DIVERSITY, DISTRIBUTION AND PROPAGATION METHODS FOR CACTI SPECIES (*CACTACEAE*) FROM ARID AND SEMI-ARID LANDS OF KENYA

OMWERI HARON ANYONA

MASTER OF SCIENCE

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Diversity, Distribution and Propagation Methods for Cacti Species (*Cactaceae*) from Arid and Semi-Arid Lands of Kenya

Omweri Haron Anyona

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Signature……..Date ……

Haron Anyona Omweri

This thesis has been submitted for examination with our approval as university supervisors.

Signature ……..Date ……

Dr. Fredah Rimberia Wanzala, PhD

JKUAT, Kenya

Signature ……..Date ……

Prof. Stephen Githiri Mwangi, PhD

JKUAT, Kenya

Signature …...……..Date ……

Prof. Daniel Ndaka Sila, PhD

JKUAT, Kenya

DEDICATION

I would like to dedicate this work to my parents for their continuous prayers and support.

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LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
AMOVA	Analysis of molecular variance
ASAL	Arid and Semi-Arid Land
CAM	Craculacean Acid Metabolism
G.O.K	Government of Kenya
JKUAT	Jomo Kenyatta University of Agriculture and Technology
ISSR	Inter simple sequence repeats
ITS	Internal transcribed spacer sequences
PCA	Principal Component Analysis
PCR	Polymerase chain reaction
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
SSR	Simple sequence repeats
UPGMA	Unweighted pair group method of arithmetic averages
UPOV	International union for the protection of new varieties of plants

ABSTRACT

Cactaceae is an important family of plants of Arid and Semi-Arid Lands (ASALs) in the world. It is useful as an ornamental plant (*Cereus peruvianus* and *Thrixanthocereus blossfeldiorum*), food, fodder and industrial purposes (*Opuntia spp*). There is minimal documentation about Cacti growing in the ASALs of Kenya. The objective was to enhance understanding and utilization of Cacti in Kenya through determination of distribution, diversity and propagation methods. A field survey of the ASAL areas was carried out and samples taken for molecular analysis and propagule evaluation. There were at least three different species of Cacti in Baringo, Laikipia, Machakos and Makueni Counties as opposed to Nakuru County that had two species (*Opuntia ficus-indica* and *Opuntia exaltata*). Majority of Cacti in the studied counties were either grown as fencing material (*Thrixanthocereus blossfeldiorum, Opuntia stricta, Opuntia monacantha, Opuntia exaltata, Opuntia ficus-indica*) in farms or as ornamentals (*Thrixanthocereus blossfeldiorum, Cereus peruvianus*) while some grew intermittently in uncultivated lands (*Euphorbia ingens, Euphorbia abyssinica*) . Sixty nine distinct populations of Cacti were characterized *in-situ* using a list of descriptors by the International Union for the Protection of New Varieties of Plants (UPOV). Results indicated that eight species namely, *Opuntia exaltata, Opuntia monacantha, Opuntia ficus-indica, Opuntia stricta, Thrixanthocereus blossfeldiorum, Euphorbia abyssinica, Euphorbia ingens and Cereus peruvianus* were present in Kenya. *Opuntia ficus-indica*

was the most diverse and was found in four of the five counties studied. *Euphorbia abyssinica* was found in four counties while *Opuntia stricta* and *Thrixanthocereus blossfeldiorum* were found in a single county each. The results indicated that vegetative propagation through cladodes; immature fruits and stem cuttings of Cacti are effective planting material and this can be enhanced by curing of the propagules for more than seven days. The results indicated significant variations among Cacti species in Kenya. The eleven simple sequence repeat (SSR) markers used were not polymorphic and did not sufficiently distinguish the *Opuntia* species investigated. The low genetic distances for some of the populations between counties call for further investigation and confirmation. Further research is needed through molecular characterization using more informative markers. This should be carried out to cover other parts of the country so as to identify other species available in these areas, their mode of distribution and their productivity to influence the choice species for utilization.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Cacti belong to the family *Cactaceae* and the order *Caryophyllales* and is classified into four sub-families namely; *Opuntioideae*, *Cactoideae*, *Maihuenioideae* and *Pereskioideae* (Mauseth, 2006) with *Opuntia* being the largest genus in the *Cactaceae* family (Segura *et al.,* 2007) . Cacti are xerophytic and thus found in hot and dry parts of Eastern, North Eastern and Rift valley regions of Kenya.

Cactaceae is endemic to the Western hemisphere (Ramawat, 2010) and believed to have originated from central Andean region of northern Chile, northwest Argentina, Bolivia, and Peru (Defelice, 2004; Edwards *et al.*, 2005). *Cactaceae* such as *Opuntia* species was introduced in other parts of the world as cladodes by European ships in the 15^{th} century (Casas & Barbera, 2002). Hence, their occurrence in Kenya is due to introduction of seeds or cuttings.

The Arid and Semi-Arid Lands (ASALs) of Kenya form well above three quarters of the country's total land mass (Orodho, 2006). They have little, unreliable and poorly distributed rainfall. ASALs can be utilized by planting of Cacti which is drought tolerant. The characteristic erratic rainfall, poor soils and high temperatures in the semi-arid and arid lands are challenging to conventional cropping systems (Le Houérou, 1996). Thus, production of crops like *Opuntia* species that can tolerate poor soils, high

temperatures and water-limited conditions will help improve productivity and crop diversification in these areas.

Among the commonly used species in the *Cactaceae* family is *Opuntia ficus-indica* (L) (FAO, 2013). This has been diversely utilized as fodder, fruit, vegetable, medicine, hedges and even in the reinforcement of white wash on homes in parts of Latin America (Ervin, 2012). It is a globally important species with a long history of human use. It has also served a role in the production of cochineal, a once important textile dye. Cacti pear fruits have high flavors with outstanding nutritive properties (Piga, 2004). The fruits are used for manufacture of food products including, natural liquid sweeteners, juices, jams and alcoholic drinks (Yeddes *et al*., 2013). Cacti fresh fruits are important vitamin C content contributors to the diet (Chiteva & Wairagu, 2013). Without fertilization and annual rainfall between 200mm to 400mm Le Houérou, (2002), recorded yields of between 20-60 metric tons/ha of fruit on arid areas while 246 tons/ha of cladodes were reported in irrigated and fertilized commercial plantations (FAO, 2002).

Vegetative propagation of Cacti is deemed to contribute to the establishment of commercial plantations for production of fresh fruits or fruits utilized as a source of important chemical compounds (López-Gómez *et al.,* 2000). A lack of know-how in Cacti pear fruit processing informs the limited commercialization of Cacti-pear in ASALs (Moßhammer *et al.,* 2006). Although Cacti are available and used as fodder for animals in Nyeri (Kang'ara & Gitari, 2008), there is no sufficient data on the amounts of Cacti available in the ASALs of Kenya.

There is a lack of a well-defined standard for varieties in *Opuntiodeae* subfamily which has resulted to clonal populaces thriving in some countries such as Spain, Italy, Chile and South Africa. In most cases the varieties are distinguished based on some morphological structures of their fruits and cladodes (Chiteva & Wairagu, 2013).

The greatest fruit harvest has been associated with large number of vegetative structures that accumulate the necessary reserves to reach minimum fruiting weight. Thus, the study of the morphological traits is necessary in indicating the productivity of available species.

Morphological diversity or similarity in the *Opuntia* species is not enough in their classification. This has been spelt out by studies that indicated significant differences in the fruits of morphologically similar *Opuntia* ecotypes. Therefore the need for molecular analysis of the available species and their classification.

Cacti in ASALs of Kenya have not been characterized. Adequate characterization for morphological traits of the Cacti in Kenya is necessary to facilitate future utilization of germplasm for breeding purposes. To achieve this, germplasm accessions of the crops need to be characterized for agronomic traits and genetic diversity over time. To attain a more objective quantification of genetic variation than do traditional morphological assessments, molecular characterizations by use of SSRs are important, as well as facilitate the identification of duplicates in the collections made.

Cacti can be propagated through various asexual methods including, divisions, cuttings and grafting or through micro propagation in tissue culture (Finti *et al*., 2012; Biosci *et*

al., 2013; Bayat *et al*., 2015). Sexual propagation through seed has been utilized (Chalak *et al.,* 2014). However, for some Cacti species it was found to face various challenges namely, genetic segregation, slow growth and development and seedling infestation (Khalafalla *et al.,* 2007). Therefore, there is need to identify the most appropriate propagules for rapid mass multiplication and production of the crop.

1.2 Statement of the problem and justification

Cacti exits in Kenya's ASALs, but, there is no information on how diverse or similar they are.

Considering that identification of some Cacti species to the level of species is quite difficult given actual specimens (Ervin, 2012), there is need to undertake the necessary procedures that will be useful in facilitating their easy identification. This is also coupled with the fact that while in habitat, there is extreme variation that plants belonging to the same species can have, due to environmental influences. These too may lead to a single species being given several names as observed in *Gymnocalycium bodenbenderianum* (Rivera, 2013). Hence, the importance of extensive field knowledge and research for the understanding of Cacti diversity, and its evolutionary modifications.

There is an absence of well-defined standard for variety identification which has led to clonal populations being developed in some countries like Spain, Italy, Chile and South Africa. Such populations are distinguished on the basis of some morphological characters of the fruits and cladodes. Other characteristics such as sugar content,

vegetative vigor, cladode fiber and content of protein among others have also been used to determine how the variety can be used (Chiteva & Wairagu, 2013)

Over three quarters of semi-arid or arid regions of Kenya that have little, unreliable and poorly distributed rainfall (Orodho, 2006) could be utilized for planting of Cacti which are drought tolerant. Given that sexual propagation of edible Cacti has been exploited, but posed serious practical problems like genetic segregation and slow growth and development, difficulty in obtaining Cacti seeds (and susceptibility of plantlets to damping-off), alternative propagation methods need to be sort for.

Due to difficulty in the identification of Cacti varieties, use of molecular markers is needed to enhance identification for breeding efforts to improve the productivity (Mihalte *et al.,* 2011; Mashope, 2007) and general utilization.

Adequate characterization of morphological traits of the Cacti in Kenya is necessary to facilitate future utilization of germplasm by breeders. To achieve this, accessions of the crops need to be characterized for morphological traits and genetic diversity over time. To attain a more objective quantification of genetic variation than do traditional morphological assessments, molecular characterization by use of SSRs will help, as well as facilitate the identification of duplicates in the collections made.

1.3 General objective

1. To contribute to enhanced understanding and utilization of Cacti in Kenya through determination of distribution, diversity and propagation methods.

