the accessions.

Table 9. SSR primers used in the study

Primer Code	Forward Sequence	Reverse Sequence	Repeat Motif
SSR1	ATTTTCAGCCAATGGAGCTCA	TATCACATTACTTCCAGCACAC	(CAA)7
SSR2	TGAAAATCTGGTCAAAATGCTATC	TGTACTCATGATAAGTTGCCTAC	(GTT)11
SSR3	TTCCTGTACCTTTGGGCCTCA	AAACACACTCAAGTAGTTCGCA	(GA)13
SSR4	ATTTAAGATGCCAGCCATTCCA	AAACACACTCAAGTAGTTCGCA	(GT)17
SSR5	GGCCAATAAAATACAAGGGACA	GATGGTTATATCACCTGAGGCA	(GT)19
SSR6	GTACAAACAACTTTATTAACATAC	CCTATAACCCAAATTTGATACTAC	(CA)13
SSR7	TGATGATAAACCATCCTTCACCA	GTCTACACTCTGAAGTAGCTTCA	T(12)(TC)17
SSR8	GCCAAAATTGTGGGAAGCAC	TGGTGTCGATTCGTTTCTAC	(GTT)8
SSR9	CTTTTCGAGCTTGATCAGTTACCA	GACTTTACTTGTACCCATCTCCA	(CTAT)18
SSR10	CCATACTTGCGTTCTGAGGTGCA	AATCCTTCCCATACTGGAGATGA	(GATA)17GA>82
SSR11	TCAGTTGAGGAGGCAGAACC	CACAATATCGACCACAGTATATCC	(TA)5(AG)13
SSR12	AATCAGAGTCAGACAGAAGGGA	GTCTTACCCATCATCTCAGACA	(CT)14
SSR13	GATTGAATGGTTCTGGGTTTCA	CAATGTAAGCGCATTCATCAATAG	(AG)21
SSR14	TCATTGTGGTTAATTTGCTTGCAAC	TTCCATGCATGGTTGGCTAAAGAC	(AG)19
SSR15	GTTACCAACATAAAAATAGCAATCAC	ACCATGAAAGATTGTTGCTGGAC	(AG)20
SSR16	CTCTGTTTTTACATGGTTACTTCGC	TCAGCGATTGATGCATATAGTCC	(AG)22
SSR17	CCTTCCCATACTGGAGATGAGA	TACTTGCGTTCTGAGGTGCA	(AG)23
SSR18	GATCTGGCTATCGGATTACTTCA	CTTCAAAACGGAGCTATTGTGTC	(AG)25
SSR19	GGATTTGGTGAGGAGAATATATTC	TCCCGTCACTCTCACCTTCA	(AG)27
SSR20	CACTTTGCATTAAAGAACACCA	AAGTCTCTCTATATATAGCAGCA	(AG)29
SSR21	CTTTGTTGAGTTTGAAGCCGC	GAGAGTAGACAATGATTTACCA	(CT)12
SSR22	TATGATGTCATGTAGGTGAAC	GGTCATATTTACACTCCTGAC	(GA)12
SSR23	CTACAGAGAAATGCTGTTCCCA	GAAATGTTGCAATGGAAGCCA	(CT)13
SSR24	AGCCCAACATGCCCATCAACCA	TGTTCCCCACACAAAACTGACTTGC	(TC)21
SSR25	ACGCCTCCTAAGAGGGATGCCA	TGTGGCCTTTTGAGGAGGTGC	(GAA)5

4.2.4 Polymerase Chain Reaction

DNA amplification was carried out with 10 µl reaction mixture, each carrying 50ng genomic DNA. The reaction mixture consisted of 2µl of DNA template, 0.5µl of dNTPs (25mM), 2.5µl of 10x one Taq standard buffer, 0.93µl of nuclease free water, 2.0µl of forward and reverse primer, and 0.5U of one Taq polymerase (Inqaba Biotech, South Africa). The reaction mixture was loaded in 96 well PCR plate in Bio-rad thermo cycler according to the following reaction conditions: pre heating at 95^{o}C for 5 minutes followed by denaturation at 95^{o}C for 1 minute,

Annealing at 50^{o}C – 64^{o}C for 1 minute (according to primers annealing temperature), extension at 72^{o}C for 1minute and final extension at 72^{o}C for 5 minutes.

4.2.5 Electrophoresis and gel staining

PCR products were checked on 2% agarose gel stained with EZ – Vision (Amresco, fountain parkway solon, OH USA) in 0.5x TBE (Tris Borate Ethylenediaminetetraacetic acid) buffer. The sizes of the PCR products were estimated by comparing with 100 bp ladder. The Gels were viewed in a Bio-rad Gel Doc EZ imager.

4.3 Statistical Analysis

After DNA amplification the fragments were scored as '0' and '1' for absence and presence of a band at each level, respectively for all accessions under the study. Polymorphism information content (PIC) was calculated at each level by using the following formula;

$\mathrm{PIC} = 1 - \sum(Pi)2$ (Botstein *et al.*, 1980)

Where: Pi is the frequency of the ith band phenotype detected.

Analysis of molecular variance (AMOVA) was calculated by using GenAIEx 6.5 software (Peakall, 2012). Nei's gene diversity and Shannon's information index were calculated using POPGENE version 1.32 software (Yeh *et al.,* 1997). The obtained matrix was then used to calculate principal coordinate analysis (PCoA) using PAST software version 1.93. The cluster analysis and construction of the unweighted pair group method with arithmetic average (UPGMA) dendrogram was performed by using NTsys –pc version 2.1 software (Rohlf, 2000).

4.4 Results

4.4.1 SSR Polymorphism

A total of 25 primers were screened, 19 primers were found to be polymorphic. The number of alleles per locus ranged from 2 to 4 with an average of 2.63 alleles per locus (Table 10). Polymorphic information content (PIC) values ranged from 0.278 in primer SSR 17 to 0.780 in

primer SSR 24. The primers with PIC value >0.5, were classified as highly informative. Consequently, six primers SSR 1, SSR 2, SSR 5, SSR 7, SSR 14 and SSR 24 were highly informative.

Table 10. SSR primer sets used in this study and their amplification results

Primer Number	Repeat Motif	Number alleles per locus	PIC
SSR1	$(CAA)_7$	3	0.592
SSR2	$(GTT)_{11}$	3	0.572
SSR3	$(GA)_{13}$	3	0.468
SSR4	$(GT)_{17}$	2	0.490
SSR5	$(GT)_{19}$	4	0.568
SSR6	$(CA)_{13}$	2	0.490
SSR7	$T_{(12)}(TC)_{17}$	3	0.631
SSR8	$(GTT)_8$	3	0.458
SSR9	$(CTAT)_{18}$	2	0.490
SSR12	$(CT)_{14}$	2	0.500
SSR14	$(AG)_{19}$	3	0.644
SSR15	$(AG)_{20}$	2	0.444
SSR16	$(AG)_{22}$	2	0.408
SSR17	$(AG)_{23}$	2	0.278
SSR18	$(AG)_{25}$	2	0.320
SSR19	$(AG)_{27}$	2	0.470
SSR21	$(CT)_{12}$	3	0.499
SSR24	$(TC)_{21}$	4	0.720
SSR25	$(GAA)_5$	3	0.469
Average Per primer		**2.631**	**0.501**

4.4.2 Genetic diversity

Table 11 shows Nei's gene diversity (*h*) and Shannon's information index (*I*) for seven populations from different parts of the world. The Nei's gene diversity (*h*) ranged from 0.262 in

population 7 from West Africa to 0.444 in population 4 from South Africa with an average of 0.348. Similarly, the Shannon's information index (*I*) ranged from 0.387 in West African accessions to 0.631 in South African accessions, with average of 0.557.

Table 11. Nei's gene diversity and Shannon's information index for different populations of *Corchorus spp*.

Population	Number of Individuals	Nei's gene diversity (*h*)	Shannon's information index (*I*)
1	18	0.387	0.557
2	16	0.325	0.475
3	4	0.266	0.393
4	11	0.444	0.631
5	20	0.407	0.592
6	9	0.344	0.504
7	5	0.262	0.387
Average	**11.86**	**0.348**	**0.505**

Note: Population 1 – East Africa, 2 – East Asia, 3 – North Africa, 4 – South Africa, 5 – South East Asia, 6 – Unknown, 7 – West Africa.

Nei's unbiased measure of genetic distance is summarized in Table 12. The highest pairwise genetic distance (0.666) was observed between North African and West African accessions, while the lowest (0.038) was obtained between East African and South African accessions. Other lowest pairwise genetic distance (0.072) was between population from East Asia and population from South East Asia. Overall, West African accessions showed highest pairwise genetic distance across all other populations.

Table 12. Nei's measure of unbiased genetic distance in *Corchorus spp*.

Population	1	2	3	4	5	6	7
1	****						
2	0.199	****					
3	0.196	0.188	****				
4	0.038	0.159	0.140	****			
5	0.208	0.073	0.216	0.148	****		
6	0.208	0.214	0.199	0.151	0.174	****	
7	0.328	0.320	0.666	0.249	0.214	0.405	****

Note: *Population 1 – East Africa, 2 – East Asia, 3 – North Africa, 4 – South Africa, 5 – South East Asia, 6 – Unknown, 7 – West Africa.*

The analysis of molecular variance (Table 13) showed that 41% of the variation was distributed among the individuals in the populations. Variations among the populations from different parts were 30%. The remaining variation was confined within individuals. Highest genetic differences (F_{ST} = 0.713) were observed within individuals and among individuals (F_{ST}=0.59). Estimate of gene flow (N_m) among all populations was 1.382 indicating high gene flow and little differentiation among the populations.

Table 13. Results of analysis of molecular variance (AMOVA).

Source	Df	SSD	MSD	Variance Component	% of Total Variance	Genetic Differences (*F*st)	*P* value
Among population	6	11.99	1.99	0.076	30	0.3	0.001
Among Individual	76	21.33	0.281	0.104	41	0.59	0.001
Within Individual	83	6	0.072	0.072	29	0.713	0.001

Note: *P value estimations were tested through 999 permutations. Df – Degrees of freedom, SSD - sums of squared deviations, MSD – mean sums of squared deviations.*

4.4.3 Cluster Analysis and Principal Coordinate Analysis

Principal coordinate analysis (PCoA) results from the SSR markers for the 83 accessions are presented in Fig. 5. PC1 and PC2 axis explained 15.3% and 12.5 % of total variation, respectively. Accessions from Africa except those from North Africa were grouped together in the positive Y- axis in the first quadrant. North African accessions were found in the third quadrant, mixed with few accessions from Asia and East Africa. Accessions from East Asia and South East Asia were grouped in the fourth quadrant and partially in the third quadrant. The Unknown accessions were found in all quadrants of the PCA. Generally, first and fourth quadrant contained accessions from Africa and Asia, respectively. The locations of Asian and African accessions on the first and fourth quadrant in the PCoA coordinate plane match their geographical distribution. The second and third quadrant contained mixed accession

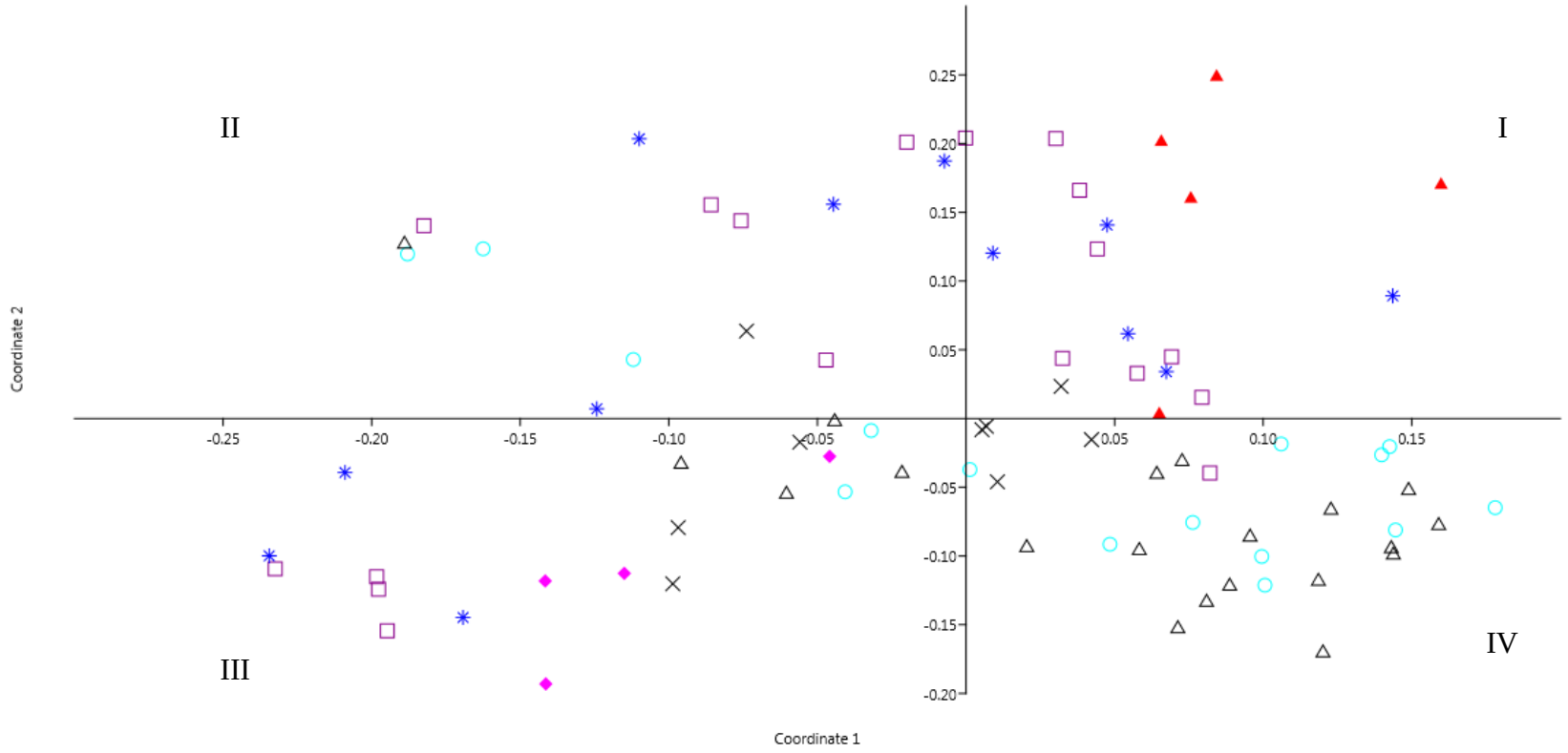

Figure 5: Scatter plot of a PCoA showing the distribution of *Corchorus spp*. accessions based on SSR data

Key: The populations are presented in different symbols based on their origin. ♦ - North Africa, ▲ – West Africa, □ – East Africa, ○ – East Asia, Δ - South East Asia, * - South Africa, X – Unknown.

UPGMA tree showed five main clusters at coefficient of 0.52 within the collection of 83 accessions from this germplasm Fig. 6. Cluster 1 contained 5 accessions; three from Asia and one from South Africa, and one accession was from unknown origin. Cluster 2 contained 9 accessions, 8 from East and South Africa and one from East Asia. Cluster 3 contained 25 accessions, the upper part of this cluster contained 10 accessions from Africa, the middle cluster contained 6 accessions from Asia and the lower part contained 8 accessions from unknown origin and 1 from East Africa was grouped with this cluster. The fourth cluster was the largest consisting of 26 accessions. Twenty four accessions were from East and South East Asia and two more accessions from West Africa and from East Africa. Cluster 5 contained 18 accessions all originated from Africa.