1.3.1 Specific objectives

1. To document distribution and uses of wild populations of cactus in Arid and Semi-Arid Lands (ASALS) of Kenya.
2. To characterize Cacti species from Rift Valley and Eastern regions of Kenya.
3. To evaluate the different methods of propagation that could be utilized in the production of the edible Cacti in Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 Description of Cacti

Cacti are perennial plant species which is xerophytic, adapted and capable of surviving in hot and dry environments. Cacti are classified in the plant family *Cactaceae*. They belong to the order Caryophyllales (Kang'ara & Gitari, 2008) with four sub-families namely Pereskioideae, Maihuenioideae, Opuntioideae and Cactoideae (Anderson, 2001).

Generally Cacti are slow growing plants which at times have limited reproductive capabilities and often have specific conditions for seed productivity, germination and flowering (Khalafalla *et al*., 2007). Plants of the *Cactaceae* family are adapted to desert conditions by having thickened cuticles, increased water-storage tissues (Salgado & Mauseth, 2002), Craculacean Acid Metabolism (CAM) (García de Cortázar & Nobel, 1992; Khalafalla *et al*., 2007), asynchronous reproduction (Bariagabre *et al*., 2016) and shallow rooting systems (Kang'ara & Gitari, 2008).

Most Cacti are hermaphrodites with herkogamy and dichogamy (Webb & Lloyd, 1986). Selfing in these plants is avoided by inbreeding depression and self-incompatibility (Ramawat, 2010). Most Cacti flowers have inferior ovaries (epigynous) while a few have superior flowers. Cacti plants are entomophilous since

their pollination mechanism is by organisms like insects like bees, birds (Böhm, 2008), bats and others.

All Cacti have the major tissues and organs of commonplace dicots (Mauseth, 2006) and are characterized by the presence of areoles, which usually develop the spines. Some Cacti bear sharp spines of up to 5 cm in length and a cluster of troublesome miniature barbed stickers termed glochids (Kang'ara & Gitari, 2008). Cacti are uniquely identified by the presence of areoles which are highly reduced branches (Anderson, 2001). It is in the areoles that tubular and multipetaled flowers arise. The presence of spines in place of leaves, shallow fibrous roots, enlarged stems that are ribbed or fluted and able to photosynthesize form part of the crucial anatomical and physiological adaptations for Cacti survival (Kang'ara & Gitari, 2008).

Cacti can be tree-like (arborescent) as seen in the genus *Pereskia,* with a race of *BrasiliOpuntia,* of 20–25 m high, forming the tallest freestanding Cacti (Gorelick, 2009). Some Cacti grow shrubby, like *Stenocereus thurberi*, while *Cephalocereus senile* is columnar. Globuse Cacti include *FeroCacti latispinus* whereas epiphytic forms are seen in *Rhipsalis paradoxa* and *Pfeiffera iantothele* (Calvente *et al.,* 2008). Some develop barreliform (*Denmoza rodacantha*), or articulated like the *Opuntia quimilo* (Ramawat, 2010). *Opuntia spp.* are characterized with having cladodes, modified photo synthetic stems and aerioles (Mashope, 2007).

Seemingly, there is no plant family which surpasses *Cactaceae* family in structural diversity; which may structurally grow as vines and trees, dwarfs and giants, epiphytes

and geophytes. Various Cacti produce different types of anatomies and/or morphologies at different stages of their lives hence referred to as dimorphic (Mauseth, 2006).

The breeding systems and variety of fertilization, enhanced with the genetic diversity within *Cactaceae*, denotes the intricate evolution of Cacti and the flexibility of their reproductive response to the spatially and temporally erratic habitats where they occur (Ramawat, 2010).

Increased vegetative traits (such as cladode size) and reproductive vigor signifies high ploidy levels in *Opuntia* species (Mashope, 2007). The basic number of chromosomes in *Cactaceae* is x=11 (Mihalte *et al.,* 2011). Figueredo *et al.,* (2010) established that tetraploid and hexaploid taxa have bigger and weightier seeds while Oselebe and Tenkouano (2009) have shown that diploids, in comparison with the triploids, are smaller plants.

The use of Isozymes is the earliest molecular marker technique applied in Cacti pear (*Opuntia* species) in investigating its genetic diversity (Uzun, 1997). However, isozymes are not used currently because they generate a low number of markers; they are affected by the environmental conditions and developmental stage of the plant and tissue type (Mashope, 2007).

Some Cacti are typically huge, leafless, long-living, fleshy stems of diverse shapes and sizes, with clusters of spines. Their flowers are usually large, solitary, and colorful, with numerous segments. The flowers are bisexual with both a pistil and several stamens (Anderson, 2001). Karle and Boyle (1999) recorded that flower senescence was hastened

in self-compatible genotypes of Easter Cacti that self-pollinated early in floral development, but was not affected by the timing of self-pollination in self-incompatible genotypes.

The described habits of Cacti growth forms include; globular, climbers, pendent, leaflike, columnar and clustering. Some had felted areoles in the axils of persistent leaves, cylindrical stems, deciduous leaves, terete, spineless, flattened stems, spherical to shortly cylindrical stems, ribbed stems, and some with tubercled stems (Anderson, 2001). The organization of the internal structure of the Cacti stem resembles a typical dicotyledonous or broad-leaved plant stem, which is composed of epidermis, cortex, vascular system, and the pith (Anderson, 2001; Bobich & Nobel, 2001).

The Plastids in *Mammillaria gracillis* (*Cactaceae*) are very sensitive organelles to an artificial hyperhydric environment as well as to Agrobacteria-mediated cell transformation (Poljuha *et al*., 2003). Additionally, majority of Cacti have thin-walled epidermal cells except for a few taxa, such as species of Armatocereus, Jasminocereus, Cereus and Mammillaria, whose periclinal (external) wall is thicker than the internal and radial walls (Nobel, 2002).

Many Cacti have a shallow root stem, while others like *Ariocarpus and Lophophora* have large taproots for water storage, some have short compact lateral roots as is the case with many globuse Cacti (Anderson, 2001; Nobel, 2002). The pollen and seed morphologies of some Cacti species have been documented (Kurtz, 2009; Cota-Sánchez & M-Patrício, 2010).

Cacti fruits may be formed on the stems (*Thrixanthocereus blossfeldiorum*), on the edges of cladodes (*Opuntia monacantha*) or on edges of its branches (*Cereus peruvianus*). They have fruits of varied sizes and with glochids (*Opuntia stricta*) while some do not have glochids (*Euphorbia abyssinicca*). The colors of ripe fruits range from light green (*Opuntia exaltata*), to orange (*Opuntia ficus-indica*), to purple (*Opuntia stricta*), violet-red (*Cereu peruvianus*) to red (*Thrixanthocereus blossfeldiorum*) (Omweri *et al.*, 2016) among others.

In South Africa varieties, cladodes of different shapes are present while in Chile the commonly available varieties have fruits which remain green even when ripe. Vegetative vigor, fiber of cladodes and, protein and sugar contents, among others, determine how *Opuntia* species can be utilized (Chiteva & Wairagu, 2013).

2.2 Origin and distribution of Cacti

Cactus is not native to Kenya. All the species in Kenya may have initially been introduced into the country by white settlers in the years between 1940 and 1960 (Githae & Nyangito, 2010; Kunyanga, Strum, Graham, Sipitiek, & Imungi, 2009) It is believed to have originated from the central Andean region of northern Chile, northwest Argentina, Peru and Bolivia (Defelice, 2004), and the new world (Nobel, 2002). Among the Cacti species, only *Opuntia ficus-indica* species has been cultivated on all continents apart from the Antarctica (Ervin, 2012). The species has been grown in hot, arid and semi-arid regions in various counties including Brazil, Argentina, Tunisia, South Africa, Italy, Chile, Algeria and Mexico (Caloggero & Parera, 2004)

Among the ASALs of Kenya where Cacti are grown as hedges or in the wild are Baringo (around Lake Baringo), Nyeri North, Laikipia North and Laikipia East (Mukogodo and Lamuria divisions, respectively) (Kang'ara & Gitari, 2008). The species is said to have been introduced in Laikipia areas in Kenya in 1940s from Australia by a colonial administrator who used the plant for fencing to keep off predators (Kang'ara & Gitari, 2008). These plants have also been established in farms for prevention of long-term ecosystem degradation, to check soil erosion (Mulu, 2015; Bariagabre *et al.*, 2016), and ultimately enhance restoration of vegetation (Zoghlami *et al.*, 2007).

Previous studies have shown that the phenols in Cacti species are region-specific. For example, some phenolic compounds have been identified in Tunisian *Opuntia ficus-indica* flowers and not exist in *Opuntia ficus-indica* flower cultivated in Sicily (Italy) (Yeddes *et al.*, 2013). Mexico hosts the highest Cacti diversity in the world (Ortega-Baes & Godínez-Alvarez, 2006).

2.3 Importance of Cacti

Adaptation and moving to more drought-tolerant crops is an important strategy within a diverse portfolio of livelihood responses to climatic stress. Of the approximated 200-300 genera (Chiteva & Wairagu, 2013) in the *Cactaceae* family, *Nopalea* and *Opuntia* genera are the most import to man (Khalafalla *et al.*, 2007). *Opuntia* in particular is an important crop for developing countries in ASALs because of its multiple purposes (Mashope, 2007).

Cacti produce large quantities of forage throughout the year when properly managed. Thus, productivity is increased with improved management conditions of the crop (Le Houérou, 2002). These can be utilized as livestock feed and it provides the much needed water, vitamins and energy in the dry seasons (Kang'ara & Gitari, 2008). However, *Opuntia* cladodes lack sufficient crude protein and protein supplementation is necessary (Mashope, 2007). Other important uses of Cacti include fruits that are food during dry periods (Chiteva & Wairagu, 2013), food products like juices, liquid sweeteners and alcoholic drinks (Yeddes et al., 2013). Young cladodes are used as vegetable and fodder (García de Cortázar and Nobel, 1992), firewood (Nilsen *et al.,* 2005), cochineal production, medicine, hedges and in reinforcement of whitewash on homes in parts of Latin America (Ervin, 2012), pharmaceutical products (FAO, 2013). Some utilized their spines in making fishhooks, utilized for religious purposes (Anderson, 2001) and biogas production with cow dung (Gebrekidan, 2014).

In addition to their nutritional properties, Cacti contain bio compounds such as betalain, and phenolic compounds with antioxidant properties that protect humans against degenerative diseases (Yeddes *et al*., 2013). The betalain pigments contained in these Cacti pears have shown beneficial effects on the redox-regulated pathways involved in cell growth and inflammation (Sumaya-Martínez et al., 2011).

Mexico, Chile, Israel, Italy and South Africa, have wholly developed Cacti industries and use the plant for various purposes (Caloggero & Parera, 2004). Japan is among the world's major consumers of Cacti products while prickly pear Cacti are sometimes preferred as natural fence barriers (Khalafalla *et al*., 2007). In Ethiopia, its production is

an important source of income (Ervin, 2012). This species has also been grown in Ethiopia for soil and water conservation (Belay *et al*., 2011) and to combat desertification (FAO, 2002).

Although local communities in Baringo Kenya believed that Cacti were causing degradation of land and desertification (Kang'ara & Gitari, 2008), the many benefits accrued from the plant in other countries could be realized through dissemination of information on its utilization.

2.4 The genome of Cacti

Cacti are polyploids of different levels, with a basal chromosome number of eleven (x=11) and has DNA content similar to that of small genome crops such as sorghum and tomato (Nobel, 2002), with majority of them being diploid (2n = 2x=22) for instance *Opuntia heliabravoana*. Fewer than 20% of these are polyploids (2 n > 22) that occur in the subfamily Opuntioideae. Some are triploid like *Haageocereus tenuis* (Arakaki *et al.*, 2013), tetraploids such as *Opuntia robusta* (4x = 44), hexaploids (2n = 3x =66) including *Opuntia oligacantha* and octoploids (8x - 88) (Anderson, 2001; Segura et al., 2007). Polyploidization results in a reduction in cell surface: volume ratio in reproductive and vegetative structures of these plants while cell sizes and organs enlarge (Cota-Sánchez & M-Patrício, 2010).

Segura *et al*,. (2007) proposed the use of flow cytometry technique for genome quantification developed first for biomedical researches and adapted for genetic plant analysis. The technique provides an estimation of the volume and intensity of

fluorescence of the nuclei of isolated cells which in turn enables a great number of nuclei to be analyzed with ease.

2.5 Cacti production

Cacti are grown for their fruits called Tuna or edible pads referred to as nopales or nopalitos (Segura *et al.*, 2007) among other domestic/cultural, commercial and industrial functions including medicine (Anderson, 2001).

Commercial plantations have been established in the Mediterranean region, Mexico, Argentina, Brazil, Chile, Algeria and South Africa (Anderson, 2001). In Mexico, about 428,763 tons of fresh fruit are harvested from 53,876 ha of commercial plantations (7.96 ton/ha) while in Ethiopia only 128,660 tones could be harvested from 30,520 ha (4.22ton/ha) (Dulume, 2010).

The productivity of some *Opuntia* species in Kenya that was determined by counting the average number of fruits per square meter at three different sample plots stood at 11.38kg/m^2 for *Opuntia ficus-indica* and 6.43kg/m^2 for *Opuntia monacantha* (Mutwa *et al.*, 2015). The main constrain of the use of some Cacti species in Kenya is cultural factors, with a complexity in adjusting the eating habits while some regard them as poisonous, invasive and with dangerous spines (Githae & Nyangito, 2010).

2.6 Cacti breeding

Genetic resources are important in providing material for selecting and improving plants through breeding hence safeguard food security needs of the world's fast rising population. Conservation and utilization of plant genetic resources are imperative components of ex-situ collections (Fu, 2003). Combining conservation and establishment of ex situ field collections of accessions that express potentially useful traits can be designed to be a source of germplasm enhancement and breeding for commercial cultivation, especially in the areas of introduction, (Chessa, 2009).

An American plant breeder, Luther Burbank, is reported to have developed many cultivars of *Opuntia ficus-indica*, thus, popularizing the plant in the first part of the twentieth century (Anderson, 2001). Over twenty, and approximately up to forty Mexican cultivars of *Opuntia ficus-indica* which is among the most important Cacti, have been developed through horticultural breeding and selection (Ervin, 2012).

Nonetheless, there is barely any documented information on the strategies employed in multiplication and improvement of the Cacti in the ASALs of Kenya. Sharing of stem cuttings and cladodes to establish borders/ fencing or/and for ornamental purposes is the main distribution method in Kenya. Dispersal of the Cacti may also be occurring through animals and birds (Kang'ara & Gitari, 2008).

2.7 Characterization of Cacti

2.7.1 Morphological characterization

In general, characterization of some Cacti like *Opuntia matudae* is incomplete. There are a few descriptions using either vegetative or reproductive traits. Descriptors lack botanical references and other data, which makes it difficult to properly document. These incomplete descriptors limit their application in formal rescue, conservation and utilization (Gallegos–Vásquez *et al.*, 2010). Cacti are underrepresented in herbaria, most likely due to the difficulty in creating dried samples due to the succulent nature (Rivera, 2013).

Both the spiny and spineless Cacti occur in Kenya. No comprehensive survey to identify and characterize the *Opuntia* species and varieties is available in Kenya and a database created (Kang'ara & Gitari, 2008). Reports indicate that the morphological variability among accessions, when they are growing in their native place, is unknown (Peña-Valdivia *et al.*, 2008). Morphological traits such as presence of spines and cladode dimensions (length and width), as well as number of areoles on the face of the cladode, allowed the characterization of some accessions of Cacti pear from north central Mexico (Boke, 2016; Caloggero & Parera, 2004). Some other traits useful in the separation of Cacti pear accessions are fruit dimensions and total sugar content. On the other hand, In Chile, prickly pear ecotypes were characterized and found significant differences between their fruits, although the plants were morphologically similar (Caloggero & Parera, 2004).

2.7.2 Molecular characterization

Management and utilization of plant genetic resources, molecular techniques play a vital role in the characterization of plant germplasm (Fu, 2003), Cacti included. Molecular characterization of different genera within the *Cactaceae* family has demonstrated significant genetic variability at different regions where the plants exist.

Amongst the studied genera, *Opuntia*s form a significant majority of the existing literature. The molecular characterization of the species was undertaken using different molecular tools such as internal transcribed spacer sequences (ITS) (Lyra *et al.,* 2013) inter simple sequence repeats (ISSR) (Ernestina *et al*., 2014), AFLPs, Random amplified polymorphic DNA (RAPDs) and SSRs. Generally genetic diversity information in wild species has come from allozyme surveys which exhibit simple inheritance, codominance, complete penetrance, and consistency of expression under a wide range of environmental conditions (Nobel, 2002).

Zoghlami *et al,*.(2007) documented considerable genetic diversity of 36 Tunisian *Opuntia ficus- indica* (L.) Mill. ecotypes using RAPD and used this outcome to recommend the genotype to be preserved in a reference collection.

2.8 Cacti genetic marker

DNA-based genetic markers have been utilized in characterizing germplasm of different Cacti species. They are helpful in estimating genetic diversity of species, varietal identification, crop improvement programs, and intellectual property protection and

quality control of seed production. They are also convenient since plant DNA is not subject to environmental modifications (Bachmann *et al.*, 2001)

In the analysis of the genetic diversity and similarity of the species Amplified fragment length polymorphism (AFLPs) technique has been utilized. This is based on the fact that AFLPs are known to map throughout the genome; this high-volume DNA fingerprinting techniques gives fast and efficient measurements of genome-wide similarity/distance (Nilsen *et al.*, 2005). The use of Isozymes (O'Leary & Boyle, 2000) and random amplified polymorphic markers(RAPDs) (Gordon & Kubisiak, 1998) have been documented. In addition to the above markers, inter simple sequence repeats (ISSR) (Ernestina *et al*., 2014) markers and simple sequence repeats (SSR) (Otero-Arnaiz *et al*,. 2004) have been developed and applied to classify some Cacti species.

The current study considers the use of simple sequence repeats (also called microsatellites) in the estimation of the genetic diversity among the Cacti species in Kenya. They are important molecular markers in both animals and plants. Simple sequence repeats (SSR) are short stretches of nucleotide units repeated in tandem and randomly spread in eukaryotic genomes. Their high mutation rate that affects the number of repeat units makes them very polymorphic (Fu and Chakraborty, 1998). Hence, easily detected on high resolution gels (e. g. sequencing gels), by running Polymerase chain reaction (PCR) amplified fragments obtained using a unique pair of primers flanking the repeat. The advantages of SSR over other molecular markers include; a) they are co-dominant, b) They allow the identification of many alleles at a single locus, (c) they are evenly distributed in the genome, (d) the analysis can be semi-

automated and performed without the need of radioactivity and, (e) little DNA is required.

This technology also offers the potential that data acquisition can be more cost effective as compared to other technologies. SSRs are also important for the genetic analysis of quantitative traits (QT), in order to evaluate the relative importance of different QT alleles in different genetic backgrounds and environments. The SSR technology requires DNA sequence data that are species specific and development of SSR primers. The SSRs may be used to develop profiles that are highly discriminative among cultivars for many species. SSRs on the other hand can be mapped to discrete loci, which are stably inherited within a given species.

CHAPTER THREE

MORPHOLOGICAL CHARACTERIZATION AND DISTRIBUTION OF CACTI IN KENYA

3.1 Introduction

Cacti grow in different forms such as trees or shrubs with conspicuous persistent leaves (Pereskia Mill.) or most often branched or unbranched, columnar to globular stem succulents. Cacti can be scandent, epiphytic, or epilithic and have either slender, terete stems or flattened, leaflike cladodes (Nyffeler, 2002). Some Cacti also grow as vines, dwarfs, giants, epiphytes and geophytes. Other Cacti produce different types of anatomy or morphology at different stages of their development hence dimorphic (Mauseth, 2006).

The highest diversity of Cacti is recorded in Mexico, followed by Brazil, Argentina, Bolivia and Peru (Ramawat, 2010), with varied and unique morphologies. *Opuntia* genera is characterized with cladodes (pads), modified photosynthetic stems, that resembles leaves, numerous areoles that may have glochids and with or without leaf spines (Mashope, 2007), stamens shorter than tepals (Gallegos–Vásquez *et al.*, 2010). Cacti species can have cylindrical, globular or flat stems leading to different life forms, such as arborescent, columnar, globular, barreliform, and articulate (Salgado & Mauseth, 2002). The fruits of Cacti take diverse colors, shapes and sizes (Gallegos-Vázquez *et al.*, 2012; Chalak *et al.*, 2014)

Brasili*Opuntia* that grows up to 25m tall is among the tallest freestanding Cacti (Gorelick, 2009). The developmental morphology of leaves, tubercles, roots, shoot axis, flowers and areoles in *Cactaceae* family has been documented by Boke (2016). The Cacti in Kenya have not been characterized and hence this study was undertaken.