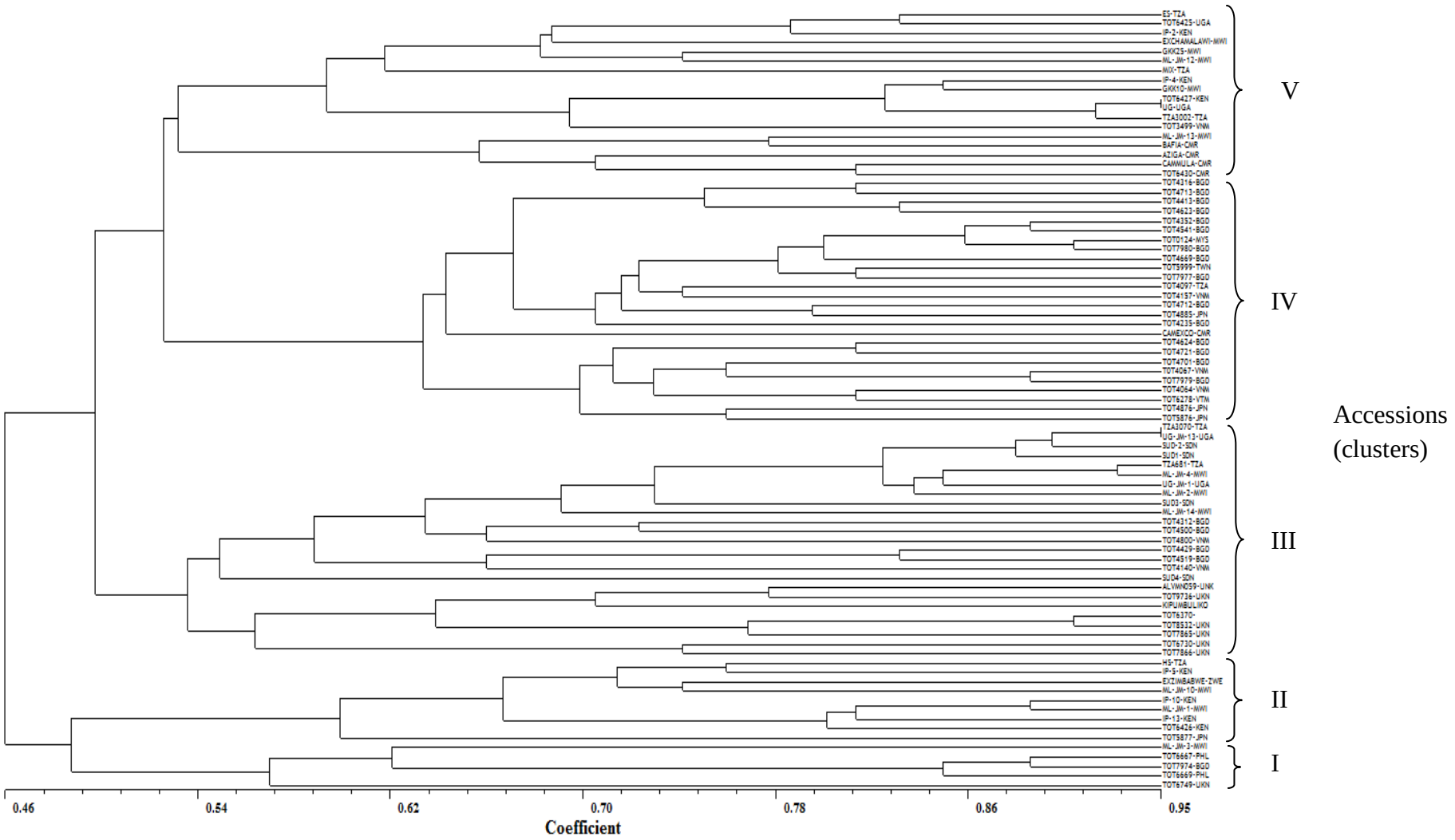

Figure 6: Dendrogram constructed from cluster analysis based on UPGMA using the SM coefficient option

Key: Country of Origin of each individual is given with 3 – digit international codes after accession name.

4.5 Discussion

Foundation for crop improvement and conservation of genetic resources is determined by genetic diversity in the germplasm collected. In this study, 83 accessions of Jute mallow mainly from Africa and Asia were characterized to assess genetic diversity and relatedness among them. SSR markers specific for Jute mallow were selected to study the genetic diversity of this germplasm.

The average number of polymorphic bands per primer was 2.63 (range 1-4) among all accessions irrespective of which species they belonged to. Similar findings are reported by (Mir *et al.*, 2009), where alleles per SSR locus of 2.56 were obtained for both *C. olitorius* and *C. capsularis* species. In another traditional vegetable (Spider plant), K'Opondo *et al*. (2009) reported average number of polymorphic band per primer of 3.1 using RAPD markers. The average number of alleles per locus is reported to be higher in *C. olitorius* than in *C. capsularis* indicating higher diversity in *C. olitorius* (Mir *et al.*, 2008). The average PIC value for individual SSRs was 0.50 (range 0.28 – 0.72) indicating high allelic diversity in these primers and possession of a large level discriminatory power. This is higher compared with PIC value of 0.198 and 0.203 for *C. capsularis* and *C. Olitorius* reported by (Banerjee *et al.*, 2012). A higher PIC value of 0.71 was reported by Khaing *et al*. (2013) in a core set of 63 accessions of Amaranthus by using SSR markers. Six SSR primers had PIC >0.5, these primers were very informative and they can be used to differentiate all accessions in this study. Similar to our study, a close PIC values of 0.45 and 0.43 for *C. olitorius* and *C. capsularis* species were also reported by (Ghosh *et al.*, 2014) using 19 SSR primers to study 63 accessions.

Average Nei's gene diversity index was 0.387. Highest gene diversity was observed in population from South Africa and lowest gene diversity was observed in population from West Africa. The high gene diversity in South African population could be attributed to high species diversity as reported by (Edmonds, 1990). Only four accessions from West Africa were used in this study and this could possibly explain the low gene diversity observed in this population. The higher the value of Nei's gene diversity found in populations, the higher the value of Shannon's information index and vice versa.

North African and West African accessions were highly different according to Nei's unbiased

measure of genetic distance (0.666). This indicates the potential of these materials to be used as parental lines for breeding of improved genotypes. East African and South African accessions demonstrated high similarities as compared with all other populations. Similar results were also observed in PCoA.

The molecular analysis showed that variation among populations from different regions was low (30%) indicating low allelic diversity. Diversity of individuals in same region was high (41%) compared with variation among individuals within populations and among populations. This may be attributed to species dominance within regions where by most of accessions from Asia were from *C. capsularis* species whereas those from Africa were mainly from *C. olitorius* species. Low genetic diversity at species level were reported in previous studies using AFLP markers (Benor *et al*., 2012), AFLP and SSR markers (Basu *et al*., 2004). The low value (F_{ST} = 0.3) of genetic differences among populations in this study indicates the accessions in the same region had narrow genetic base probably due to sharing of the same genetic materials among farmers or less breeding activities. In addition, Jute mallow is a self-pollinated crop.

PCoA Fig. 5 displayed the differences of Asian accessions from the rest of other accessions. Most of these accessions were found in the fourth quadrant and partly in the third quadrant of the PCoA plane. This distinctiveness may be due to continued breeding and specific selection of accessions with superior qualities and fiber yield. It is reported that during breeding of commonly grown varieties in Asia, only few genotypes were used as parents (Roy *et al.*, 2006; Mir *et al.*, 2008). Contrary to our findings, Benor *et al*. (2012) found that, the Asian accessions clustered together with those from East Africa. Moreover, highest similarity was observed between South African and East African accessions which clustered together in the first and second quadrant. Both in East Africa and South Africa commercial farming of Jute mallow is practiced to a very limited extent, thus most of these accessions are under their natural conditions with no efforts of breeding for improvement. There is high diversity of species in both areas but the species occurring in these areas are common (Edmonds, 1990). Accessions from West Africa displayed highest diversity than other African accessions and they were found in the first quadrant of PCoA. The level of domestication of the accessions in West Africa is higher as opposed to other parts of Africa (Nyadanu *et al*., 2016). This topology depicts both natural selection and breeding for leaf yield improvement.

Cluster analysis grouped the accessions into five major clusters at Nei's distance coefficient of 0.52. The Nei's distance coefficients ranged from 0.46 to 0.95. Small clusters were observable among the major clusters. The grouping of accessions based on their geographical origin was only observed in cluster 4 and 5. African accessions were found in cluster 5 and cluster 4 contained Asian accessions, a pattern which is also supported by results of PCoA. Most of accessions from Africa were from *C. olitorius* species while those from Asia were from *C. capsularis*. The clustering pattern based on the two species in our study is similar to those obtained in earlier studies using chloroplast-SSR, genomic-SSR, ISSR, AFLP and RAPD (Hossain *et al*., 2004; Basu *et al*., 2004; Roy *et al*., 2006; Mir *et al*., 2008) The remaining clusters were admixture groups and did not reflect geographical distribution. Substantial diversity existed in cluster 3 indicating the potential movement of germplasm across boundaries. Similar findings were reported by Roy *et al*. (2006).

4.6 Conclusion

This study demonstrated that there is high genetic diversity in the Jute mallow germplasm collected under the current study. Accessions in populations from different geographical locations as designated in this study can be a potential source of allele diversity for breeding activities and improved genotypes due to differences observed in gene diversity. Accessions such as Cameroon Mula, Bafia and Aziga from West Africa displayed highest diversity in the PCoA plane. Other accessions with high diversity include Sudan 2 from North Africa, UG-JM-13, ES, HS and TZA 681 from East Africa, ML-JM-4 and Ex-Zimbabwe from South Africa and TOT 6667 and TOT 7974 from Asia. For future conservation and breeding programs of this neglected and underutilized leafy vegetable crop, the high level of genetic diversity observed in this germplasm could be exploited.

CHAPTER FIVE

Genetic Diversity of Jute mallow (*Corchorus spp*.) accessions using ISSR markers[4]

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Abstract

Jute mallow is a nutritious leafy vegetable. The leaves are rich in proteins, vitamins and essential amino acids. Molecular characterization of Jute mallow as vegetable is scarcely reported. In the present study, inter simple sequence repeats (ISSR) molecular markers were employed to assess genetic diversity and relationships of 83 accessions of Jute mallow from different parts of Africa and Asia conserved at the World Vegetable Center in East and Southern Africa. A total of 89 bands were amplified by 8 ISSR primers. Number of polymorphic bands per primer ranged from 2 to 6 with an average of 2.75 bands per primer. Polymorphic information content (PIC) values ranged from 0.390 to 0.760 with average of 0.53. Average Nei's gene diversity (*h*) and Shannon's information index (*I*) was 0.335 and 0.494, respectively. The highest pair wise genetic distance was 0.431 observed in a population from East Africa accessions. PC1 and PC2 axis explained 21.69% and 11.66% of the total variation, respectively. UPGMA cluster analysis grouped the accessions into five main clusters at genetic similarity coefficient of 0.53 as standard value for classification. These results have important implications for Jute mallow breeding and conservation.

Key words: *Corchorus spp*., Genetic diversity, ISSRs, Jute mallow, Leafy vegetable

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5.1 Introduction

Jute mallow (*Corchorus spp*.) is a traditional leafy vegetable that is used in many households in Africa, Middle East and Latin America as both vegetable and medicinal plant. It is an annual herb belonging to the family Malvaceae which is comprised of 50-60 species which are distributed in the tropics, subtropics and warm temperate regions of the world (Merlier, 1972; Morakinyo 1997; Dansi *et al*., 2008). The leaves are well known as emollient, diuretic, tonic and purifying to the body (Fagbohoun and Ibrahim, 2011; Adebo *et al*., 2015). The leaves contain on average 85-87g H_2O, 5.6g protein, 0.7g oil, 5g carbohydrate, 1.5g fiber, 250-266mg Ca, 4.8mg Fe, 1.5mg vitamin A, 0.1mg thiamine, 0.3mg riboflavin, 1.5mg nicotinamide and 53-100mg ascorbic acid per 100g (Ndlovu and Afoyalayan, 2008). Jute mallow also contains high amount of all essential amino acids and antioxidants needed for a good health (Tulio *et al*., 2002; Kumawat *et al*., 2012; Barku *et al*., 2013).

Though Jute mallow has significant contribution to food security; its cultivation in many parts of Africa is very limited. The crop grows as volunteer crop in farmer's fields and the leaves are collected during rainfall season, dried and stored for use during the dry seasons. Jute mallow is still classified as neglected and underutilized crop. It is neglected by researchers and national agricultural development policies (Dansi *et al*., 2012; Nyadanu and Lowor, 2015). Thus, there are few improved varieties of Jute mallow in Africa and those which are grown by farmers are locally obtained from germplasm maintained by farmers (Olanrewaju and Nwangburuka, 2012).

In order to increase utilization of Jute mallow, it is crucial to make availability of improved genotypes to farmers a priority. To achieve this goal, the information on the genetic diversity of Jute mallow germplasm stored in the gene banks plays a key role (Sultan *et al*., 2012; Nyadanu *et al*., 2015).

Morphological data coupled with the use of appropriate molecular markers are more reliable and informative of genetic diversity of any species (Roy *et al*., 2006). Moreover, molecular markers are not affected by environment and they have been used successfully in different plant species for genetic characterization of germplasm at different levels (Mandal *et al*., 2013).

The use of molecular markers to study genetic diversity of Jute mallow has been reported by several authors. Recently, various molecular markers such as random amplified polymorphic DNA (RAPD) (Mandal *et al*., 2013; Roy *et al*., 2006), simple sequence repeats (SSR) (Mir *et al*., 2009; Mir *et al*., 2008; Huq *et al*., 2009; Banerjee *et al*., 2012; Akter *et al*., 2008), amplified fragment length polymorphism (AFLP) (Benor *et al*., 2012; Das *et al*., 2011), sequence-related amplified polymorphisms (SRAP) (Chen *et al*., 2014; Soliman *et al*., 2014) and sequence tagged microsatellite sites (STMS) (Roy *et al*., 2006) have been used to detect genetic diversity in different germplasms of Jute mallow. Most of these studies focused on Jute mallow as a fiber crop and the major emphasis were on improvement of fiber related traits. Also in many researches the plant materials used were of Asian origin. The results of these studies indicated high diversity among the species but low diversity within the species. Only few studies have been conducted with focus on Jute mallow as vegetable with emphasis on improvement of leaf yield. Collections from East, West and Southern Africa largely remain unexploited for genetic diversity studies particularly with traits related to leaf yield which is the main use of the crop in Africa.

ISSR markers are important molecular markers possessing different genome coverage (Noormohammadi *et al*., 2012). ISSR use microsatellite sequences as primers to amplify genomic regions flanked by microsatellite repeats (Roy *et al*., 2006). ISSR markers are simple and cost effective to use with high degree of reproducibility as compared with RAPD markers (Eftekharian *et al*., 2016). The present study was conducted to assess genetic diversity among accessions of Jute mallow germplasm maintained at the World Vegetable Center using ISSR markers.

5.2 Materials and Methods

5.2.2 Plant Materials

DNA from each of the 83 accessions was extracted from the leaves (28-days old seedlings). The Jute mallow collection represent one of the different types of traditional vegetables collected from farmers' fields and preserved *ex-situ* for breeding, other research activities or direct use by farmers. The list of these accessions is presented in Table 14.