3.2 Materials and methods

3.2.1 Collection sites of Cacti

The study was conducted in two zones in the ASALs of Kenya. The Eastern zone comprised of Machakos and Makueni counties, and the Rift Valley zone comprised of Baringo, Laikipia and Nakuru. The counties are located above 1000 m but below 3098 m of altitude and receiving rainfalls of between 150 mm and 1800 mm (Table 3.1).The areas were selected in consideration of their climatic conditions and presence of the Cacti species. The characterization of Cacti *in situ* was carried out different locations in the above counties.

3.2.2 Experimental design

Morphological characterization of identified Cacti species growing in ASALs was carried out in their respective locations. Selected vegetative characteristics of the plants including, cladodes, flowers, areole numbers, seeds and fruits of each accession was undertaken based on the UPOV descriptors list. A GPS receiver (extrex 10) was used to show the specific geographical coordinates (Table 3.1) of locations where samples were collected.

A purposeful sampling procedure which targeted locations where Cacti were present was followed to define the sampling units. The Cacti sampled were either growing in farmers' fields as fencing/border material or those growing in uncultivated lands. Clusters of Cacti that were growing along the major roads and off the roads were also sampled.

3.3 Data collection

All the morphological traits observed in the field were recorded based on (UPOV, 2006) derived descriptors list (Appendix 2). Three replications of each parameter were collected. These included the plant growth characteristics and habits, the floral traits, stem characteristics, spines, fruit traits and setting, areole characters, glochids, cladodes and seed characteristics.

3.3.2 Data analysis

Morphological data obtained was converted to binary data format and cluster analysis done using Unweighted Pairwise Group Method with Arithmetic Averages (UPGMA) to determine the variations among the Cacti.

3.4 Results

3.4.1 Distribution of Cacti species in the study areas

Eight different Cacti species (Table 3.2) were found growing in Baringo, Lakipia, Nakuru, Machakos and Makueni Counties at an altitude from 1000 meters above sea

level to 3098 meters above sea level. The lowest mean temperature was 9.1^0C while the highest was 35^0C. The annual rainfall ranged between 150 mm to 1800 mm (Table 3.1).

Table 3.1: Geographical distribution of the twenty locations with Cacti species in Kenya

Machakos County

Species	Location	Altitude (m asl)	Longitude	Latitude	Temperature	Rainfall Range (mm p.a)
Thrixanthocereus blossfeldiorum	Daystar	1000-1600	037.03706^{0}E	01.47686^{0}S	9.1^0-26.7^{0}C	500-900
Thrixanthocereus blossfeldiorum	Lukenya	1000-1600	037.04763^{0}E	01.04763^{0}S	9.1^0-26.7^{0}C	500-900
Opuntia exaltata	Arthi River	1000-1600	036.99327^{0}E	01.44386^{0}S	9.1^0-26.7^{0}C	500-900
Opuntia ficus-indica	Lukenya 2	1000-1600	037.04918^{0}E	01.45531^{0}S	9.1^0-26.7^{0}C	500-900
Opuntia monacantha	Masimba	1000-1600	037.60233^{0}E	02.15351^{0}S	9.1^0-26.7^{0}C	500-900
Cereus peruvianus	Lukenya 2	1000-1600	037.06279^{0}E	01.49396^{0}S	9.1^0-26.7^{0}C	500-900
Euphorbia abyssinica	Arthi River	1000-1600	037.04729^{0}E	01.46043^{0}S	9.1^0-26.7^{0}C	500-900
Cereus peruvianus	Lukenya 1	1000-1600	037.04765^{0}E	01.46028^{0}S	9.1^0-26.7^{0}C	500-900
Euphorbia abyssinica	Green park	1000-1600	037.01513^{0}E	01.46534^{0}S	9.1^0-26.7^{0}C	500-900

Baringo County

Species	Location	Altitude (m asl)	Longitude	Latitude	Temperature	Rainfall (mm p.a)
Cereus peruvianus	Kures	1000-2600	035.91872^{0}E	00.08334^{0}N	10^0-35^{0}C	600-1500
Euphorbia ingens	Radat	1000-2600	035.89096^{0}E	00.05399^{0}S	10^0-35^{0}C	600-1500
Opuntia monacantha	Marigat	1000-2600	035.97977^{0}E	00.47060^{0}N	10^0-35^{0}C	600-1500
Opuntia monacantha	Marigat	1000-2600	035.94146^{0}E	00.38866^{0}N	10^0-35^{0}C	600-1500

Laikipia County

Species	Location	Altitude (m asl)	Longitude	Latitude	Temperature	Rainfall (mm p.a)
Opuntia ficus-indica	IDP-Wiyumerere	1500-2611	036.65597^{0}E	00.05776^0 S	16^0-26^{0}C	400-750
Opuntia ficus-indica	Matunda	1500-2611	036.67084^{0}E	00.01241^0 S	16^0-26^{0}C	400-750
Euphorbia abyssinica	Nairuti	1500-2611	036.71133^{0}E	00.14193^0 S	16^0-26^{0}C	400-750
Opuntia exaltata	Jikaze	1500-2611	036.61605^{0}E	00.07926^0 S	16^0-26^{0}C	400-750

Makueni County

Species	Location	Altitude (m asl)	Longitude	Latitude	Temperature	Rainfall (mm p.a)
Opuntia monacantha	Utini	1000-1600	037.58617^{0}E	02.10618^0 S	9.1^0-26.7^{0}C	500-900
Euphorbia abyssinica	Salama	1000-1600	037.25404^{0}E	01.83437^0 S	9.1^0-26.7^{0}C	500-900
Opuntia stricta	Sultan Hamud	1000-2100	037.36682^{0}E	02.00916^0 S	12^0-28^{0}C	150-650

Nakuru County

Species	Location	Altitude (m asl)	Longitude	Latitude	Temperature	Rainfall (mm p.a)
Opuntia ficus-indica	Delamere 1	1530-3098	036.41130^{0}E	00.68800^0 S	12^0-29.3^{0}C	500-1800
Opuntia exaltata	Naivasha	1530-3098	036.41100^{0}E	00.68802^0 S	12^0-29.3^{0}C	500-1800
Euphorbia ingens	Kiongororia	1530-3098	036.35695^{0}E	00.56759^0 S	12^0-29.3^{0}C	500-1800
Opuntia ficus-indica	Dalmare farm	1530-3098	036.41121^{0}E	00.68857^0 S	12^0-29.3^{0}C	500-1800
Cereus peruvianus	Pema Victorius	1530-3098	036.23124^{0}E	00.37987^0 S	12^0-29.3^{0}C	500-1800
Opuntia exaltata	Kikopei	1530-3098	036.41100^{0}E	00.68802^0 S	12^0-29.3^{0}C	500-1800
Opuntia ficus-indica	Kiongororia	1530-3098	036.41130^{0}E	00.68800^0 S	12^0-29.3^{0}C	500-1800
Opuntia exaltata	Dalmare farm	1530-3098	036.43187^{0}E	00.70041^0 S	12^0-29.3^{0}C	500-1800

Sixty two percent of the Cacti species were found growing in rows as fencing/border material in farms (Plate 3.1a-d), along main roads and some were dispersed intermittently in uncultivated lands. *Cereus peruvianus, Euphorbia abyssinica* and *Euphobia ingens,* were grown as ornamentals, but were also found growing in the wild. *Thrixanthocereus blossfeldiorum* was grown ornamental and as fencing material (Plate 3.1c). There were no sighted plantations for fruit or vegetable production despite the fruits of *Opuntia ficus-indica, Opuntia monacantha and Opuntia stricta* being edible.

Plate 3.1: (a-d): Morphological differences among Cacti species in the ASALs of Kenya. a) *Cereus peruvianus,* b) *Opuntia monacantha,* c) *Thrixanthocereus blossfeldiorum,* d) *Opuntia exaltata,* e) *Opuntia stricta,* f) *Euphorbia ingens,* g) *Euphorbia abyssinica,* h)*Opuntia ficus-indica*

Table 3.2: The distribution of Cacti species in the ASALs of Kenya

Location	Nakuru	Baringo	Laikipia	Machakos	Makueni
Species					
Opuntia monacantha					
Opuntia ficus-indica					
Opuntia exaltata					
Opuntia stricta					
Thrixanthocereus blossfeldiorum					
Euphorbia abyssinica					
Cereus peruvianus					
Euphorbia ingens					

The highest clonal populations of the *Opuntia* species were found in Marigat (Baringo County) and Matunda (Laikipia County). Apart from trimming the *Opuntia*s grown as border markers and life hedges, there were no other management practices undertaken for these growths.

Opuntia ficus-indica was found in Nakuru, Baringo, Machakos and Laikipia Counties; *Opuntia stricta* was only sighted in Makueni County, a frequency similar to that of *Thrixanthocereus blossfeldiorum* that appeared only in Machakos County. *Opuntia monacatha* was present in Baringo, Makueni and Machakos County. *Opuntia exaltata* in Nakuru Laikipia and Machakos Counties while *Euphorbia abyssinica* was found in all counties studied except Nakuru. *Cereus peruvianus* and *Euphorbia ingens* were present in Nakuru and Baringo County (Fig. 1).

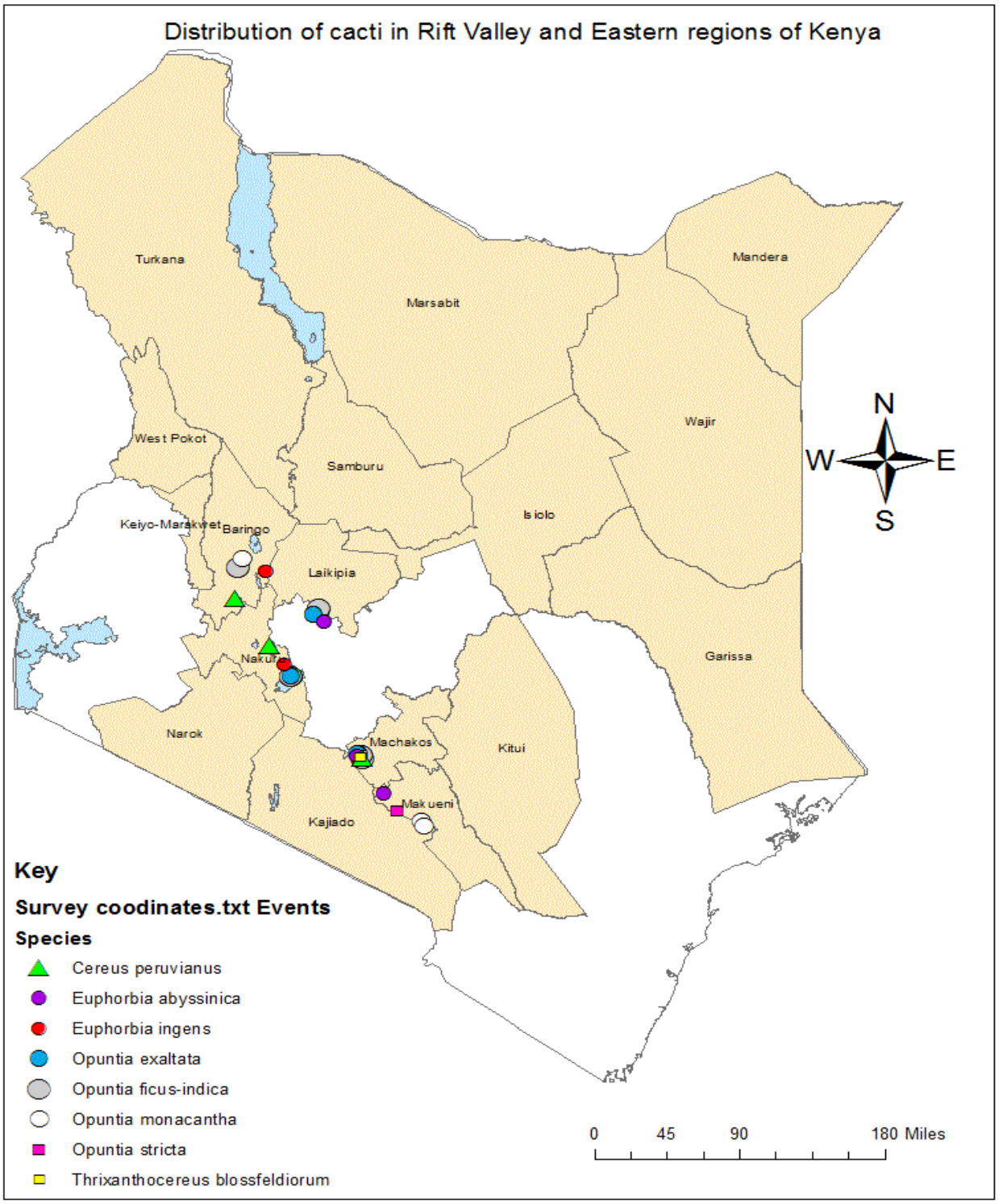

Figure 3.1: Distribution of Cacti species in the Rift Valley (Laikipia, Baringo and Nakuru) and Eastern (Machakos and Makueni) regions of Kenya

3.4.2 Morphological diversity: Plant characteristics

The species identified were either arborescent or shrubby with majority of them having elongated shape such as in *Opuntia exaltata* (AR01) and *Opuntia ficus-indica* (DP03). Thirty three populations composed of seven species bore round plant shapes (Appendix 3). Examples include *Opuntia monacatha* (UT01), *Euphorbia ingens* (ENN02) and *Opuntia ficus-indica* (DP03). Majority of the stems produced were flattened whereas all the *Opuntia exaltata* accessions had round shaped stems. Only two populates of *Opuntia ficus-indica* (UK02, UK03) produced plants with flat shape.

The diversity of the Cacti was also evidenced by the differing cladode characteristics among the identified species. Spines were present in all Cacti cladodes but, there were no glochids in *Opuntia exaltata* accessions. There were relatively small or no spines on cladodes of old *Oputia ficus-indica* species. Twenty seven of the 69 accessions had white spines (*Opuntia ficus-indica* (DP01, DF03), *Opuntia monacantha* (UT01)), ten of them had light yellow spines (*Opuntia exaltata* (JL02), *Thrixanthocereus blossfeldiorum* LK01, LK02), while *Opuntia stricta* and some *Opuntia exaltata* bore golden spines. Black spines were produced by *Euphorbia abyssinica* while the rest produced either brown or grey spines. Cladodes were present in *Opuntia* genera alone. Seventeen of the *Opuntia* species had elliptic cladodes (*Opuntia ficus-indica* (DP01), *Opuntia monacantha* (VK03), 14 were ovate (*Opuntia stricta* (SH03)) and cylindrical cladodes were produced by the ten *Opuntia exaltata* species.

Spines were present in all the species with the lower range of 1-2 spines per areole in *Euphorbia ingens*, 1-7 spines in *Opuntia exaltata* and the highest (*Thrixanthocereus blossfeldiorum*), of more than ten spines per areole. However, cladode glochids were observed in all the *Opuntia* species except for *Opuntia exaltata.*

Plate 3.2(a-f): Different corolla colors among Cacti species in the ASALs of Kenya. a) *Opuntia exaltata,* b) *Thrixanthocereus blossfeldiorum,* c) *Opuntia stricta,* d) *Opuntia monacantha,* e) *Opuntia ficus-indica,* f) *Euphorbia ingens*

3.4.3 Morphological diversity: Corolla and fruit characteristics

Six corolla colors were recorded namely; pink, yellow, cream white, cream yellow, orange and yellow with purple strips (Plate 3.2). All the *Opuntia exaltata* accessions had pink flowers, while orange flowers were produced by *Opuntia ficus-indica*. *Opuntia stricta* (SH01, SH02 and SH03) produced bright yellow flowers and of the remaining *Opuntia monacantha* had yellow flowers with purple strips. *Euphorbia abyssinica*, *Thrixanthocereus blossfeldiorum* and *Cereus peruvianus* flowers were cream to white whereas those of *Euphorbia ingens* were cream but with a yellow shade.

Plate 1.3(a-f): Morphological differences in fruit characteristics of Cacti species in Kenya. a) & b) ***Opuntia exaltata, c) Thrixanthocereus blossfeldiorum.*** **d) & e)** ***Opuntia stricta*** **f)** ***Opuntia ficus-indica***

Sixty eight percent of fruits produced were oval in shape except for *Opuntia stricta* and *Euphorbia abyssinica* that produced globuse fruits. None of the *Opuntia exaltata, Euphorbia abyssinica,* Euphorbia *ingens* or *Cereus peruvianus* accessions had fruit glochids whereas the rest produced fruits with glochids. Mature ripe fruits with purple coloration were produced by *Opuntia monacantha, Opuntia stricta, Euphorbia ingens and Euphorbia abyssinica* while *Opuntia ficus-indica* accessions produced orange fruits (Plate 3.3). All the *Opuntia exaltata* produced light green fruits; *Thrixanthocereus blossfeldiorum* had red fruits and *Cereus peruvianus* violet-red fruits (Appendix 4).

The Cacti were morphologically grouped into two main clusters A and B (Figure 3.2). All the species in the *Opuntia* genera clustered together in group A most probably due to the presence of cladodes that were missing in the second cluster of species. *Opuntia ficus-indica* had the most divers morphological traits resulted in the distribution within the different branches in the main cluster. The other genera clustered separately (B) which majorly was attributed to their plant sizes with mean heights of more than 5meters and that they grew arborescent apart from *Cereus peruvianus* which may also columnar.

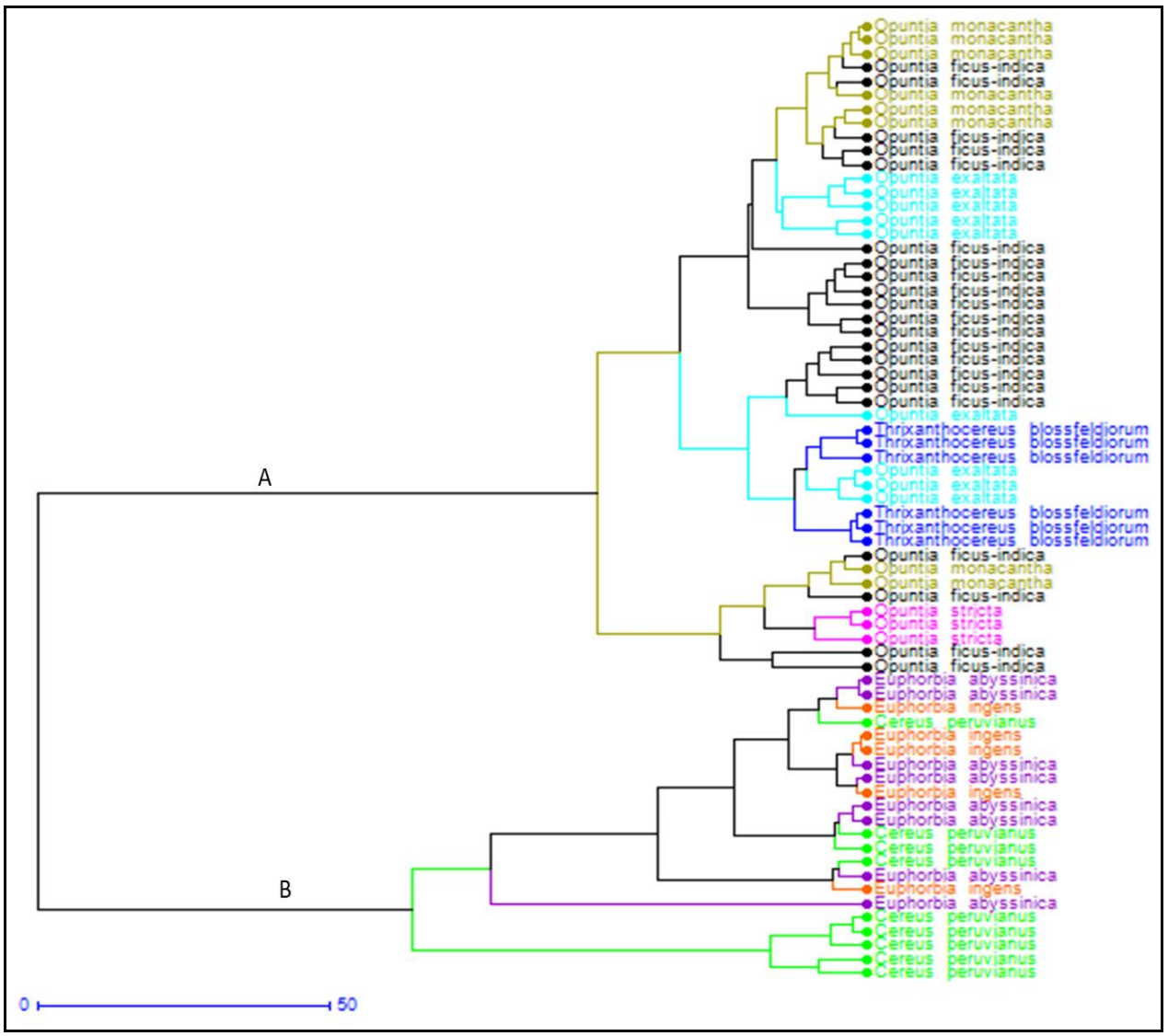

Figure 3.2: Dendrogram constructed based on morphological characters showing the diversity of Cacti species in Kenya. Key: *Opuntia monacantha.* *Oputia ficus-indica.* *Thrixanthocereus blossfeldiorum* *Opuntia exaltata.* *Euphorbia ingens.* *Opuntia stricta.* *Euphorbia abyssinica.* *Cereus peruvianus*

3.5 Discussion

Cacti in the two ASAL areas of Kenya studied are evidently diverse and distinct. Their morphology varies in their growth forms, shape of their stems, presence or absence of cladodes, spines characteristics and fruit characteristics. Based on these characters, eight distinct species of Cacti exist in the Rift Valley and Eastern regions of Kenya's.