Table 14. List of *Corchorus spp*. accessions used in the molecular characterization using ISSR

SN	Accession Name	Country of Origin	SN	Accession Name	Country of Origin
1	ALV MN 059	Unknown	42	TOT 4413	Bangladesh
2	AZIGA	Cameroon	43	TOT 4429	Bangladesh
3	BAFIA	Cameroon	44	TOT 4500	Bangladesh
4	CAM EX CO	Cameroon	45	TOT 4519	Bangladesh
5	CAM MULA	Cameroon	46	TOT 4541	Bangladesh
6	ES	Tanzania	47	TOT 4623	Bangladesh
7	EX CHAMALAWI	Malawi	48	TOT 4624	Bangladesh
8	EX ZIMBABWE	Zimbabwe	49	TOT 4669	Bangladesh
9	GKK 10	Malawi	50	TOT 4701	Bangladesh
10	GKK 25	Malawi	51	SUD - 2	Sudan
11	HS	Tanzania	52	TOT 4712	Bangladesh
12	IP - 10	Kenya	53	TOT 4713	Bangladesh
13	IP - 13	Kenya	54	TOT 4721	Bangladesh
14	IP - 2	Kenya	55	TOT 4800	Vietnam
15	IP - 5	Kenya	56	TOT 4876	Japan
16	IP -4	Kenya	57	TOT 4885	Japan
17	MIX	Tanzania	58	TOT 5876	Japan
18	ML-JM-1	Malawi	59	TOT 5877	Japan
19	ML-JM-10	Malawi	60	TOT 5999	Taiwan
20	ML-JM-12	Malawi	61	TOT 6278	Vietnam
21	ML-JM-13	Malawi	62	TOT 6370	Unknown
22	ML-JM-14	Malawi	63	TOT 6425	Uganda
23	ML-JM-2	Malawi	64	TOT 6426	Kenya
24	ML-JM-3	Malawi	65	TOT 6427	Kenya
25	ML-JM-4	Malawi	66	TOT 6430	Cameroon
26	SUD 1	Sudan	67	TOT 6667	Philippines
27	SUD 3	Sudan	68	TOT 6669	Philippines
28	SUD 4	Sudan	69	TOT 6730	Unknown
29	T0T 4067	Vietnam	70	TOT 6749	Unknown
30	TOT 0124	Malaysia	71	TOT 7865	Unknown
31	TOT 3499	Vietnam	72	TOT 7866	Unknown
32	TOT 4064	Vietnam	73	TOT 7974	Bangladesh
33	TOT 4097	Tanzania	74	TOT 7977	Bangladesh
34	TOT 4140	Vietnam	75	TOT 7979	Bangladesh
35	KIPUMBULIKO	Unknown	76	TOT 7980	Bangladesh
36	TOT 4157	Vietnam	77	TOT 8532	Unknown
37	TOT 4235	Bangladesh	78	TOT 9736	Unknown
38	TOT 4312	Bangladesh	79	UG	Uganda
39	TOT 4316	Bangladesh	80	TZA 3002	Tanzania
40	TOT 4352	Bangladesh	81	TZA 3070	Tanzania
41	TZA 681	Tanzania	82	UG-JM-1	Uganda
83	UG-JM-13	Uganda			

5.2.3 DNA Extraction

DNA extraction was done using modified Cetyltrimethyl ammonium bromide (CTAB) method according to Doyle and Doyle (1990). The DNA was purified by RNase treatment followed by Sodium acetate and ethanol. The quality and concentration of DNA was checked on 0.8% agarose gel. Fifteen ISSR primers namely 814, 811, 815,817, 824, 812, 821, 813 and 816, 820, 822, 823, 825, 818 and 819 were used in this study.

5.2.4 PCR and gel Electrophoresis

After initial screening of the 15 ISSR primers, 8 primers with good and clear banding pattern were used for analysis of genetic diversity (Table 15). The PCR was performed in a 10µl reaction mixture as follows. A 2µl of 50ng DNA template, 2µl of primer (Inqaba Biotech, South Africa), 0.5µl of dNTPs, 2.5µl of 10X one Taq standard buffer, 2.93µl of nuclease free water and 0.5U of one Taq polymerase. The reaction mixture was loaded in a 96 well plate initially denatured at 94^{o}C for 5minutes; followed by 35 cycles of 94^{o}C for 1 minute. Annealing temperature was according to primer for 1 minutes and 72^{o}C for 1 minute followed by final extension at 72^{o}C for 5 minutes. The amplified products were separated on 1.5% agarose gel stained with EZ-Vision (Amresco, fountain parkway solon OH USA) in 0.5 TBE (Tris Borate Ethylenediaminetetraacetic acid) buffer. To estimate the sizes of the bands, 100 bp ladder was loaded with the samples. The gels were viewed in a Bio-rad Gel Doc EZ imager.

5.3 Data Analysis

ISSR amplified bands in the gel were manually scored as present (1) or absent (0). Only the consistently clear bands were scored and used to create 1/0 matrix. This matrix was then used to assess the genetic diversity of *Corchorus spp*. accessions. Polymorphism information content (PIC) was calculated for each band according to Botstein *et al*. (1980) using the formula;

$$\text{PIC} = 1 - \sum (Pi)2$$

Where: Pi is the frequency of the ith band phenotype detected.

Nei's pairwise genetic distance, Nei's gene diversity and Shannon's information index (*I*) were calculated using computer program POPGENE version 1.32 (Yeh *et al*., 1977). The obtained matrix was also used to calculate principal coordinate analysis (PCoA) using PAST software version 1.93 and to perform cluster analysis and construct the unweighted pair group method with arithmetic average (UPGMA) dendrogram using NTsys – pc 2.1 software.

5.4 Results

5.4.1 ISSR Polymorphism

Of the 15 primers used, 8 primers showed good and clear banding pattern. The number of polymorphic bands per primer ranged from 2 to 6 with an average of 2.75 bands per primer. Regarding the average number of bands amplified, 11.13 bands were amplified per primer and a total of 89 bands for all primers. The total number of bands per primer ranged from 5-18. Polymorphic information content (PIC) values ranged from 0.390 in primer 815 to 0.760 in primer 817. The primers with PIC value >0.5, were classified as highly informative. Three primers namely, 817, 818 and 825 were highly informative (Table 15).

Table 15. ISSR primers used in this study and their amplification results

Primer Name	Repeat Motif	Number of polymorphic Bands	Number of amplified Bands	Polymorphic information content (PIC)
814	$(GA)_6GG$	2	6	0.5
815	$(CAG)_3GC$	2	5	0.39
817	(GAC)TCC5	6	17	0.76
818	$(AG)_8G$	3	18	0.586
819	$(GATA)_8$	2	9	0.493
821	$(AG)_8T$	2	10	0.48
824	$TGTA(CA)_7$	2	9	0.494
825	$(CTT)_6$	3	15	0.567
Average Per primer		**2.75**	**11.13**	**0.53**

5.4.2 Genetic Diversity

Table 16 shows Nei's gene diversity (*h*) and Shannon's information index (*I*) for seven populations from different parts of the world. The Nei's gene diversity ranged from 0.164 in population 1 from East Africa to 0.417 in population 2 from East Asia with an average of 0.335 across all populations. Similarly Shannon's information index ranged from 0.245 in population 1 from East Africa to 0.605 in population 2, from East Asia with average of 0.494 across all the populations.

Table 16. Nei's gene diversity and Shannon's information index for different populations of *Corchorus spp*.

Population	Number of Individuals	Nei's gene diversity (*h*)	Shannon's information index (*I*)
1	18	0.1636	0.2447
2	16	0.4167	0.6045
3	4	0.3403	0.4977
4	11	0.3949	0.5769
5	20	0.3278	0.4966
6	9	0.2936	0.4490
7	5	0.4089	0.5877

Note: *Population 1 – East Africa, 2 – East Asia, 3 – North Africa, 4 – South Africa, 5 – South East Asia, 6 – Unknown, 7 – West Africa*.

Nei's measure of original genetic distance is summarized and presented in Table 17. The highest pairwise genetic distance (0.431) was observed between population 1 from East Africa and population 6 from unknown. The lowest pairwise genetic distance (0.0216) was recorded between population 2 from East Asia and population 4 from South Africa. Population from East Africa had the highest pairwise genetic distance as compared with other populations. This was also observed in population from North Africa. Other populations with lowest pairwise genetic

distance (0.0223) was observed between population 4 from South Africa and population 5 from South East Asia.

Table 17. Nei's measure of original genetic distance in *Corchorus spp.*

Populations	1	2	3	4	5	6	7
1	****						
2	0.1908	****					
3	0.2685	0.1135	****				
4	0.2180	0.0216	0.1264	****			
5	0.2812	0.0445	0.1517	0.0223	****		
6	0.4308	0.0671	0.1760	0.0557	0.0368	****	
7	0.2638	0.0554	0.1423	0.0486	0.0775	0.0701	****

Note: *Population 1 – East Africa, 2 – East Asia, 3 – North Africa, 4 – South Africa, 5 – South East Asia, 6 – Unknown, 7 – West Africa*.

Principal coordinate analysis results from the ISSR markers for the 83 accessions are presented in Fig. 7. PC1 and PC2 axis constituted 21.69% and 11.66 % of total variation, respectively. Accessions from East Africa were grouped in the right side of both positive and negative Y-axis in the first quadrant and fourth quadrant. Also accessions from East Asia and South East Asia were grouped with accessions from East Africa. The second quadrant contained mixed accessions but mainly from East and South East Asia. The unknown accessions were mainly found in second quadrant and third quadrant. The highly different accessions in their clustering pattern were four accessions from East Asia which clustered far from the rest in each quadrant. Two accessions from West Africa also clustered separately in the third quadrant as well as South African accessions in the fourth and second quadrant.

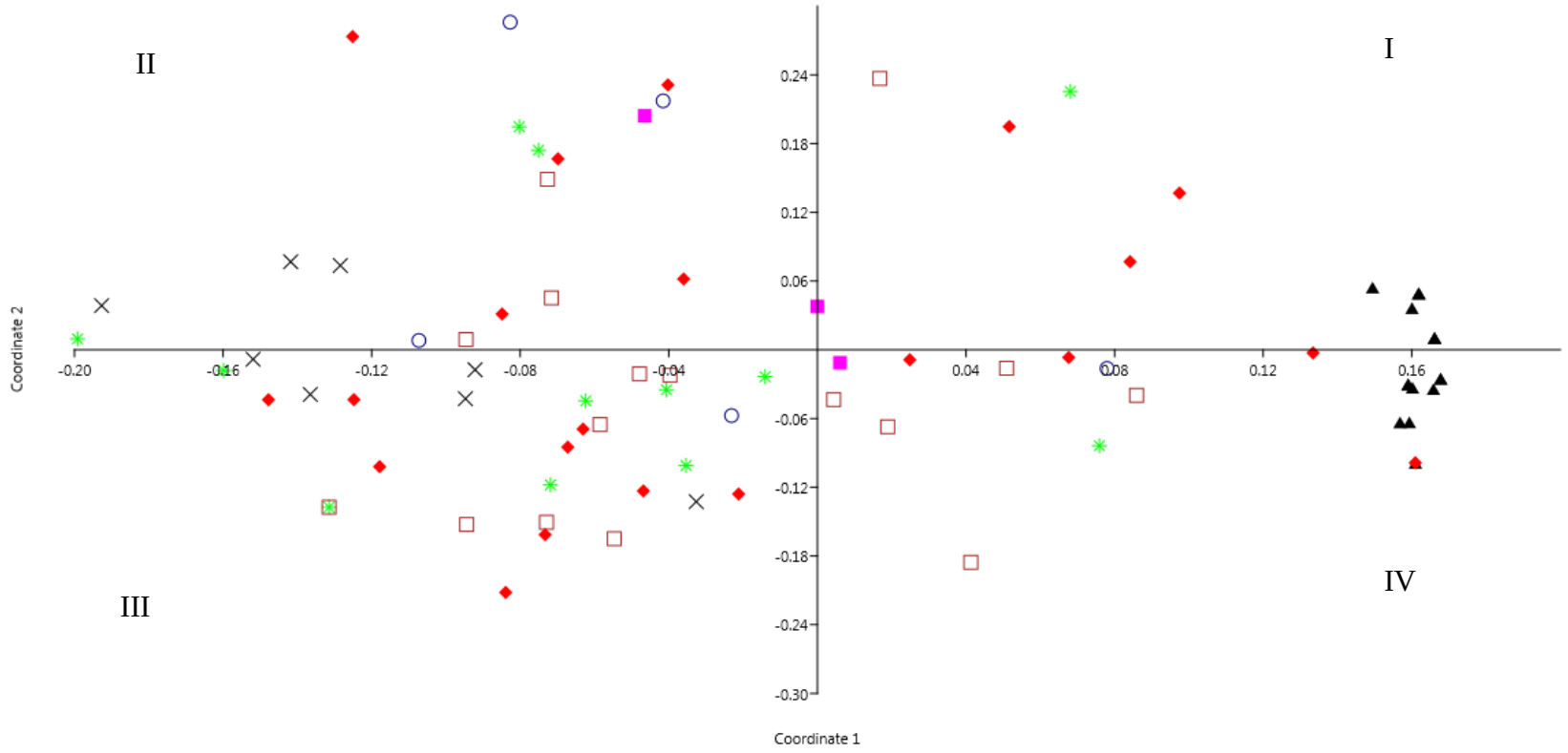

Figure 7: Scatter plot of a PCoA showing the distribution of *Corchorus spp*. accessions based on ISSR data

Key: The populations are presented in different symbols based on their origin. ♦ - East Asia, ▲ – East Africa, □ – South East Asia, ■ – North Africa, ○ - West Africa, * - South Africa, X – Unknown

5.4.3 Cluster Analysis

UPGMA tree showed 5 main clusters in the collection of 83 accessions from this germplasm Fig. 8. The first cluster contained 2 accessions, one from Sudan and one from Bangladesh. The second cluster contained 6 accessions, 4 from Bangladesh, 2 from Cameroon. Cluster three contained 31 accessions. This was the largest cluster with mixed accessions from different countries but mainly from Asia (19 accessions). Cluster four contained 15 accessions, 5 from South Africa, 7 from South East Asia, 2 accessions from South Africa, one from North Africa and one from West Africa. Cluster five contained 29 accessions, 17 from East Africa (the upper sub cluster), 8 from South East Asia, 2 from South Africa, 1 from North Africa and 1 from West Africa.