The presence or absence of cladodes distinguished the species into the *Opuntia* (cluster A) and the non-*Opuntia* genera (cluster B) (Figure 3.2). All the *Opuntia* species had cladodes, while the other genera such as *Cereus* and *Thrixanthocereus* do not bear cladodes. Cladode characteristics for the *Opuntias* were also useful in distinguishing species into cylindrical (*Opuntia exaltata*), ovate (*Opuntia stricta,* or elliptic (*Opuntia monacatha* and *Opuntia ficus-indica* (Chalak *et al.,* 2014; Peña-Valdivia *et al.,* 2008)). Thirteen percent of the *Opuntia ficus-indica* in Nakuru bore ovate cladodes as was the case for *Opuntia monacatha* in Baringo County. Hence, there is need to use more than cladodes in distinguishing the two species.

Cladode characteristics contribute to characterization as documented by Peña-Valdivia *et al.* (2008) who recorded that leaf length could be reliable as a morphological marker in characterization of *Opuntia* accessions since small differences of this characteristic were statistically significant. Additionally, they reported differences in the areole characteristics and presence or absence of spines in the cladodes as relevant distinguishing morphological characteristics.

The floral traits at a glance would easily distinguish the Cacti of Kenya. *Opuntia exaltata* produces pink petals, *Opuntia stricta* have yellow flowers (Majure & Ervin, 2007), *Opuntia ficus-indica* with orange flowers while *Opuntia monacatha* produces yellow flowers with purple strips. *Euphorbia ingens, Thrixanthocereus blossfeldiorum* and *Euphorbia abyssinica* cream yellow in color whereas *Cereus peruvianus* bore white flowers.

The corolla color did not directly correspond to the final color of the mature fruit produced. This is because the mature fruit traits were varied. *Opuntia ficus-indica* produced orange fruits; *Opuntia exaltata* had yellowish green fruits, *Cereus peruvianus* violet-red fruit, *Thrixanthocereus blossfeldiorum* had red fruits while *Opuntia stricta* and *Opuntia monacantha* produced purple fruits. The mature fruit color and the fruit's pulp color were the same in all the identified species except for *Cereus peruvianus* whose pulp color was white. Nevertheless, the mature fruit color of *Opuntia* species is a direct indicator of the pulp color and the color of juice produced by the species. Chalak *et al*., (2014) recorded similar characteristics in the prickly pears (*Opuntia ficus-indica*) cultivated in Lebanon which included orange fruits, elliptic cladodes, round and ovoid shaped fruits.

High growth densities of *Opuntia monacantha* and *Opuntia ficus-indica* observed in Baringo and Laikipia respectively were influenced by low vegetative covers in these areas. With minimal disruptions by human activities, the densities may likely spread to cover majority of uncultivated areas in these regions. Erre *et al.,* (2009) observed a reduced competitiveness of *Opuntia* in places with dense vegetative cover. This is a

pattern observed for *Thrixanthocereus blossfeldiorum*. This trend is mostly as a result of the ease of growth through vegetative plant parts.

3.6 Conclusion and recommendations

Cacti are an available and important family of crops in the ASALs of Kenya but largely neglected with respect to their many uses. These plants are mainly shared across borders as fencing/border materials by farmers and some as ornamental. The distribution of Cacti in Kenya ASAL area is mainly through human activity.

The Cacti species in the ASALs of Kenya were morphologically distinct from one another. The major diagnostic characters of the Cacti in Rift Valley and Eastern ASAL areas of Kenya are presence or absence of cladodes and their growth forms; however, other key features include different corolla and mature fruit colors, type of stems and spine characteristics. The use of morphological descriptors should be complemented with molecular characterization so as to ascertain that the genotypes are genetically distinct.

Generally, the invasiveness of the *Opuntia* species in Baringo and Laikipia is potentially detrimental and if unchecked may in turn lead to destruction of useful productive land areas. Nevertheless, these two counties pose as being among the most ideal places for both small and large scale establishment of commercial farms for *Opuntia* species. Finally, based on the prolific nature and ability to form dense populations in Baringo and Laikipia, *Opuntia monacantha* and *Opuntia ficus-indica* are the forerunner-candidate species for these locations.

The effect of altitude and differences in climatic conditions on the distribution of Cacti in the study areas was not established in the current study. This relationship could be evaluated in future studies to identify the influence of altitude on the distribution of Cacti species in the country.

CHAPTER FOUR

ASSESSMENT OF GENETIC DIVERSITY OF THE CACTI SPECIES IN KENYA

4.1 Introduction

Mexico has the highest diversity of the *Cactaceae* family (Ramawat, 2010). The genetic diversity studies of different Cacti have been documented for *Opuntia spp* in South Africa ((Mashope, 2007), *Opuntia pilifera* in Mexico (Nilsen *et al.,* 2005), *Haegeocereus tenuis* in Peru (Arakaki *et al.,* 2013) and *Opuntia ficus-indica* in Ethiopia (Zoghlami *et al.,* 2007). These studies were based on the use of AFLP and RAPDs to describe the molecular variations of the respective species evaluated.

Although molecular markers are plagued by a number of shortcomings, namely; long time used in development of primers, inconsistent reproducibility in different laboratories, and failure to cross-amplify in related taxonomic groups (Seeb *et al.,* 2011), they are useful in distinguishing between *Opuntia* species (Nilsen et al., 2005). This has been demonstrated by (Ernestina *et al*., 2014) who documented the high genetic variation among genotypes of xoconostles using ISSRs.

The use of molecular marker methods proved a more valuable method to the use of taxonomic methods that resulted in poor and inexact outcomes (Mihalte *et al.*, 2011). Microsatellites were used in distinguishing the variability of *Haageocereus tenuis*, RAPDs for documenting the genetic structure of *Opuntia ficus-indica* in Morocco.

The current study utilized SSR markers to distinguish the Cacti species identified in the Rift Valley and Eastern regions of Kenya. The use of SSR markers has been deemed essential for describing the genetic structure of Cacti populations and aiding in conservation of wild ecotypes (Hughes et *al.,* 2008). SSRs are also useful in complementing phenotypic characterization to enhance accuracy of genotype identification (Mashope, 2007). Little information on similar studies has been documented on the Cacti found in Kenya's ASALs.

Given that some Cacti in the *Opuntia* genera have many morphological similarities and undergo drastic changes in different environments (Mashope, 2007), the use use of SSR makers would be essential in identifying the species in the ASALs of Kenya. This reduces the use of duplicates in conserved accessions (Chapman *et al.,* 2002) for future breeding purposes.

4.2 Materials and methods

4.2.1 Sample collection

Meristematic tissues (young leaves, buds, cladodes) from healthy plants were collected from sampled plants in the fields. These samples were kept in labeled polythene zip bags

containing silica gel then taken to JKUAT phytotechnology laboratory for DNA extraction. Assessment of the genetic diversity of the sampled species was done using simple sequence repeats (SSR technique).

4.2.2 Extraction of Cacti genomic DNA

The sampled meristematic tissues kept in zip bags were dried by use of silica gel. This was done by placing the samples in zip bags then a layer of cotton wool placed between the sample and silica gel then these were kept for a period of 12 hours. The silica gel was then replaced whenever it had turned in color from blue. The silica gel was replaced at intervals of 12 hours till there were no more changes in the color of the silica gel and the samples confirmed to have dried.

DNA extraction from the collected samples was done using a DNeasy plant mini-kit (Www.qiagen.com/handbooks, 2012). The dried samples of 0.04g each were crushed in a mortar and pestle. Then 400 µl Buffer AP1 and 4 µl RNase A were added. The contents were vortexed and incubated for 10 min at 65°C. The tubes were inverted 2–3 times during incubation. This was followed by addition of 130 µl Buffer P3, the contents mixed and incubated for 5 min on ice. Centrifugation of the lysate was done for 5 min at 14,000 rpm. The lysate was pipetted into a QIAshredder spin column placed in a 2 ml collection tube. Contents were centrifuged for 2 min at 14,000 rpm.

The flow-through was transferred into a new tube without disturbing the pellet present, then 1.5 volumes of Buffer AW1 added and mixed by pipetting. Part of the mixture (650 µl) was transferred into a DNeasy Mini spin column placed in a 2 ml collection tube and

centrifuged for 1 min at 6000 x g (8000 rpm). The flow-through was discarded and this step repeated with the remaining sample. The spin column was placed into a new 2 ml collection tube and 500 µl Buffer AW2 added, and centrifuged for 1 min 8000 rpm. The flow-through was discarded and another 500 µl Buffer AW2 added followed by centrifuged for 2 min at 8000 rpm.

The spin column was removed from the collection tube carefully so that the column does not come into contact with the flow-through then transferred to a new 1.5 ml or 2 ml microcentrifuge tube. Buffer AE (100 µl) was added for elution and the contents incubated for 5 min at room temperature (15–25°C). This was then centrifuged for 1 min at 8000 rpm.

Eleven microsatellite markers were used to characterize the Cacti species (Table 4.1).