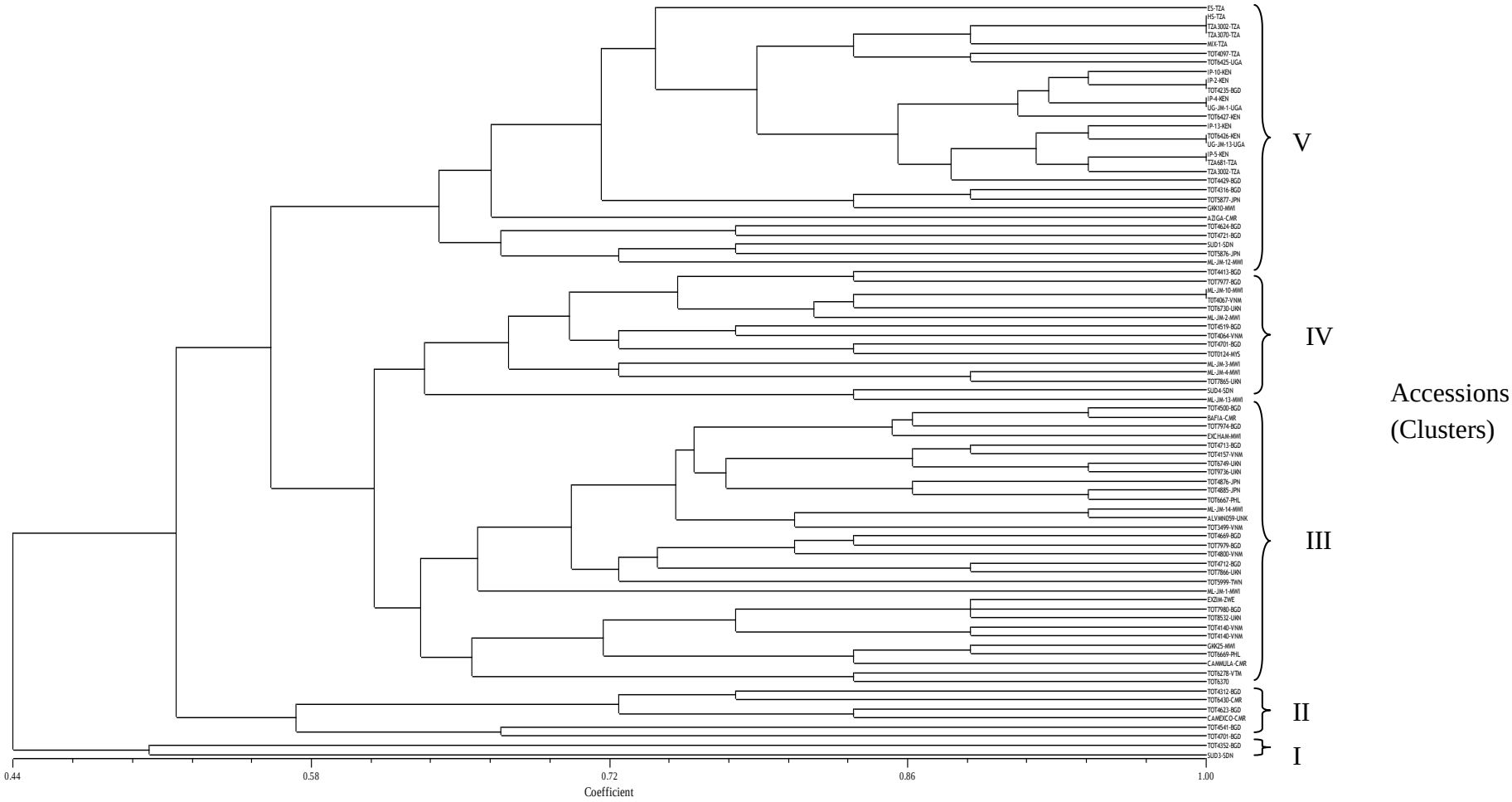

Figure 8: Dendrogram constructed from cluster analysis based on UPGMA using the SM coefficient option for 83 accessions of *Corchorus spp.*

Key: Country of Origin of each individual is given with 3 – digit international codes after accession name.

5.5 Discussion

Assessment of genetic diversity within cultivated crops has important implications in breeding for improvement and conservation of genetic resources. Molecular markers can be employed as a tool to reveal the diversity within crop species. In this study ISSR markers were used to assess the diversity of 83 accessions of Jute mallow which is an indigenous vegetable.

In the current study, the number of polymorphic bands ranged from 2-6 with an average of 2.75 bands per primer. The number of bands amplified ranged from 5 to 18 bands. Roy *et al*. (2006) reported a relatively high range of amplified fragments which ranged from 12 to 21 by using four ISSR primers of Jute mallow. High PIC values in this study clearly indicated usefulness and applicability of the markers used in establishing the diversity within the studied accessions. A PIC value of 0.34 from primer $(CCT)_6$ is reported as the highest when polymorphism was considered across all species by Roy *et al*. (2006). This was in contrast to this study where the highest PIC value was 0.76 from primer (GAC) TCC5. This was the most informative primer compared with the remaining primers in present study. This was attributed to primer differences as well as number and types of accessions used in each study.

The highest Nei's gene diversity (0.42) was observed in population from East Asia and West Africa. High number of accessions from East Asia was used in this study and that could be a reason for the observed high diversity. Relatively higher genetic diversity was detected in African populations by Benor *et al*. (2012) using AFLP markers. This is similar to study where the overall genetic diversity detected in African populations was higher except for a population from East Africa. Low gene diversity was recorded in East African accessions indicating high similarity of the accessions in the region. In all these observations, Shannon's information index values were relatively higher than Nei's gene diversity. Apart from gene diversity based on different origins; average gene diversity (*h*) for complete set of all accessions was 0.34 and Shannon's information index was 0.49. This shows that there is relatively high level of genetic variation among these accessions. Similar results were reported in *Nothapodytes nimmoniana*, an endangered medicinal plant in India ($h = 0.3$; $I = 0.44$) (Kareem *et al*., 2011). Wang *et al*. (2012) reported a gene diversity of 0.29 in goat's rue accessions using ISSR markers.

Highest pairwise genetic distance was observed between the unknown accessions and the East African accessions. This indicates that East African accessions were more distinguished from the unknown accessions and the rest of other accessions. These results corroborated with the Nei's gene diversity recorded in this study. Higher genetic distances in populations were recorded from East and North Africa populations than from the rest of other populations under the present study. The remaining populations were relatively similar. This similarity may be due to sharing of genetic materials between different regions or possibly due to common parents especially for accessions from East Asia and South East Asia where there is long history of domestication and breeding of Jute mallow (Kundu, 1951).

In PCoA where PC1 and PC2 constituted 33.4%, accessions from East Africa were distinctly found in the first and fourth quadrant separated from other accessions. This pattern of clustering is also supported by the results of Nei's pairwise genetic distance reported in this study. In that it showed the highest distance between these accessions and the rest. There was no clearly defined pattern of clustering for the remaining accessions as they overlapped in different quadrants of the PCoA. Accessions such as Cameroon Ex. Co, TOT 4623, TOT 4624, GKK 10, ES and ML-JM-10 displayed the highest diversity across all four quadrants. In cluster analysis, 5 clusters were obtained at genetic similarity coefficient of 0.64. However, the clustering of geographically closer accessions was not clearly reflected in the dendrogram except for cluster 5 which contained accessions mainly from Africa. This showed that the association between genetic similarity and geographical location was insignificant. Similar results were reported in goat's rue (Wang *et al*., 2011) and in Azuki bean (Yee *et al*., 1999). Although there was no clearly defined clustering pattern reflecting geographical distribution of the accessions in the five clusters generated, generally accessions from Africa and Asia were grouped separately. This could be attributed to species differences where most of Asian accessions were from *C. capsularis* whereas those from Africa were mostly from *C. olitorius*.

5.6 Conclusion

Our results indicated the presence of high genetic diversity among Jute mallow collection. Genetic variation among this germplasm as revealed by ISSR markers could be useful in selection of parental lines that can be crossed to generate populations that are suitable for

breeding. The findings of this study can also be used in conservation of this underutilized vegetable.

CHAPTERSIX

Leaf and seed yield of Jute mallow (*Corchorus spp*.) accessions under field conditions for two consecutive growing seasons[5]

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Abstract

Jute mallow (*Corchorus spp*.) is a nutritious leaf vegetable. It represents a cheap but quality source of nutrition for many people in urban and rural areas in Africa. In this study the purpose was to select accessions with high leaf yield and seed yield. We investigated seven agronomic traits that are related to leaf yield and seed yield and how they correlate with each other. The results indicated significant differences among the accessions in all traits. Leaf fresh weight ranged from 18.3g/plant to 121.3g/plant in accessions TOT 6747 and TOT 8532, respectively. Highest variability between the accessions was also observed in seed yield. It ranged from 1.0g/plant to 35.5g/plant in accessions TOT 7980 including eight others and TOT 7866, respectively. Significant and positive correlations were observed between leaf fresh weight and leaf dry weight (r= 0.84), leaf area (r=0.33) and number of leaves per plant (r=0.40). Significant correlation was observed in seed yield and weight of pods per plant (r= 0.83). Evaluation of these agronomic traits for leaf and seed yield in this germplasm is insightful and has revealed important information useful for breeders in their efforts to improve the yield of this vegetable as well as selection of accessions with good agronomic traits.

Key words: *Corchorus spp*., Jute mallow, Germplasm, Leaf area, Leaf yield, Seed yield

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6.1 Introduction

Corchorus spp. is known in tropics and widely grown as vegetable for viscosity of its leaves either freshly prepared or sun dried. It is one of the popular tropical leafy vegetables in Africa, Asia, some parts of Middle East and Latin America (Adediran *et al*., 2015). The young leaves of many species with short and branched stems are widely used as leaf vegetables (Velempini *et al*., 2003). When boiled or cooked, the leaves produce mucilage, a property useful in making sauces to accompany the coarse starchy foods (Benor *et al*., 2010).

Jute mallow like other traditional leafy vegetables represents a cheap but quality nutrition for large segments of the population in urban and rural areas (Freiberger *et al.*, 1998; Kinabo *et al.*, 2006; van Rensburg Willem *et al.*, 2007; Lewu and Mavengahama, 2010). Nutritionally, Jute mallow leaves contain an average of 15% dry matter, 4.8 g of protein, 259 mg of calcium, 4.5 mg of iron, 4.7 mg of vitamin A, 92 µg of folate, 1.5 mg of nicotinamide and 105 mg of ascorbic acid per 100 g of leave (Grubben and Denton, 2004; Odhav *et al.*, 2007; Dansi *et al.*, 2008).

In many African countries, particularly Tanzania, commercial farming of Jute mallow is practiced to a very limited extent compared with other vegetables. However, for a long time, it has been semi-domesticated, and sometimes grows as a volunteer crop in farmers' fields and on fertile soils close to and around most homesteads (Mnzava, 1997). The status of Jute mallow as a volunteer or wild plant renders it unappealing for consideration in crop development programs and is often seen as an orphaned crop. In Kenya, for instance, limited research identified Jute mallow as a vegetable that could be developed for commercial production (K'opondo *et al.*, 2005).

In order to improve this vegetable production in Tanzania, efforts must be directed towards selection of promising accessions with good adaptation to the local enviroment and including them inbreeding programmes for improvement of desirable traits. The most important aspect that will facilitate production relies on the ability of these accessions to produce high amount of leaves which is the harvestable part as well as high seed yield for its propagation. Lack of seeds is reported as a problem limiting production of Jute mallow (Opabode and Adebooye, 2005). Little information is reported on the availability of improved genotypes of Jute mallow with

good leaf and seed yield as well as with good adaptation to local environments.

A number of studies on Jute mallow under different environments have been conducted to assess the leaf yield and its components (Olaniyi and Ajibola, 2008; Fasinmirin and Olufayo, 2009; Makinde *et al.*, 2009; Adediran *et al.*, 2015). Plant spacing and leaves harvesting frequency significantly affects leaf yield and seed yield (Madakadze *et al.*, 2007). Aluko *et al.* (2014) observed that plants treated with fertilizer (2.5 t/ha of organic fertilizer mixed with 75kg of NPK) had thicker stems, more leaves and higher dry matter as opposed to unfertilized plant. Similar results were reported by Adediran *et al.* (2015) in which poultry manure significantly improved all growth parameters of Jute mallow as compared with other nutrient sources.Successful production of Jute mallow seeds is influenced by factors such as season of crop growth, disease incidence and severity and difficult in drying fruits after harvest during rain (Tindall, 1965; Van Epenhuijsen, 1974).

In many studies conducted, the main focus has been the effect of environments factors on yield of leaves and seeds. No information is availabe on selection of Jute mallow accessions for seed set ability. However, for the purpose of germplasm characterization and selection of promising accesions adapted to local environment, varietal or accessions differences in leaf and seed production under similar environment is crucial for meaningful selection. The present study therefore aims to explore the variation in accessions in terms of leaf production and seeds under similar environment and then assess whether significant correlations exist between leaf yield and seed yield related attributes.

6.2 Materials and Methods

6.2.1 Study location and Experimental Material

Field experiments were carried out at World Vegetable Centre, Eastern and Southern Africa based at Arusha Tanzania. Experimental field was located at Madiira farm with an altitude of 1262m above the sea level, latitude 03.38^{o}S and longitude 36.87^{o}E. Experiments were carried out for two growing seasons in 2015 and 2016 (from February to June). The soils are well drained. The monthly total rainfall and monthly average temperature during the growing season are presented in Fig. 9.

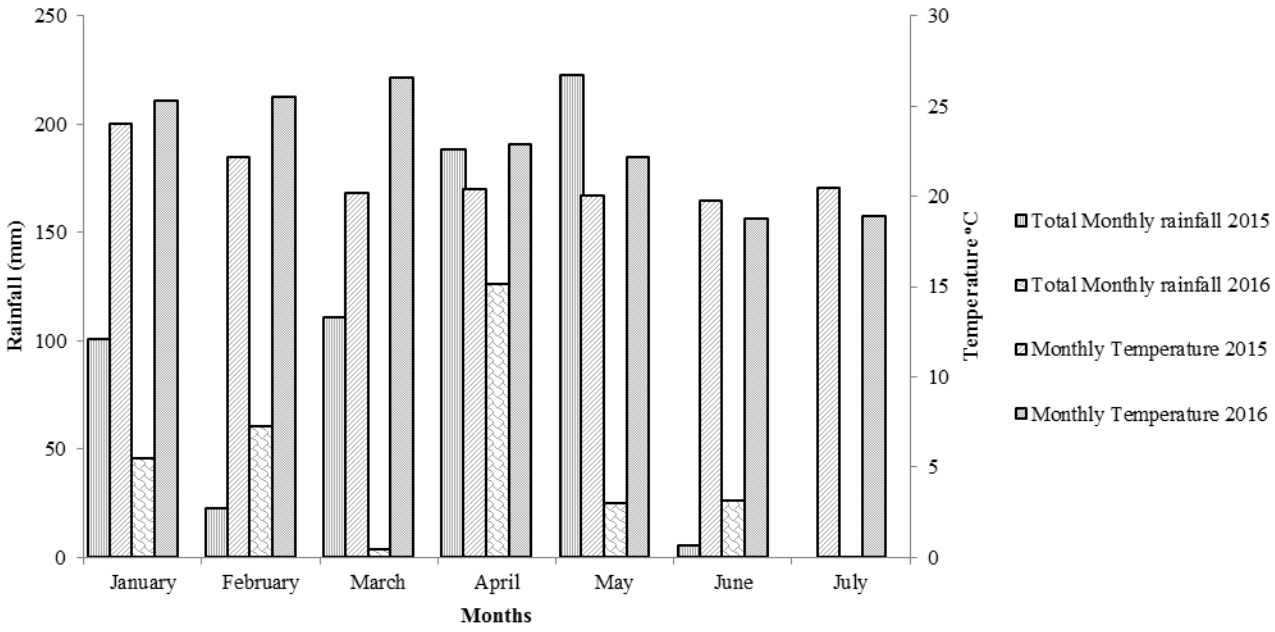


Figure 9: Meteorological data on monthly average temperature and total monthly rainfall during the two growing seasons

The experimental materials comprised of 90 accessions of Jute mallow (*Corchorus spp.*) provided by seed repository of the World Vegetable Centre, Eastern and Southern Africa. These accessions originated from fourteen countries; Tanzania, Cameroon, Malawi, Kenya, Uganda, Sudan, USA, Japan, Zimbabwe, Vietnam, Malaysia, Bangladesh, Philippines and Taiwan. Six accessions out of these had unknown origins. The accessions represent a part of the center's germplasm of different types of traditional vegetables that are locally grown by farmers.

6.2.2 Experimental Design and Layout

Seeds of each accession were sown in a separate plastic tray (PVC) with 66 holes (4cm diameter and 4cm height) by using sterilized soil. The trays were kept in a screen house to raise the seedling at temperature of 27-30^{o}C and irrigated by water cane twice a day. The relative humidity of the screen house on average was 80%. After 28 days, the seedlings were transplanted to the field. The plot size was 3m x 0.6m with three replications in a complete randomized block design (CRBD). Each accession was planted in two rows and the space

between plants was 0.25m with 12 plants per row. Fertilizer application was done one week after transplanting. Diammoniumphosphate (DAP) at application rate of 143.8 kgP/Ha and Urea at application rate of 55.2 kgN/Ha was used. The experiments were conducted during the rainfall season; the plants were only supplemented with irrigation when necessary. Manual weeding was carried out regularly to maintain weed free plots.

6.3 Data collection and Analysis

Five plants per plot were selected randomly and the following data were taken from them; number of leaves per plant (counted), leaf fresh weight (harvested simultaneously three times at an interval of 14 to 21 days), leaves dry weight (oven dried at 80^{o}C for 24 hours until constant weight attained). Leaf moisture content was estimated by using the following formula;

$$MC\ \% = \frac{\text{Leaf fresh weight - Leaf Dry weight}}{\text{Leaf fresh weight}} \times 100$$

Where: MC = Moisture content

Other morphological characters included number of pods per plant (counted from five plants and averaged to one plant), weight of pods per plant, weight of seeds per plant, measurement of leaf length and width for ten leaves selected from the five plants and averaged to one value for calculation of Leaf area.

Leaf area was calculated according to (Peksen, 2007).

$$LA = 0.919 + 0.682LW$$

Where: LA = Leaf Area

L= Leaf length

W= Leaf width

Each of the five plants value was averaged to get a single value for each plot. Then the average value for each plot in each replication for both seasons was calculated. This pooled mean of the

two growing seasons from each plot was subjected to analysis of variance (ANOVA). Descriptive statistics i.e mean, minimum, maximum and standard error was calculated for the eight traits.

Model and Analysis of (RCBD) design was as follows:-

$Y_{hi} = \mu + \theta h + ri + \varepsilon hi$

Where:

Y_{hi}= is the random variable representing response of accession *i* observed in block *h*

μ = Overall mean

θh = Block Effect

ri = Treatment effect (Accession)

εhi = Random error for i^{th} accession in h^{th} block.

Pearson's correlation method was used to compute correlation coefficients so as to investigate the degree of association between the traits. These computations were done by using statistical software program STATISTICA version 12, edition 2013 (Statsoft inc., Tulsa, OK, USA). Fisher least significance was used to compare means at P=0.05 level of significance.

6.4 Results

6.4.1 Leaves fresh weight and dry weight

The results of analysis of variance indicated significant ($p<0.001$) differences among the accessions in all traits studied (Table 18). Both leaf fresh weight yield and leaf dry weight yield was lowest in accession ML-JM-7 and highest in accession TOT 8532. Leaf area was lowest in accession TOT 9736 and highest in accession Bafia. Accession TOT 6730 had lowest number of leaves per plant and highest number of leaves per plant was found in TOT 4051. The highest moisture content was found in accession Kipumbuliko and TOT 4519 had lowest moisture content (Table 18).

The smallest leaf area (value given per leaf) among the top five accessions was observed in TOT 4051. This accession had highest number of leaves compared with all other accessions in this study (Table 18). It was observed that most of these accessions with small leaves also contained many primary and secondary branches. Accessions with highest leaf area were Bafia, TOT 7974, Aziga, TOT 8532 and Kipumbuliko (Data not shown). These accessions had lowest number of leaves (124, 272, 101, and 158) well below the average, as compared with the rest of other accessions. These accessions had few primary and secondary branches. Exception was found TOT 8532 which had highest number of leaves among them (623) well above the average for all accessions, and it had highest fresh leaves yield. Thus the combination of both high number of leaves and large leaf area resulted into high leaf yield. Accessions with highest leaf yield among all accessions under the study are presented in Figure (10a-e).

(a) TOT 8532 (b) TOT 4051 (c) GKK 25 (d) TOT 7980 (e) TOT 4713

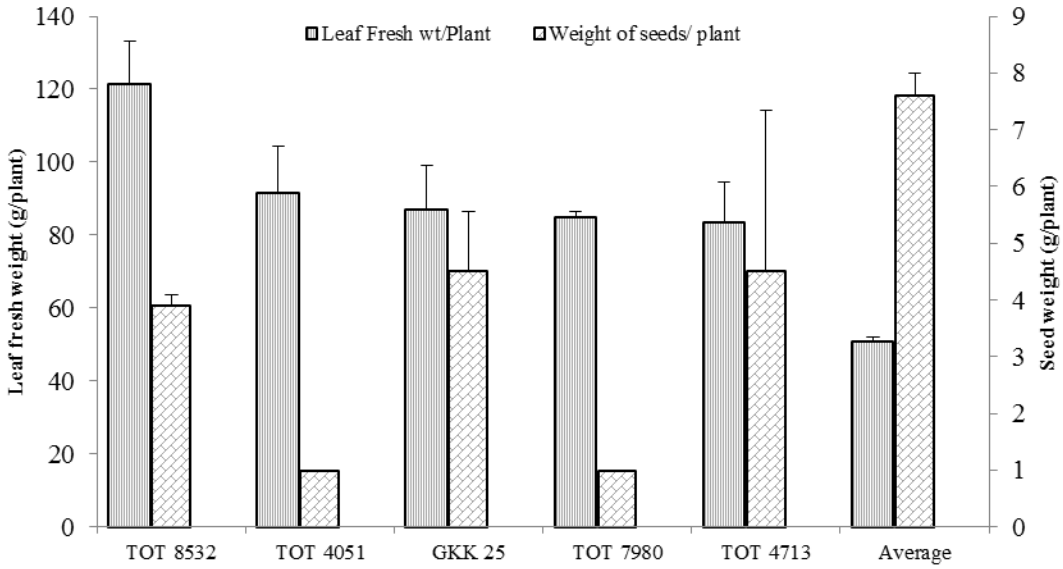
Figure 10: (a) – (e) Accessions with high leaf yield

Table 18. Descriptive statistics and analysis of variance of eight leaf and seed yield related traits of the 90 accessions

	Leaf fresh weight/plant	Leaf dry weight/plant	Leaf Area (value per leaf)	Number of leaves/plant	Weight of pods/plant	Weight of seeds/plant	Number of pods	Moisture Content (%)
Mean	50.68***	14.10***	44.66***	289.58***	19.53***	7.61***	112.24***	71.9**
Maximum	121.3	32.6	106.97	1089.67	55.03	35.53	430.33	80.6
Minimum	9.2	3.4	14.07	31.00	4.33	1.00	9.3	59.1
S.E	1.24	0.39	1.07	11.42	0.79	0.39	6.49	0.40

= Significant at P<0.01, *=Significant at P<0.001

Fig. 11 shows five accessions with highest leaf yields compared with all other accessions in this study. Fig. 11(a) shows five accessions highest for fresh leaf yield. In Fig. 11(b) five accessions highest for dry leaf yield are presented. Leaf yield in both figures was compared with respective seed yield of individual accession. Accession GKK 25 produced highest seed yield among the top five accessions in leaf yield, this seed yield was below the average seed yield of all accessions and statistically significantly different from the average seed yield.



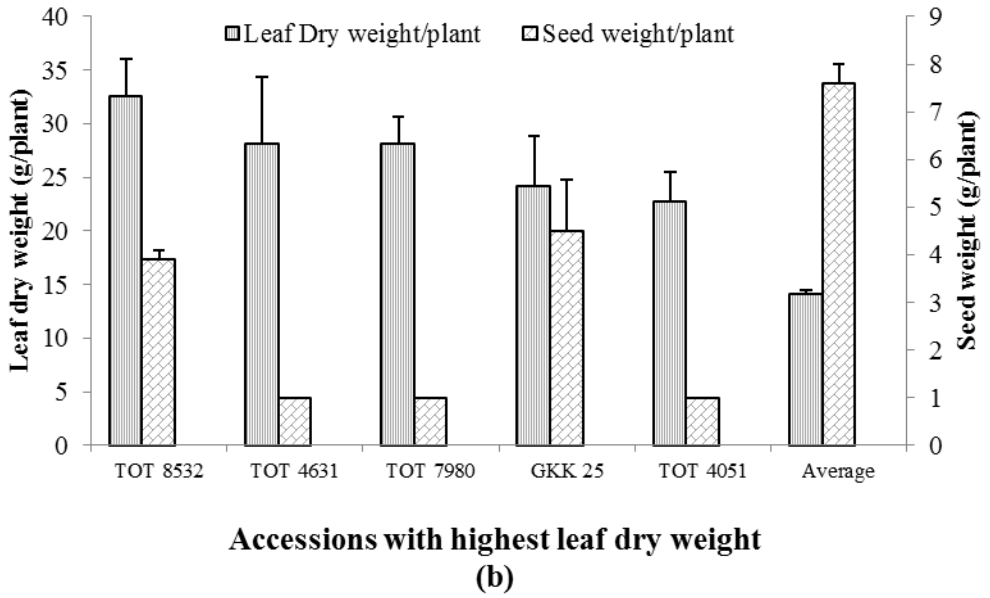
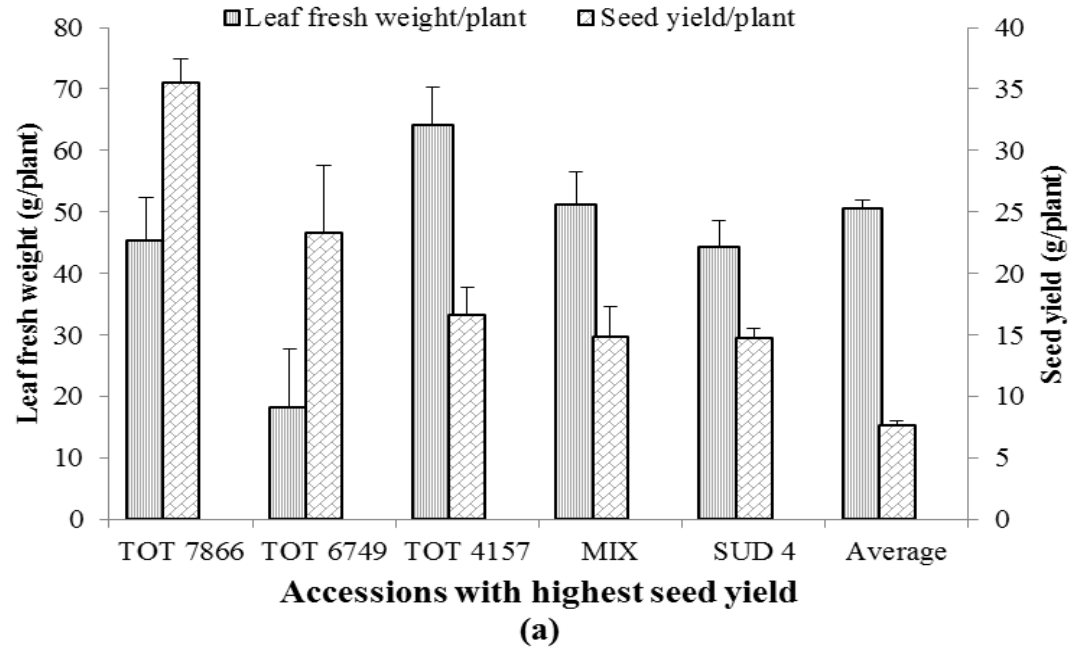


Figure 11: (a) Accessions with highest leaf fresh weight yield and their seed yield and (b) Accessions with highest dry weight and their seed yield

6.4.2 Seed yield

Seed yield varied significantly across all accessions. TOT 7866 had the highest seed yield compared with all other accessions, while accession TOT 7980 had lowest seed yield (Table 18).Other accessions with lowest seed yield include TOT 4051, TOT 4631, TOT 0124, TOT 4708, TOT 3499, TOT 4670, Kipumbuliko and TOT 6730 (Data not shown).

Five accessions with highest seed and pods yields compared with all other accessions in this study are shown in Fig. 12. Fig. 12 (a) shows five accessions highest for seed yield. Five accessions highest for pods yield are presented in Fig. 12 (b). Seed yield in Fig. 12 (a) was compared with leaf fresh weight and in Fig. 12 (b) pods yield was compared with seed yield.



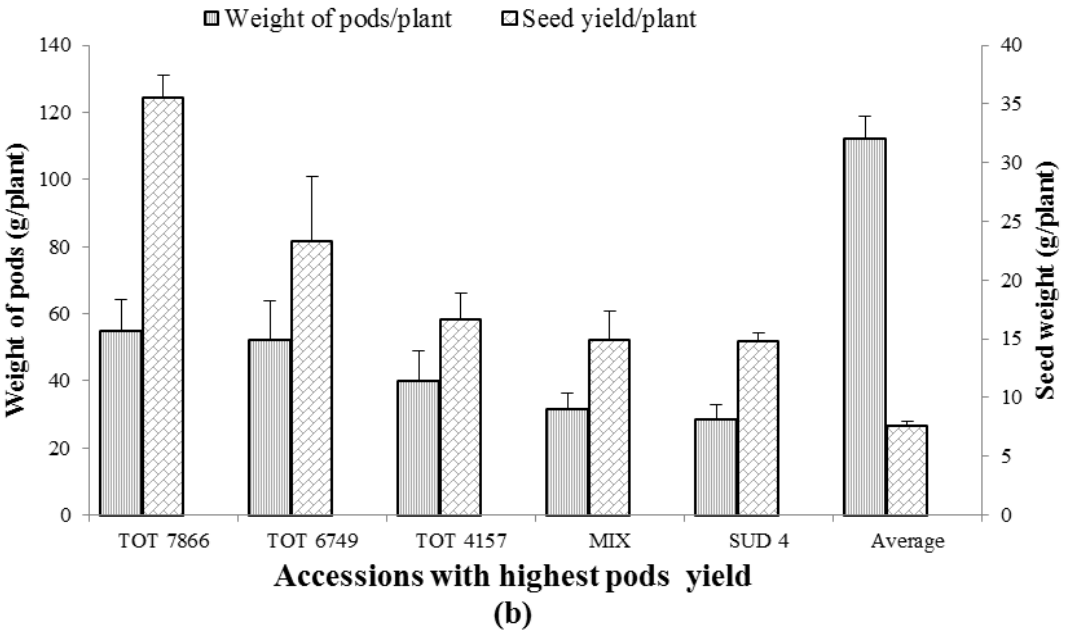


Figure 12: (a) Accessions with highest seed yield and their leaf fresh weight yield and (b) Accessions with highest dry weight and their seed yield

The accessions highest for seed yield showed low leaf yield, three of them well below the average leaf yield for all accessions Fig. 12(a). In Fig. 12(b), high weight of pods is associated with high seed yield i.e the higher the pods weight the higher the seed yield.

6.4.3 Simple correlations between leaf and seed yield related attributes

Fresh leaf yield per plant was significantly and positively (r=0.84, <0.01) correlated with leaf dry weight (Table 19)**.** Leaf area was positively (r=0.33, <0.001.) correlated with leaf fresh weight. Number of leaves per plant was significantly correlated with fresh leaf yield (r=0.40, <0.001). Leaf fresh weight was negatively correlated with weight of pods per plant and weight of seeds per plant. Number of pods per plant and weight of seeds per plant were negatively correlated in this study because some locules and seed sockets in these pods had brownish and poorly developed seeds with light weight and shrunken.