Table 4.1: SSR markers used to genotype Cacti genotypes collected from ASALs of Kenya

Marker name	Primer sequences 5′–3′	Multiplex (T_a in °C)	Size range (bp)	Prop. unique genotypes	Freq. most prevalent genotype	GenBank Accession no.
*Opuntia*4	F: Cy5-GATGATTCCGCCATTCACC R: GTTT-CGTCGATCTGACTCACACC	2 (53.5)	105–152	0.90	0.023	DQ914850
*Opuntia*8	F: Cy5-ACCGCCATCACCAGCTATC R: GTTT-CTCACCCACAATTCCAAACC	4 (57)	136–178	0.93	0.064	DQ914854
*Opuntia*12	F: Cy5-TAATCTTATTCTCAGGTCAGTTAC R: GTTT-GGTATCTTGTTATTCGTTCG	4 (57)	226–294	1.0	0.005	DQ914858
*Opuntia*9	F: Cy5-CTAGGCTTCATCCCACATTAGG R: GTTT-TCCAAATTCACCTCCTCTGC	5 (59.3)*	147–185	0.87	0.019	DQ914855
*Opuntia*16	F: Cy5-GTCAATCCCGAGCAATTTAGG R: GTTT-CTCATTAGTGAGGCCCAACG	5 (59.3)*	322–350	0.70	0.062	DQ914862
*Opuntia*21	F: Cy5-AAAGGGAAGACCTTGCTCTC R: GTTT-TCTATTCTCAGCCCTCCTCTC	6 (59)	75–144	0.98	0.012	DQ914852
*Opuntia*10	F: Cy5-ACCAACATCAAACCTTCAATACC R: GTTT-CATGCTTCATCTTGTTCATTGG	6 (59)	191–247	0.30	0.13	DQ914856
*Opuntia*3	F: Cy5-GTGAGTGCCCAGATGAAACT R: GTTT-TCCTCAACTTTATTGTAGCAAGAG	6 (59)	317–344	0.67		DQ914849

*Opuntia*1	F: Cy5-CCATCTACTTCCCACTTTGC R: GTTT-CTCCTGTGTTTCTCTGTGCTC	3 (53)*	110 –138	0.92	0.014	DQ914847
*Opuntia*11	F: F: Cy5-CCTACACCTGCTGCCAATC R: R: GTTT-CGAGACAAACATCAGAGGAG	7 (61.5)	352–367	0.10	0.49	DQ914857

Source: (Helsen *et al.*, 2006)

Marker name	Primer sequences 5′–3′	Repeat type	Size range (bp)	Alleles	Alleles per ind.	Annealing (Ta in °C)
Opufic17	F: ATGGATCGTCTTCGTCCTTG R: GATGTCACCCCATTCCATTC	(AG)13	158-181	14	1-6	56

Source: Erre *et al.*, 2011

4.2.3 Agarose electrophoresis

Three grams of agarose powder was placed in a 500ml Erlenmeyer flask and 100ml of 1X TBE (TRIS 89Mm, boric acid 89Mm and EDTA 2.5Mm, Ph 8.3) buffer was added. The mixture was heated in a microwave for a few minutes for the agarose gel to dissolve.

The solution was then cooled to 50^0 then 0.5µg/ml of ethidium bromide added. The gel tray was prepared as the gel cooled by sealing the open edges of a clean dry glass tray with autoclave tape to form a mold. The edges were also sealed with some agarose solution. The rest of the agarose solution was poured into the gel tray and combs inserted to form sample slots which was then allowed to polymerize for an hour before removing the autoclave tape.

The gel was then immersed into an electrophoresis tank containing 0.5 TBX buffer. The combs were removed and 3µl of each DNA samples mixed with 1µl of loading dye on parafilm loaded in the first lane. The gel was then run for one hour at 100V until the bromophenol blue reached the end of the gel. The gel was viewed in a UV trans-

illuminator and the gel images captured using a digital camera. The presence or absence and number of bands were recorded.

4.2.4 Scoring of the SSR bands

The bands obtained were scored as discrete variables, using “0” when absent and “1” if they were present for a particular SSR band. The data was kept as allele frequencies in MS Excel and later used to calculate the number of alleles, genetic diversity and polymorphism information content (PIC).

4.2.5 Data analysis

The distance matrices, AMOVA and banding patterns among populations were calculated using GenAlEx 6.502. Binary data obtained from scored alleles was used to construct dendogram using DARwin 6.0.12. The ability of the primers to distinguish between morphologically distinct groups was determined by using power marker. Similarly, the genetic diversity and polymorphic information content (PIC) was computed using PowerMarker.

Analysis of variance, multiple comparisons of means, and multivariate analysis by cluster and principal components were used.

4.3 Results

Each of the eleven markers produced bands that were monomorphic for *Opuntia* species (figure 4.1). There was no amplification in *Cereus, Euphorbia and Thrixanthocreus* genera. A total of 168 bands were obtained with an average of 15.27 bands per primer

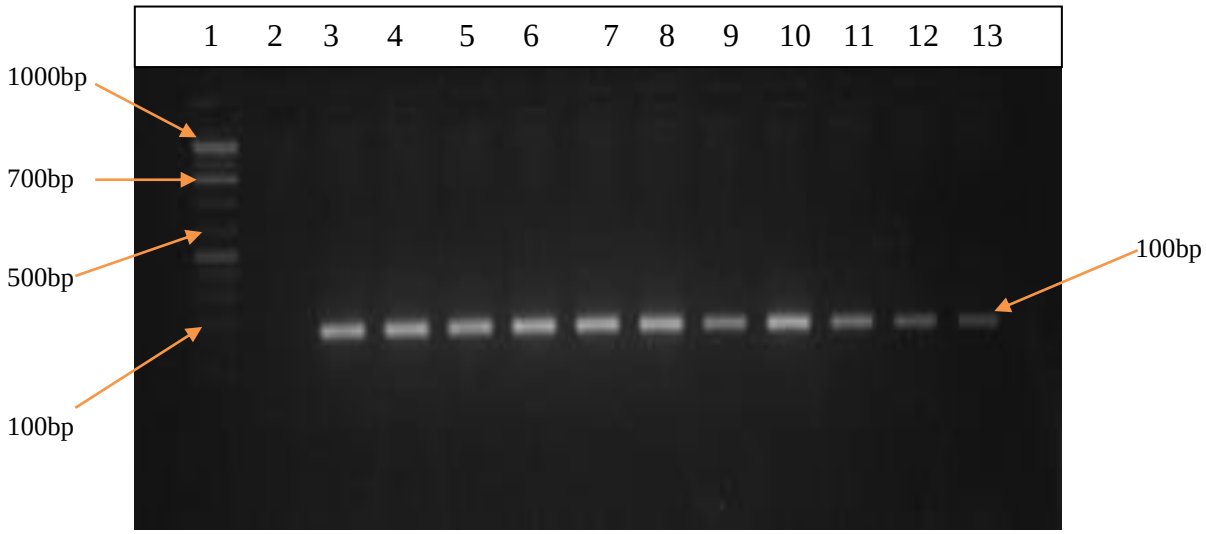

Figure 4.1: PCR profile of eleven Cacti samples using *Opuntia*8 SSR marker. Lane 1 is 1kb molecular weight marker, Lane 2 negative control and Lane 3, 7, and 8 *Opuntia monacantha* samples. Lane 4, 5, 6, 9-13 *Opuntia ficus- indica*

4.3.1 Characteristics of the SSR markers

The polymorphic information content (PIC) values of the eleven SSR markers used ranged from 0.199 for *Opuntia*4 to 0.375 for *Opuntia*1, *Opuntia*3 and *Opuntia*10 with a mean of 0.337 (Table 4.2). Only *Opuntia*4 and *Opuntia*12 produced PIC values below 0.3 whereas the remaining markers produced values above 0.335. The highest major allele frequency value was 0.872 produced by *Opuntia*4 followed by *Opuntia*12 with

0.846. Major allele frequency was lowest at 0.513 for *Opuntia*1, *Opuntia*3 and *Opuntia*10 (Table 4.2).

Table 4.2: Characteristics and genetic parameters of eleven SSR markers used in assessing genetic diversity of 39 *Opuntia* collections from Kenya ASALs

Marker	Major Allele Frequency	Sample Size	Gene Diversity	PIC
Opufic17	0.5897	39.0000	0.4839	0.3668
***Opuntia*21**	0.6154	39.0000	0.4734	0.3613
***Opuntia*10**	0.5128	39.0000	0.4997	0.3748
***Opuntia*11**	0.6154	39.0000	0.4734	0.3613
***Opuntia*4**	0.8718	39.0000	0.2235	0.1986
***Opuntia*8**	0.6923	39.0000	0.4260	0.3353
***Opuntia*9**	0.5385	39.0000	0.4970	0.3735
***Opuntia*1**	0.5128	39.0000	0.4997	0.3748
***Opuntia*16**	0.6154	39.0000	0.4734	0.3613
***Opuntia*12**	0.8462	39.0000	0.2604	0.2265
***Opuntia*3**	0.5128	39.0000	0.4997	0.3748

4.3.2 Genetic diversity and relationship among *Opuntia* collections

One allele was amplified for each of the eleven markers. The total number of bands amplified per marker ranged from 5 for *Opuntia*4 to 23 for Opufic17 with a mean of 15.27 bands and the total number of bands present for all the 36 accessions was 168 (Appendix 5). The gene diversity ranged from 0.22 to 0.50 with a mean of 0.43 (Table 4.2). A summary statistics for various genetic diversity parameters are presented in Table 4. *Opuntia*4 produced the lowest gene diversity (0.22) while *Opuntia*1, *Opuntia*3 and *Opuntia*10 had the highest gene diversities (0.5) among the eleven markers.

4.3.3 Analysis of molecular variance (AMOVA)

Table 4.3: Analysis of molecular variance (AMOVA) of eleven SSR markers for 39 *Opuntia* species in Rift Valley and Eastern regions of Kenya

Source	df	SS	MS	Est. Var.	%
Among Populations	4	17.989	4.497	0.302	12%
Within Populations	34	75.806	2.230	2.230	88%
Total	38	93.795		2.532	100%

There was a significant difference ($P \leq 0.037$) in molecular variance among populations within counties. Most of the genetic variability was due to differences among individuals within counties contributing to 88% of the variations. Only 12% were due to variations within Counties.

4.3.4 Genetic distance and genetic identity

The average Nei's unbiased genetic distance indicated among and within the location of germplasm collections is presented in table 4.4. The analysis showed the greatest distance for genotypes sampled between the areas of Makueni and Laikipia (0.700) (Table 4.4). There was zero Nei Unbiased Genetic Distance between Baringo and Laikipa, Baringo and Nakuru as well as between Machakos and Nakuru.