But significant and positive correlation was observed between number of pods per plant and number of leaves per plant as well as leaf fresh weight (r=0.51, <0.001 and r=0.18, <0.01), respectively. Weight of pods per plant was positively and significantly correlated with seed yield per plant (r=0.83, <0.01). Leaf area was negatively correlated with seed yield per plant (r= -0.03) and positively correlated with pods yield per plant (r=0.69), but in both cases there was no significant correlation.

Table 19. Correlation coefficient matrix of leaf yield and seed yield related traits

	Leaf Fresh weight per Plant	Leaf Dry weight per Plant	Weight of pods per plant	Weight seeds per plant	Leaf Area	Number of Leaves per plant	Number of Pods per plant
Leaf Fresh weight per Plant							
Leaf Dry weight per Plant	0.84**						
Weight of pods per plant	-0.16**	-0.07^{ns}					
Weight seeds per plant	-0.22***	-0.15*	0.83**				
Leaf Area	0.33***	0.30***	0.69^{ns}	-0.03^{ns}			
Number of Leaves per plant	0.40***	0.33***	-0.21***	-0.19**	0.21**		
Number of Pods per plant	0.18**	0.18**	-0.25***	-0.27***	-0.28***	0.51***	

*= Significant at P<0.05, **=Significant at P<0.01, ***=Significant at P<0.001, ns= Non-significant.

6.5 Discussion

Significant variations in leaf yield and its attributes were observed among the accessions in this study. This variation is attributed to differences between accessions in terms of other leaf yield related attributes. It is unfortunate that high fresh leaf yield was associated with significant low seed yield well below average.

Significant positive correlation was also observed between fresh leaf yield and dry leaf yield. The top five accessions with high leaf fresh yield with exception of one accession TOT 4713 had high dry leaf yield as well. The differences observed are likely due to variations in moisture

content among the accessions. In terms of leaf area (value per leaf), of the top five accessions with high leaf fresh weight, three accessions (TOT 8532, GKK 25 and TOT 4713) had a leaf area of 74.13, 50.9 and 50.8cm^2, respectively well above the average leaf area (44.6) of all accessions. This indicates that leaf area is important trait in selection of accessions with high leaf yield. Leaf area is affected by plant spacing (Makinde *et al.*, 2009) and it can also be affected by frequency of leaf harvesting (Madakadze *et al.*, 2007). TOT 4051 had a smallest leaf area (33.33) of the five accessions with highest fresh leaf yield but had highest number of leaves (1089.7) compared with all other accessions in this study. High number of leaves has contributed to the high yield in leaf fresh weight of this accession. Thus not only leaf area can play part in contributing to leaf yield but also the number of leaves. Similarly, other top five accessions had higher number of leaves well above the average of all accessions. Fasinmirin and Olufayo (2009) noticed significant differences in number of leaves in their study of Jute mallow resulting from different soil moisture regimes. Good soil moisture content was associated with high leaf formation.

In terms of weight of pods per plant, the top five accessions in seed yield had high weight of pods which corresponds to their seed yield except two accessions (MIX and SUD-4). These two accessions showed low weight of pods per plant but reasonably high seed yield. This indicates that the pods from these accessions had high amount of seeds and less biomass compared with the other three accessions. This is similar to observation of Madakadze *et al* .(2007) in Jute mallow, where significant increase in seed yield which was associated with decreased number of pods per plant was recorded. The highest seed yield in this study (35.5g/plant) is equivalent to 2.9t/ha. Seed yield of 2.78t/ha has been attained by Madakadze *et al.* (2007) as the highest in their study by using a spacing of 0.5m x 0.1m. The authors found that variation in seed yield is cultivar and cultural management dependent. Other accessions had very low leaf yield. This may be attributed to poor adaptation of the accessions to the cultivation environment as this is known to affect seed production adversely (Hartmann and Kester, 1963). In our study, the main variation resulted from the differences in accessions; plant spacing was maintained at 0.6m x 0.25m.

Strong correlation between leaf yield per plant and dry leaf weight per plant suggests that the higher the fresh weight the higher the leaf dry weight though there is some slight variation in leaf moisture content among the accessions. Moisture content varied from one accession to another

indicating that leaf dry weight will be affected with inherent moisture content of the respective accession as compared with another accession with the same leaf fresh weight but with different moisture content. Leaf area was positively correlated with leaf fresh weight indicating that leaf area is important yield component and it should be considered during selection of promising accessions. Leaf surface area contributes to quantity of food synthesized by plant during photosynthesis (Kisua *et al.*, 2015). Accessions with high number of leaves are likely to have higher photosynthetic ability that can contribute to growth and development of the plant. Leaf fresh weight was negatively correlated with weight of pods per plant and weight of seeds per plant; this shows that it is important to strike a balance between seed yield and leaf yield for sustainable production of this vegetable. The number of leaves per plant is negatively and significantly correlated to both weights of pods per plant and seed yield per plant. These findings are similar to results of Mills and Jones (1979) with bell peppers that indicated low pod yield as result of excessive N treatment which stimulated more vegetative growth and reduced flowering. Contrary to our results, Mathowa *et al.* (2014) reported high number of leaves per plant which was correlated with higher pod weight in *Corchorus olitorius*. However, strong and positive correlation was observed between numbers of leaves per plant and number of pods per plant. For crops grown for their leaves, good vegetative growth before flowering and seed set results into high seed yield (Tindall, 1965; Van Epenhuijsen, 1974; Grubben *et al.*, 1977). This corroborate to what Johnson and Decoteau (1996) reported on Jalapeno pepper where plant biomass and pod productions were found to be highly correlated. These results did not match with our study because despite having positive correlation between number of leaves per plant and number of pods per plant like Mathowa *et al.* (2014), some pods in our study had locules and seed sockets with brownish and poorly developed seeds.

Normally high number and high weight of pods could contribute to the amount of seeds produced by a particular accession while big leaf area and high number of leaves can contribute significantly to leaf yield. Generally, the strong correlation observed between leaf yields related traits such as leaf area and number of leaves and seed yield related traits such as weight of pods per plant indicates the importance of these agronomic traits in selection of good accessions with promising vegetative and seed yield potential.

6.6 Conclusion

The variation in leaf yield of these accessions resulting from their genotypic differences was significant indicating the potential of useful selection out of this germplasm for accessions with good agronomic traits. The strong and positive correlations between leaf yields related traits indicated the importance of focusing to more than one agronomic trait during selection process. Seed yield also varied significantly among the accessions. By considering both seed yield and leaf yield as an important agronomic traits for selection of promising accessions, the challenge becomes how to balance leaf yield and seed yield as these were observed to be inversely related that is accessions with high leaf yield had relatively low seed yield. Nevertheless, leaf yield as a harvestable part remains more important than seed yield. Seed yield could be optimized through breeding. More studies are recommended for evaluation of how seed yield can be affected by leaf harvesting and how seed yield can be increased in case the aim is to produce seeds.

CHAPTER SEVEN

7.1 General Discussion, Conclusion and Recommendations

In this study, chapter one showed background information and importance of this underutilized and neglected crop. Chapter two is the review of literature, where the significance of Jute mallow as vegetable crop with potential to widen the nutritional base of vegetables is discussed. Despite the nutritional importance of Jute mallow, there is no commercial production of this crop in many parts of Africa including Tanzania. Among the constraints encountered by farmers include lack of information about nutritional importance of the crop (ethno-botanical knowledge) as well as availability of improved varieties which are adapted to specific environment. Little is known about the variation and diversity of the available accessions. Thus the crop grows as volunteer crop in farmers' fields. In areas where cultivation is practiced to a limited extent, the different accessions that the farmers are using are traditionally inherited and locally shared among farmers (Denton and Nwangburuka, 2012).

This study is therefore aimed to create a platform for breeding of improved varieties of Jute mallow with high leaf yield and adapted to specific environment. To achieve this goal, 90 accessions of Jute mallow collected from farmers' fields as well as from different gene banks and preserved *ex-situ* for breeding and other research purposes were used for morphological and molecular characterization. The same accessions were evaluated for their leaf and seed yield under field conditions for two consecutive growing seasons.

An insight was gained with regard to both morphological and molecular diversity of these accessions as well as their adaptation for seed and leaf yield. Multivariate analysis of both quantitative and qualitative traits showed that accessions were morphologically very diverse. These variations could be attributed to differences in genetic makeup of the accessions. High variation was observed in harvest index, biomass yield and 1000 seed weight. Traits such as primary branches, secondary branches and leaves per plant accounted for highest variability observed in the accessions according to Principal Component Analysis (PCA).Similar results are reported by Nyadanu *et al.* (2016). High plant height and high number of primary branches was positively correlated with biomass yield indicating that these traits can be used as selection

criteria for accessions with high leaf yield. Yield is a combination of different factors and traits with strong positive correlations can be improved simultaneously during breeding.

Cluster analysis grouped the accessions into five distinct clusters mainly based on their origin. This suggested that materials from same geographical origin had common evolutionary relationship. Few exceptions were found in cluster number four where accessions from different areas were grouped together indicating the movement of germplasm materials from one place to another as previously reported by Edmonds (1990). This also suggested the diversity of accessions within same geographical area. Similar results were reported by Nyadanu *et al.* (2016) in Ghana. Though morphological markers are known to be influenced by environment (Falconer *et al.,* 1996), a meaningful selection can still be made based on these observations. Thus this morphological diversity makes this germplasm collection from different parts of the world, useful parental stock for breeding improved varieties.

Molecular analysis employed both ISSR and SSR microsatellite markers. Both markers revealed high genetic variability among the accessions. Both morphological and molecular studies indicated the distinctiveness of the accessions from Asia and Africa. High number of primary and secondary branches was detected in accessions from Africa as compared with low number of branches observed in Asian accessions which are bred for fiber production. This is in agreement with molecular study where the clustering pattern of the accessions from Africa and Asia was different. ISSR markers were more informative than SSR markers in this study based on relatively higher PIC values and number of polymorphic bands observed. This might be due to the horizontal gel (agarose) that was used for SSR. SSR markers can be more informative than ISSR markers when vertical gel (Polyacrylamide gel electrophoresis) is used due to its high resolution. Similar results were reported in Ruthenia Medic based on percent polymorphic bands by using ISSR and SSR markers (Li *et al.*, 2013). The in formativeness of markers depends on genome coverage and sequence type recognized by each marker (Powell *et al.*, 1996; Sehgal and Raina, 2005). ISSR markers are distributed throughout the genome thus revealing the diversity of the entire genome whereas SSR markers only amplified target region of the open reading frame (ORF), the functional regions (Gimenes *et al.*, 2007; Yang *et al.*, 2010). Thus this may have also contributed to the different dendrograms obtained by the two markers used in this study.

Significant variations in seed and leaf yield and its components were observed among accessions in this study. High leaf yield was associated with low seed yield. This indicated the need to select accessions with balanced seed and leaf yield. Seed is important factor to consider in food production because it is the quality of seed that determines performance of crops (Achigan–Dako *et al.*, 2010). High leaf yield was also associated with large leaf area and high number of leaves per plant. These traits can be improved through breeding simultaneously due to their positive correlation. Leaf dry weight is important factor to consider during selection of accessions with good qualities desired by farmers. Farmers tend to dry the leaves for future use. In this case, the same accessions with the highest fresh leaf yield had highest leaf dry weight except TOT 4631 which had higher dry weight than that of TOT 4051. Seed yield was highest in accessions TOT 7866, TOT 6749, TOT 4157, MIX and SUD 4. The highest seed yield per plant attained is equivalent to 2.9 t/ha. Madakadze *et al.* (2007) reported a yield of 2.78 t/ha as highest yield attained in their study at plant spacing of 0.5m x 0.1m. Seed yield per unit area is affected by plant spacing and it plays a critical role in defining seed yield. In this study, plant spacing was maintained at 0.6 m x 0.25 m.

7.2 General Conclusion

Accessions in this study can be a potential source of allele diversity for breeding of improved genotypes due to high genetic diversity. Accessions such as Cameroon Mula, Bafia and Aziga from West Africa displayed highest diversity. Other accessions with high diversity include Sudan 2 from North Africa, UG-JM-13, ES, HS and TZA 681 from East Africa, ML-JM-4 and Ex-Zimbabwe from South Africa and TOT 6667 and TOT 7974 from Asia. Genetic variation among these germplasm as revealed by both SSR and ISSR markers could be useful in selection of parental lines that can be crossed to generate populations that are suitable for breeding. In terms of leaf yield; accessions TOT 8532, TOT 4051, GKK 25, TOT 7980 and TOT 4713 had highest leaf yield. Despite their low seed yield, these accessions can be preliminarily selected for cultivation by farmers. Leaf yield and seed yield as important agronomic traits should be considered during selection of promising accessions while taking into consideration the balance between the two traits as they appear to inversely relate to each other in the present study. Nevertheless leaf yield as a harvestable part remains more important than seed yield. Seed yield could be optimized through breeding. For future conservation and breeding programs of this

neglected and underutilized vegetable, the high levels of genetic diversity observed in this germplasm can be exploited. The accessions with high leaf yield can be directly used by farmers.

7.3 General Recommendation

Jute mallow is an important vegetable that can be used to address the problem of malnutrition both in rural and urban areas. This germplasm represent just a part of all accessions currently cultivated by farmers in Tanzania as well as those from other countries. The most reliable and useful information can be generated by conducting a detailed study to document ethno-nomenclature (as the same accessions are known by different names in different places), ethno-botanical uses, progress in terms of domestication and challenges faced by farmers. This will not only be a stepping stone towards improvement of the accessions which are currently in use by farmers but also an opportunity to plant breeders to introduce better accessions which are well adapted to specific environment and preferred by farmers. The genetic diversity observed in this study is the basis for identification as well as selection of desirable accessions for farmers to use directly and selection of parents for future breeding. In the light of this study, the management and conservation strategies can be established for the accessions with good agronomic traits in the available germplasm.

REFERENCES

Achigan–Dako, E., Pasquini, M., Assogba Komlan, F., N'Danikou, S., Dansi, A. and Ambrose–Oji, B. (2010). Traditional vegetables in Benin. Institut National des Recherches Agricoles du Bénin. Cotonou: Imprimeries du CENAP.

Adebo, H.O., Ahoton, L.E., Quenum, F. and Ezin, V. (2015). Agromorphological characterization of *Corchorus olitorius* cultivars of Benin. *Annual Research and Review in Biology* **7**(4): 229-240.

Adediran, O.A., Ibrahim, H., Tolorunse, K. and Gana, U. (2015). Growth, Yield and Quality of Jute Mallow (*Corchorus olitorius* L.) as Affected by Different Nutrient Sources. *International Journal of Agriculture Innovations and Research*. **3**(5): 1544-1547.

Agong, S.G., Ojiewo, C.O., Mwai, G.N., Abukutsa-Onyango, M.O. and Nono-Womdim, R. (2013). Exploiting the genetic diversity of vegetable African nightshades.*Bioremediation, Biodiversity and Bioavailability.* **7**(1)*:* 6–13.

Akoroda, M.O. (1985). Morphotype diversity in Nigeria landraces of *Corchorus olitorius*. *Journal of Horticultural Science.* **60**:557-562.

Akter J., M.S. Islam, A.A. Sajib, N. Ashraf, S. Haque and Khan H. (2008). Microsatellite markers for determining genetic identities and genetic diversity among jute cultivars. *Australian Journal of Crop Science*. **1**: 97-107.

Alemayehu N and Becker H. (2002). "Genotypic diversity and patterns of variation in a germplasm material of Ethiopian mustard (*Brassica carinata* A. Braun),"*Genetic Resources and Crop Evolution*. **49** (6) :573–582

Ali, M.N., Ghosh, A., Sasmal, B., Sarkar, H. and Das, P. (2012). Isozyme diversity in selected leaf mutants of 'tossa'jute (*Corchorus olitorius L*.).*Indian Journal of Biotechnology*. **11**(3):333-336

Aluko O.A., Olanipekun T, O., Olasoji J.O., Abiola I.O. and O., A. (2014). Effects of organic and Inorganic fertilizer on the yield and nutrient composition of Jute mallow. *Global Journal of Agriculture Research*. **2**(3): 1-9.

Andini, R., Yoshida, S., Yoshida, Y. and Ohsawa, R. (2013). Amaranthus genetic resources in Indonesia: morphological and protein content assessment in comparison with worldwide amaranths. *Genetic Resources and Crop Evolution*. **60**(7): 2115-2128.

Armand, N.M., Martin, B.J., Célestin, D., Elmith, M.J., Bille, N.H., Ntsefong, G.N. and Akoa, A. (2013). Assessment of Some Agro-Morphological Parameters of Some Local and Exotic Varieties of Okra [*Abelmoschus Esculentus* (Moench)]. *International Journal of Biotechnology and Food Science.* **1**(1):6-12.

Attere, A.F. (1997). Promoting the conservation and use of underutilized and neglected crops.; Conservation and use, August 29-31, 1997. Gatersleben/International Plant genetic Resources Institute, Rome, Italy.

Barnerjee, S., Das, M., Mir, R.R., Kundu, A., Topdar, N., Sarkar, D., Sinha, M.K., Balyan, H.S. and Gupta, P.K. (2012). Assessment of genetic diversity and population structure in a selected germplasm collection of 292 jute genotypes by microsatellite (SSR) markers. *Molecular Plant Breeding*. **3**(1):11-25.

Barku, V.Y., Opoku-Boahen, Y., Owusu-Ansah, E., Dayie, N. and Mensah, F. (2013). In-vitro assessment of antioxidant and antimicrobial activities of methanol extracts of six wound healing medicinal plants. *Journal of Natural Sciences Research*. **3**(1):74-80.

Basu, A., Ghosh, M., Meyer, R., Powell, W., Basak, S. and Sen, S. (2004). Analysis of genetic diversity in cultivated jute determined by means of SSR markers and AFLP profiling. *Crop Science*. **44**(2): 678-685.

Bayer, C. and Kubitzki, K. (2003). *Malvaceae, Flowering Plants· Dicotyledons*. Springer, pp. 225-311.

Becher, S., Steinmetz, K., Weising, K., Boury, S., Peltier, D., Renou, J.-P., Kahl, G. and Wolff, K. (2000). Microsatellites for cultivar identification in Pelargonium. *Theoretical and Applied Genetics*. **101**(4): 643-651.

Begum, T. and Kumar, D. (2011). Usefulness of morphological characteristics for DUS testing of jute (*Corchorus olitorius* L. and *C. capsularis* L.). *Spanish Journal of Agricultural Research*. **9**(2): 473-483.

Benor, S., Blattner, F.R., Demissew, S. and Hammer, K. (2010). Collection and ethnobotanical investigation of *Corchorus* species in Ethiopia: potential leafy vegetables for dry regions. *Genetic Resources and Crop Evolution*. **57**(2): 293-306.

Benor, S., Demissew, S., Hammer, K. and Blattner, F.R. (2012). Genetic diversity and relationships in *Corchorus olitorius* (Malvaceae sl) inferred from molecular and morphological data. *Genetic Resources and Crop Evolution*. **59**(6): 1125-1146.

Benor, S., Fuchs, J. and Blattner, F.R. (2011). Genome size variation in *Corchorus olitorius* (Malvaceae sl) and its correlation with elevation and phenotypic traits. *Genome*. **54**(7): 575-585.

Biswas, B.K. (2013). Genetic diversityin Jute genotypes revealed by RAPD markers, Dissertation.

Botstein, D., White, R.L., Skolnick, M. and Davis, R.W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*. **32**(3): 314-331.

Bretting, P. and Widrlechner, M.P. (1995). Genetic markers and horticultural germplasm management. *Hort Science*. **30**(7): 1349-1356.

Burkill, H.M. (2004) *The useful plants of West Tropical Africa*, (2nd edn), vol 6, general index. Royal Botanical Gardens, Kew.

Chandra, A. (2008). Genetic relationship among accessions of *Stylosanthes hamata* based on seed proteins profile. *The Indian Journal of Genetics and Plant Breeding*. **68**(2): 208-211.

Chen, Y., Zhang, L., Qi, J., Chen, H., Tao, A., Xu, J., Lin, L. and Fan, P. (2014). Genetic linkage map construction for white jute (*Corchorus capsularis* L.) using SRAP, ISSR and RAPD markers. *Plant Breeding*. **133**(6): 777-781.

Choudhary, S.B., Sharma, H.K., Karmakar, P.G., Kumar, A., Saha, A.R., Hazra, P. and Mahapatra, B.S. (2013). Nutritional profile of cultivated and wild jute ('*Corchorus*') species. *Australian Journal of Crop Science*. **7**(13): 1973.

Chweya, J.A. and Eyzaguirre, P.B. (1999). *The biodiversity of traditional leafy vegetables.* University of Nairobi. pp.181

Dansi, A., Adjatin, A., Adoukonou-Sagbadja, H., Faladé, V., Yedomonhan, H., Odou, D. and Dossou, B. (2008). Traditional leafy vegetables and their use in the Benin Republic. *Genetic Resources and Crop Evolution*. **55**(8): 1239-1256.

Das, M., Banerjee, S., Topdar, N., Kundu, A., Sarkar, D., Sinha, M.K., Balyan, H.S. and Gupta, P.K. (2011). Development of large-scale AFLP markers in jute. *Journal of Plant Biochemistry and Biotechnology*. **20**(2):270-275.

Das, B. and Maiti, S. (1998). Jute (*Corchorus* species) and allied fibres research in India. *Indian Journal of Agricultural Science*. **68**(8): 484-493.

Denton, L. (1997). A review of *Corchorus olitorius* in Nigeria. In: Schippers R, Budd L (eds) Workshop on African indigenous vegetables. Limbe, Camoroon, January 12-18, 1997, 25-30.

Denton, O.A. and Nwangburuka, C.C. (2012). Morphological Diversity among *Corchorus olitorius* Accessions Based on Single Linkage Cluster Analysis and Principal Component Analysis. *Jordan Journal of Biological Sciences*. **5**(3):191-196.

Doyle, J. and Doyle, J.L. (1990) Isolation of Plant DNA from Fresh Tissue. *Focus*, **12**:13-15.

Dziechciarkova, M., Lebeda, A., Dolezalova, I. and Astley, D. (2004). Characterization of *Lactuca* spp. germplasm by protein and molecular markers-a review. *Plant Soil and Environment*. **50**(2): 47-58.

Edmonds, J.M. (1990). *Herbarium survey of African Corchorus L. species*. IBPGR. pp 284.

Eftekharian, R., Sheidai, M., Attar, F., Noormohammadi, Z. and Ebadi-Nahari, M. (2016). Morphometric and ISSR based variability analysis to elucidate population genetic structure in *Senecio glaucus* L.(Asteraceae: Senecioneae). *The Nucleus*: 1-7.

Fagbohoun, E.D and Ibrahim, T.A. (2011). Physicochemical properties and in vitro antibacterial activity of *Corchorus olitorius* Linn. Seed oil. *Life Sciences Leaflets Plant Biologist*; **15**: 499-505.

Falconer, D.S., Mackay, T.F. and Frankham, R. (1996). Introduction to quantitative genetics (4th edn). *Trends in Genetics*. **12**(7): 280.

Fasinmirin, J. and Olufayo, A. (2009). Yield and water use efficiency of jute mallow *Corchorus olitorius* under varying soil water management strategies. *Journal of Medicinal Plants Research*. **3**(4): 186-191.

Formisano, G., Roig, C., Esteras, C., Ercolano, M.R., Nuez, F., Monforte, A.J. and Picó, M.B. (2012). Genetic diversity of Spanish *Cucurbita pepo* landraces: an unexploited resource for summer squash breeding. *Genetic Resources and Crop Evolution*. **59**(6): 1169-1184.

Freiberger, C., Vanderjagt, D., Pastuszyn, A., Glew, R., Mounkaila, G., Millson, M. and Glew, R. (1998). Nutrient content of the edible leaves of seven wild plants from Niger. *Plant Foods for Human Nutrition*. **53**(1): 57-69.

Ghosh, R.K., Wongkaew, A., Sreewongchai, T., Nakasathien, S. and Phumichai, C. (2014). Assessment of genetic diversity and population structure in jute (*Corchorus* spp.) using simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers. *Kasetsart Journal of Natural Sciences*. **48**:83-94.

Gimenes, M.A., Hoshino, A.A., Barbosa, A.V., Palmieri, D.A. and Lopes, C.R. (2007). Characterization and transferability of microsatellite markers of the cultivated peanut (Arachis hypogaea). *BMC Plant Biology*. **7**(9):1-13

Grubben, G. and Denton, O. (2004). *Plant Resources of Tropical Africa 2*. Vegetables. PROTA Foundation, Wageningen, Netherlands.

Grubben, G.J., Tindall, H.D. and Williams, J.T. (1977). *Tropical vegetables and their genetic resources***.** International Board for Plant Genetic Resources, Rome, Italy.

Haque, S., Begum, S., Sarker, R. and Khan, H. (2007). Determining genetic diversity of some jute varieties and accessions using RAPD markers. *Plant Tissue Culture and Biotechnology*. **17**(2): 183-191.

Hartmann, H.T. and Kester, D.E. (1963). Plant Propagation: Principles and Practice. *Soil Science*. **95**(1): 89.

Hillocks, R. (1998). The potential benefits of weeds with reference to small holder agriculture in Africa. *Integrated Pest Management Reviews*. **3**(3): 155-167.

Hossain, M.B., Haque, S. and Khan, H. (2002). DNA fingerprinting of jute germplasm by RAPD. *BMB Reports*. **35**(4): 414-419.

Huq, S., Islam, M.S., Sajib, A.A., Ashraf, N., Haque, S. and Khan, H. (2009). Genetic diversity and relationships in jute (*Corchorus spp*.) revealed by SSR markers. *Bangladesh Journal of Botany*. **38**(2): 153-161.

Johnson, C.D. and Decoteau, D.R. (1996). Nitrogen and potassium fertility affects Jalapeño pepper plant growth, pod yield, and pungency. *HortScience*. **31**(7): 1119-1123.

Julia, C.C., Waters, D.L., Wood, R.H. and Rose, T.J. (2016). Morphological characterisation of Australian *ex situ* wild rice accessions and potential for identifying novel sources of tolerance to phosphorus deficiency. *Genetic resources and crop evolution*. **63**(2): 327-337.

K'opondo, F., Muasya, R. and OK, K.A. (Ed.) (2005). A review on the seed production and handling of indigenous vegetables (spiderplant, jute mallow and african nightshade complex). *In: Proceedings of the third Horticulture Workshop on Sustainable Horticultural production in the Tropics. Maseno University, Maseno*, pp. 42-48.

Kar, C., Kundu, A., Sarkar, D., Sinha, M. and Mahapatra, B. (2009). Genetic diversity in jute (*Corchorus spp*) and its utilization: a review. *Indian Journal of Agricultural Sciences*. **79**(8): 575-586.

Kareem, V.A., Rajasekharan, P., Mini, S. and Kumar, T.V. (2011). Genetic diversity and structure of the threatened anti-cancerous plant *Nothapodytes nimmoniana* as revealed by ISSR analysis. *Plant Genetic Resources*. **9**(4): 506-514.

Keding, G., Weinberger, K., Swai, I. and Mndiga, H. (2007). Diversity, traits and use of traditional vegetables in Tanzania. Technical Bulletin No. 40. Shanhua Taiwan: AVRDC-WorldVegetableCenterpp.53.

Khaing, A.A., Moe, K.T., Chung, J.W., Baek, H.J. and Park, Y.J. (2013). Genetic diversity and population structure of the selected core set in Amaranthus using SSR markers. *Plant Breeding*. **132**(2): 165-173.

Khatun, M. and Alam, S.S. (2010). Confirmation of species status of *Corchorus trilocularis* and *C. pseudo-olitorius* by differential chromosome banding and isozyme assay. *Cytologia*. **75**(1): 83-88.

Khatun, M., Sultana, S.S., Ara, H., Islam, M.N. and Alam, S.S. (2011). Differential Chromosome Banding and Isozyme Assay of Three *Corchorus spp*. *Cytologia*. **76**(1): 27-32.

Kinabo, J., Mnkeni, A., Nyaruhucha, C., Msuya, J., Haug, A. and Ishengoma, J. (2006). Feeding frequency and nutrient content of foods commonly consumed in the Iringa and Morogoro regions in Tanzania. *International Journal of Food Sciences and Nutrition*. **57**(1-2): 9-17.

Kisua, J., Mwikamba, K., Makobe, M. and Muigai, A. (2015). Genetic diversity of sweet and grain sorghum populations using phenotypic markers. *International Journal of Biosciences*. **6**(9): 34-46.

Knight, C.A., Molinari, N.A. and Petrov, D.A. (2005). The large genome constraint hypothesis: evolution, ecology and phenotype. *Annals of Botany*. **95**(1): 177-190.

Krebs, G. (2001). Tiliaceae. *Hanelt P, Institute of Plant Genetics and Crop Plant Research (eds) Mansfeld's Encyclopedia of Agricultural and Horticultural Crops*. **3**:1560-1567.

Kristkova, E., Dolezalova, I., Lebeda, A., Vinter, V. and Novotná, A. (2008). Description of morphological characters of lettuce (*Lactuca sativa* L.) genetic resources. *Horticultural Science*. **35**:113-129.

Kumawat, B., Gupta, M. and Tarachand, Y.S. (2012). Free radical scavenging effect of various extracts of leaves of *Balanites aegyptiaca* (L.) Delile by DPPH method. *Asian Journal of Plant Sciences Research*. **2**(3): 323-329.

Kundu, A., Topdar, N., Sarkar, D., Sinha, M.K., Ghosh, A., Banerjee, S., Das, M., Balyan, H.S., Mahapatra, B. and Gupta, P.K. (2013). Origins of white (*Corchorus capsularis* L.) and dark (*C. olitorius* L.) jute: a reevaluation based on nuclear and chloroplast microsatellites. *Journal of Plant Biochemistry and Biotechnology*. **22**(4): 372-381.

Kundu, B. (1951). Origin of jute. *Indian Journal of Genetics and Plant Breeding*. **11**:95-99.

Lewu, F. and Mavengahama, S. (2010). Wild vegetables in Northern KwaZulu Natal, South Africa: Current status of production and research needs. *Scientific Research and Essays*. **5**(20): 3044-3048.

Li, H.y., Li, Z.y., Cai, L.y., Shi, W.g., Mi, F.g. and Shi, F.l. (2013). Analysis of genetic diversity of Ruthenia Medic (*Medicago ruthenica* (L.) Trautv.) in Inner Mongolia using ISSR and SSR markers. *Genetic Resources and Crop Evolution*. **60**(5): 1687-1694.

Loumerem, M. and Alercia, A. (2016). Descriptors for jute (*Corchorus olitorius* L.). *Genetic Resources and Crop Evolution*. **63**(7): 1103-1111.

Madakadze, R., Kodzanayi, T. and Mugumwa, R. (Ed.) (2007). Effect of plant spacing and harvesting frequency on *Corchorus olitorious* leaf and seed yields. *In: 8th African Crop Science Society Conference, El-Minia, Egypt, 27-31 October 2007. African Crop Science Society*, pp. 279-282.

Maina, S. and Mwangi, M. (2008). **Vegetables in East Africa**. Elewa Publications, Farming Resources Series.

Makinde, S.O., Oluwole, O.S., Ojekale, B.A. and Olufeyimi, S.R. (2009). Effects of intrapopulation competition on morphological and agronomic characters of Jute plant (*Corchorus olitorius* L.). *African Journal of Biotechnology*. **8**(10):2195-2201.

Mandal, A., Datta, A.K., Datta, S. and Gupta, S. (2013). Genetic assessment of eight *Corchorus spp*.(Tiliaceae) using RAPD and ISSR markers. *The Nucleus*. **56**(1): 23-30.

Mathowa, T., Madisa, M., Moshoeshoe, C., Mojeremane, W. and Mpofu, C. (2014). Effect of different growing media on the growth and yield of jute mallow (*Corchorus olitorius* L.). *International Journal of Research Studies in Biosciences*. **2**(11): 153-163.

Meena, R.K. and Shukla, P. (2013). Genetic diversity of seed storage proteins in popular rice (*Oryza sativa* L.) varieties in Chhattisgarh and its application. *Research Journal of Agricultural Sciences*. **4**(3): 329-332.

Merlier, H. (1972). Phenological studies of fallow species in Central Senegal (Synthesis). *Agronomie Tropicale*. **27**(12): 1229-1252.

Mills, H.A. and Jones Jr, J.B. (1979). Nutrient deficiencies and toxicities in plants: Nitrogen. *Journal of Plant Nutrition*. **1**(2): 101-122.

Mir, R.R., Banerjee, S., Das, M., Gupta, V., Tyagi, A.K., Sinha, M.K., Balyan, H.S. and Gupta, P.K. (2009). Development and characterization of large-scale simple sequence repeats in jute. *Crop Science*. **49**(5): 1687-1694.

Mir, R.R., Rustgi, S., Sharma, S., Singh, R., Goyal, A., Kumar, J., Gaur, A., Tyagi, A.K., Khan, H. and Sinha, M.K. (2008). A preliminary genetic analysis of fibre traits and the use of new genomic SSRs for genetic diversity in jute. *Euphytica*. **161**(3): 413-427.

Mnzava N.A. and Ngwerume, F.C. (2004). *Cleome gynandra*. In: Grubben G.J.H. and Denton O.A. (Eds.). *Plant Resources of Tropical Africa 2. Vegetables. PROTA Foundation, Wageningen, Netherlands,/Backhuys Publishers, leiden, Netherlands/CTA, Wageningen, Netherlands*. p. 191-195.

Mnzava, N. (1997). Vegetable crop diversification and the place of traditional species in the tropics. *Promoting the Conservation and Use of Underutilized and Neglected Crops (IPGRI)*.

Morakinyo, J. (1997). Morphology and cytogenetics of *Corchorus olitorius* and *Corchorus tridens*. *Bioscience Research Communications*. **9**(1): 9-13.

Nath, P. and Denton, L. (1980). Leafy vegetables in Nigeria. *Plant Genetic Resources Newsletter* 42: 14-17.

National Report of PGR, FAO (2009). Tanzania Report on the state of Plant Genetic Resources for food and Agriculture. Dar es salaam, Tanzania.

National Institute of Horticultural Research (1986). Advances in Fruit and vegetable Research at NIHORT (1976-1986): A commemoration Publication to Mark the 10th Anniversary of the National Horticultural Research Institute. NIHORT, Ibadan, Nigeria, Pages: 62.

Ndlovu, J. and Afolayan, A. (2008). Nutritional analysis of the South African wild vegetable *Corchorus olitorius* L. *Asian Journal of Plant Sciences*. **7**(6): 615-618.

Nelson, R.L. (2011). Managing self-pollinated germplasm collections to maximize utilization. *Plant Genetic Resources*. **9**(01): 123-133.

Nethra, N., Prasad, S.R., Vishwanath, K., Dhanraj, K. and Gowda, R. (2007). Identification of rice hybrids and their parental lines based on seed, seedling characters, chemical tests and gel electrophoresis of total soluble seed proteins. *Seed Science and Technology*. **35**(1): 176-186.

Nguni, D. and Mwila, G. (2007). Opportunities for increased production, utilization and income generation from African leafy vegetables in Zambia. *African Journal of Food, Agriculture, Nutrition and Development*. **7**(4): 1-20.

Noormohammadi, Z., Fasihee, A., Homaee-Rashidpoor, S., Sheidai, M., Baraki, S.G., Mazooji, A. and Tabatabaee-Ardakani, S.Z. (2012). Genetic variation among Iranian pomegranates (*Punica granatum* L.) using RAPD, ISSR and SSR markers. *Australian Journal of Crop Science*. **6**(2): 268.

Norman, J. (1972). Tropical leafy vegetables in Ghana. *World Crops*.

Ntuli, N.R., Tongoona, P.B. and Zobolo, A.M. (2015). Genetic diversity in *Cucurbita pepo* landraces revealed by RAPD and SSR markers. *Scientia Horticulturae*. **189**(192-200.

Nwangburuka, C. and Denton, O. (2012). Heritability, Character Association, Genetic Advance in Six Agronomic and Yield Related Characters in leaf *Corchorus olitorius*. *International Journal of Agriultural Research***7**(7): 365-375.

Nyadanu, D., Amoah, R.A., Kwarteng, A., Akromah, R., Aboagye, L., Adu-Dapaah, H., Dansi, A., Lotsu, F. and Tsama, A. (2016). Domestication of jute mallow (*Corchorus*

*olitorius*L.): ethnobotany, production constraints and phenomics of local cultivars in Ghana. *Genetic Resources and Crop Evolution* **64**(6):1313-1329.

Nyadanu, D. and Lowor, S. (2015). Promoting competitiveness of neglected and underutilized crop species: comparative analysis of nutritional composition of indigenous and exotic leafy and fruit vegetables in Ghana. *Genetic Resources and Crop Evolution*. **62**(1): 131-140.

Odhav, B., Beekrum, S., Akula, U. and Baijnath, H. (2007). Preliminary assessment of nutritional value of traditional leafy vegetables in KwaZulu-Natal, South Africa. *Journal of Food Composition and Analysis*. **20**(5): 430-435.

Ogunkanmi, L., Okunowo, W., Oyelakin, O., Oboh, B., Adesina, O., Adekoya, K. and Ogundipe, O. (2010). Assessment of genetic relationships between two species of jute plants using phenotypic and RAPD markers. *International Journal of Botany* **6**(2):107-111.

Oguntona, E. and Akinyele, I. (1995). Nutrient composition of commonly eaten foods in Nigeria-Raw, processed and prepared.Food Basket Foundation Publication Series 1995. 131 pp (English)

Oh, S.J., Song, J.Y., Lee, J., Lee, G.A., Ko, H.C., Stoilova, T., Krasteva, L., Kim, Y.G., Rhee, J.H., Gwag, J.G. and Ro, N.Y., 2012. Evaluation of genetic diversity of red pepper landraces (*Capsicum annuum* L.) from Bulgaria using SSR markers. *The Journal of the Korean Society of International Agriculture*.24(5):547-556.

Olaniyi, J. and Ajibola, A. (2008). Growth and yield performance of *Corchorus olitorius* varieties as affected by nitrogen and phosphorus fertilizers application. *American-Eurasian Journal of Sustainable Agriculture*. **2**(3): 234-241.

Opabode, J. and Adebooye, O. (2005). Application of biotechnology for the improvement of Nigerian indigenous leaf vegetables. *African Journal of Biotechnology* **4**(3): 138-142.

Osawaru, M., Ogwu, M., Chime, A. and Amorighoye, A. (2012). Morphological evaluation and protein profiling of three accessions of Nigerian *Corchorus* Linn. species. *Bayero Journal of Pure and Applied Sciences*. **5**(1): 26-32.

Pal, D., Mandal, M., Senthilkumar, G. and Padhiari, A. (2006). Antibacterial activity of Cuscuta reflexa stem and *Corchorus olitorius* seed. *Fitoterapia*. **77**(7): 589-591.

Palit, P., Sasmal, B. and Bhattacharryya, A. (1996). Germplasm diversity and estimate of genetic advance of four morpho-physiological traits in a world collection of jute. *Euphytica*. **90**(1): 49-58.

Palve, S., Sinha, M. and Mandal, R. (2003). Preliminary evaluation of wild species of jute (*Corchorus species*). *Plant Genetic Resources Newsletter (eng)*.

Palve, S. and Sinha, M. (2005). Genetic variation and interrelationships among fibre yield attributes in secondary gene pool of *Corchorus* spp. *SABRAO Journal of Breeding and Genetics*. **37**(1): 55-64.

Peakall, P.R.S. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics*. **28**(2537-2539.

Peksen, E. (2007). Non-destructive leaf area estimation model for faba bean (*Vicia faba* L.). *Scientia Horticulturae*. **113**(4): 322-328.

Peratoner, G., Seling, S., Klotz, C., Florian, C., Figl, U. and Schmitt, A. (2016). Variation of agronomic and qualitative traits and local adaptation of mountain landraces of winter rye (*Secale cereale* L.) from Val Venosta/Vinschgau (South Tyrol). *Genetic Resources and Crop Evolution*. **63**(2): 261-273.

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S. and Rafalski, A. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding*. **2**(3): 225-238.

Qi, J., Zhou, D., Wu, W., Lin, L., Fang, P. and Wu, J. (2003). The application of RAPD technology in genetic diversity detection of Jute. *Yi chuan xue bao= Acta Genetica Sinica*. **30**(10): 926-932.

Rohlf, F. (2000). Numerical taxonomy and multivariate analysis system version 2.1. *New York: Exeter Publishing*.

Roy, A., Bandyopadhyay, A., Mahapatra, A., Ghosh, S., Singh, N., Bansal, K., Koundal, K. and Mohapatra, T. (2006). Evaluation of genetic diversity in jute (*Corchorus* species) using STMS, ISSR and RAPD markers. *Plant Breeding*. **125**(3): 292-297.

Saha, A., and S. K. Hazra. (2008). Jute-The heritage of India.*Jute and Allied Fibre Updates: Production and Technology. Barrackpore: CRIJAF*: 1-17.

Schippers, R.R. (2000). African indigenous vegetables: an overview of the cultivated species.University of Greenwich, Natural Resources Institute, London, UK. 222 pp.

Sehgal, D. and Raina, S.N. (2005). Genotyping safflower (*Carthamus tinctorius*) cultivars by DNA fingerprints. *Euphytica*. **146**(1): 67-76.

Shukla, S., Bhargava, A., Chatterjee, A., Srivastava, A. and Singh, S. (2006). Genotypic variability in vegetable amaranth (*Amaranthus tricolor* L for foliage yield and its contributing traits over successive cuttings and years. *Euphytica*. **151**(1): 103-110.

Soliman, M.I., Zaghloul, M.S. and Heikal, Y.M. (2014). Genetic variation within and among three Egyptian *Mesembryanthemum* species using different genetic markers. *Egyptian Journal of Basic and Applied Sciences*. **1**(3): 127-135.

Sultan, M., Rabbani, M.A., Shinwari, Z.K. and Masood, M.S. (2012). Phenotypic divergence in guar (*Cyamopsis tetragonoloba* L.) landrace genotypes of Pakistan. *Pakistan Journal of Botany*. **44**:203-210**.**

Thanh, C.V. and Hirata, Y. (2002). Seed storage protein diversity of three rice species in the Mekong Delta. *Biosphere Conservation: for Nature, Wildlife, and Humans*. **4**(2): 59-67.

Thompson, J.L., Gebauer, J., Hammer, K. and Buerkert, A. (2010). The structure of urban and peri-urban gardens in Khartoum, Sudan. *Genetic Resources and Crop Evolution*. **57**(4): 487-500.

Tindall, H.D. (1965). *Fruits and Vegetables in West Africa*. Food and Agriculture Organization of the United Nations.

Tulio, A.Z., Ose, K., Chachin, K. and Ueda, Y. (2002). Effects of storage temperatures on the postharvest quality of jute leaves (*Corchorus olitorius* L.). *Postharvest Biology and Technology*. **26**(3): 329-338.

Tukey, J.W. (1977). *Exploratory data analysis*. Addison - Weslay Publishing Company, Reading, Mass.

Van Epenhuijsen, C. (1974). *Growing native vegetables in Nigeria*. Roma, Italy. pp 127.

van Rensburg Willem, J., HJ, V.I., Van Zijl, J. and Sonja, L.V. (2007). Conservation of African leafy vegetables in South Africa. *African Journal of Food, Agriculture, Nutrition and Development*. **7**(4): 1-12.

Velempini, P., Riddoch, I. and Batisani, N. (2003). Seed treatments for enhancing germination of wild okra (*Corchorus olitorius*). *Experimental Agriculture*. **39**(04): 441-447.

Wang, Z., Wang, J.-E., Wang, X.-M., Gao, H.-W., Dzyubenko, N.I. and Chapurin, V.F. (2012). Assessment of genetic diversity in *Galega officinalis* L. using ISSR and SRAP markers. *Genetic Resources and Crop Evolution*. **59**(5): 865-873.

Weinberger, K. and Msuya, J.M. (2004). Indigenous vegetables in Tanzania: significance and prospects. AVRDC-WorldVegetableCenter. T*echnical Bulletin No. 31*. *AVRDC Publication* 04-600.70pp.

Westphal-Stevels, J. (Ed.) (1985). Local vegetables in Cameroon: *Corchorus* species used as a vegetable. *In: I International Symposium on Taxonomy of Cultivated Plants 182*, pp. 423-426, 1985.

Willem, J.V.R., Ineke, V.H.J., Van Zijl, J.J.B., Sonja, V.L. (2007). Conservation of African leafy vegetables in South Africa. *African Journal of Food Agriculture Nutrition and Development*, **7**:317–326.

Xiong, H. (2008). Breeding sciences of bast and leaf fiber crops. *Chinese Agricultural Science and Technology Press, Beijing*: 319-341.

Yang, Y., Pan, Y., Gong, X. and Fan, M. (2010). Genetic variation in the endangered Rutaceae species *Citrus hongheensis* based on ISSR fingerprinting. *Genetic Resources and Crop Evolution*. **57**(8): 1239-1248.

Yee, E., Kidwell, K., Sills, G. and Lumpkin, T. (1999). Diversity among selected *Vigna angularis* (Azuki) accessions on the basis of RAPD and AFLP markers. *Crop Science*. **39**(1): 268-275.

Yeh, F.C., Yang, R.C., Boyle, T.B., Ye, Z. and Mao, J.X. (1997). POPGENE, the user-friendly shareware for population genetic analysis. *Molecular Biology and Biotechnology Centre, University of Alberta, Canada*. **10**:295-301

Zhang, L., Yuan, M., Tao, A., Xu, J., Lin, L., Fang, P. and Qi, J. (2015). Genetic structure and relationship analysis of an association population in Jute (*Corchorus* spp.) evaluated by SSR markers. *PloS one*. **10**(6):1-9.

APPENDICES

Appendix 1: The genetic diversity of leaf vegetable Jute mallow (*Corchorus spp*.): A Review

Appendix 2: Characterization of Morphological Diversity of Jute Mallow (*Corchorus spp*.)

Appendix 3: Leaf and seed yield of Jute mallow (*Corchorus spp*.) accessions under field conditions for two consecutive growing seasons