Table 4.4: Pairwise Population Matrix of Nei Unbiased Genetic Distance for *Opuntia* species

Baringo	Laikipia	Machakos	Makueni	Nakuru	
0.000					**Baringo**

0.000	0.000				**Laikipia**
0.002	0.199	0.000			**Machakos**
0.222	0.700	0.117	0.000		**Makueni**
0.000	0.183	0.000	0.038	0.000	**Nakuru**

4.4 Discussion

Given that some *Opuntia* species may differ morphologically but are genetically the same ((Helsen *et al.,* 2009), analysis of genetic diversity of the Cacti in the ASALs of Kenya was necessary. The current study indicated minimal molecular differences in the *Opuntia spp* analysed. This was with respect to the differences in the presence or absence of amplification based on eleven SSR markers (Table 4.1), Nei Unbiased Genetic Differences (Table 4.4) and a significant difference in the analysis of molecular variance among populations (Table 4.3).

All the eleven primers (Table 4.1) utilized in the current study were found to be monomorphic for the *Opuntia* genera but had no amplification for *Cereus*, *Euphorbia* and *Thrixanthocreus* genera. The lack of amplification of the non-*Opuntia* genera was because all the markers were developed from only *Opuntia* genera (Helsen *et al.,* 2006; Erre *et al.,* 2011) and the fact that some primers are more efficient than others in producing stable profiles (Zoghlami *et al.,* 2007), hence being *Opuntia* specific. This level of specificity also informs the lack of polymorphism in the current study samples

because majority of the markers were developed particularly for characterizing the endangered *Opuntia echios* (Helsen *et al.,* 2006) varieties. Thus, there is need for identification and/or development of more informative markers for the Cacti in Kenya.

The mean PIC values ranged from 0.199 to 0.375, with an average below 0.5. This indicates that the SSR markers were less informative. According to Botstein *et al.'s,* (1980) PIC guideline, *Opuntia*4 and *Opuntia*12 were only slightly informative while the remaining nine SSR markers with PIC values above 0.25 but less than 0.5 were reasonably informative. This implies that the SSR markers used for analysis were only slightly-to- reasonably informative with relatively low discriminating ability. Similar PIC values of below 0.5 were reported by (Ernestina *et al.,* 2014) for 24 xoconostle accessions (*Opuntia spp.*) and using those values, they still concluded that the DNA variability obtained represented good candidates for preservation in germplasm banks. The current finding is contrary to that of Helsen *et al.,* (2006) which implied a high discriminating ability of the markers.

There was a high percentage molecular variability (88%) of species in different counties. This is in agreement with their morphological differences described. The high variability within the populations in the counties was because of differences in the type of species present in the counties. Nassar *et al.,* (2001) reported the same trend of high genetic diversity of mixed-mating cactus (*Melocactus curvispinus*) with respect to different regions where they grew.

The differences were less at 12% among populations in different counties (Fig. 4) as a result of having similar varieties in different counties. For instance *Opuntia ficus-indica* and *Opuntia exaltata* were present in Nakuru, Machakos and Laikipia (Table 3.2). This signifies the distribution and movement across county borders of same Cacti species mainly by human for use as border material or planting them as ornaments in their farms. However, *Stenocereus eruca* clonal diversity was relatively high across the distribution ranges as reported by (Clark-Tapia & Molina-Freaner, 2003)

The varying total numbers of bands produced by the different markers (Appendix 5) signifies some level of genetic diversity among Kenyan Cacti species. The higher Nei Unbiased Genetic Distance between Makueni and Laikipia (0.700) may have been as a result of differences in the species present in those locations. This is evident in that Laikipia has *Opuntia ficus-indica*, *Opuntia exaltata* and *Euphorbia abyssinica* while Makueni has *Opuntia monacantha*, *Opuntia stricta* and *Euphorbia abyssinica.*

There was a significant difference ($P \leq 0.037$) of molecular variance among genotypes within distinct and agro-ecologies of collection. The AMOVA result suggests that a small collection within a given region may not capture the genetic diversity existing in Kenya ASALs. However, the genetic distance values for *Opuntia* species in Kenya require further confirmation by using more informative molecular markers.

4.5 Conclusion and recommendations

Cacti in the ASALs of Kenya are generally diverse in their genetic composition. There are at least three distinct species of Cacti in each of the five ASAL counties surveyed in

the current study. The marginal diversity among populations in different counties confirms that the same species have been exchanged across these borders. In order to establish further details of the genetic diversity of the cactus species in Kenya, there is need for identification and/or development of more informative and specific markers for the same.

CHAPTER FIVE

CACTI PROPAGATION

5.1 Introduction

Cacti plants can be established vegetatively through micro propagation (Ghaffari *et al.*, 2013; Giusti *et al.,* 2002; Oliveira et al., 1995) grafting, cuttings, bulbils and offsets, seed and division (Bayat *et al.,* 2015). This is normally possible because areoles in Cacti have the ability to produce roots or shoots (Böhm, 2008).

Clonal propagation allows successful genotypes to persist and eventually to colonize new environments without involving the critical phases of germination and seedling establishment. Sexual reproduction on the other hand results in new genetic recombination and the colonization of new environments through long distance seed dispersal (Del-Maria *et al.*, 2016).

The ease of propagation through vegetative parts of the plants contributes to the growth as a result of dispersion by animal coats or running water over long distances (Böhm, 2008). However, sexual propagation through seeds by modification and exposure of the seeds to various conditions has been documented by Rojas-Aréchiga and Vázquez-Yanes (2000).

In the current study, evaluation of the ideal propagation method is informed by the presence of the species, the economic importance of the species and its potential

productivity in the country. Additionally, there is no documented information on the various methods of propagation of Cacti in Kenya. There is also the case with the economic importance of *Euphorbia ingens, Euphorbia abyssinica, Cereus peruvianum* and *Thrixanthocereus blossfeldiorum* in Kenya.

5.2 Materials and methods

5.2.1 Sample collection

Based on factors such as its presence in several counties with ASAL conditions, competitive production potential and having been utilized by some communities in Kenya, *Opuntia monacantha* was selected for this study. Actively growing vegetative parts (cladodes and immature fruits) of *Opuntia monacantha* were collected in the month of June, 2014 from a farm in Juja (S 01.10319 and E 037.01001). The collected samples were grown in a greenhouse at Jomo Kenyatta University of Agriculture and Technology (JKUAT), in order to increase the quantity of vegetative propagules. Sexual propagation through seeds was not undertaken in this study.

5.2.2 Site

The experiment was conducted in JKUAT, Juja. Juja is located 1530 meters above sea level (asl) and 36 km North East of Nairobi.

5.2.3 Experimental details

The experiment was conducted in polythene pots (6in x 4in x 12in) placed in a greenhouse. The pots were filled with garden soil. A single propagule was planted per pot and four pots were used per replication. The vegetative propagules of *Opuntia monacantha* were prepared and cured by covering them with dried grass under shade for varying number of days. The first set was covered for 10 days, the second set for seven days while the third set was cured for 2 days before planting. The last set of propagules was harvested, prepared and planted on the same day. Then the propagules were evaluated in the following format;

a) Full cladodes with spines and those without (Figure 5.1a),
b) Pieces of cladodes with spines and those without(Figure 5.1b),
c) Inverted pieces of cladodes with spines and those without(Figure 5.1c),
d) Immature fruits with glochids and those without (Figure 5.1d).

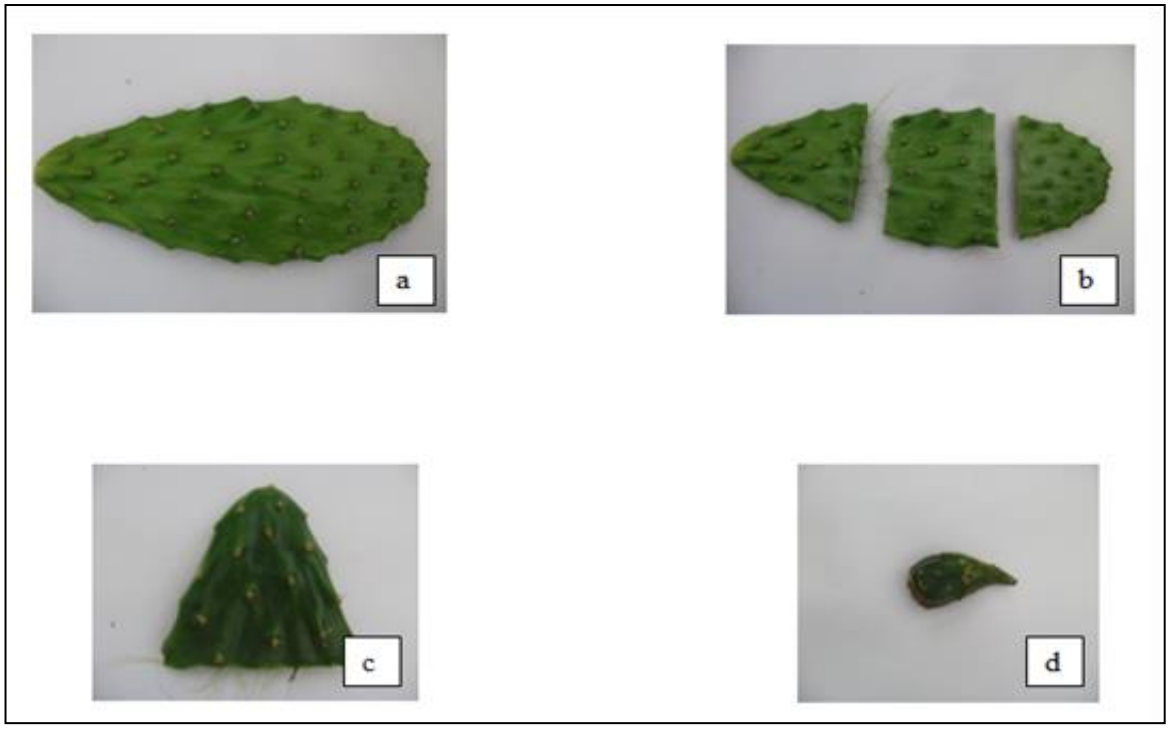

Figure 5.1: The different types of vegetative propagules that were sown to generate new cladodes; a) Full cladodes, b) Pieces of a cladode, c) Inverted piece of cladode d) Immature fruit

Table 5.1: The list of different types of propagules cured for different number of days and their respective codes

Propagule Name	Code	Propagule Name	Code
Fruits with glochids cured for 10days	FSD	Fruits with glochids cured for 7days	FSB
Fruits without glochids cured for 10days	FLD	Fruits without glochids cured for 7days	FLB
Full cladodes with spines cured for 10days	FPSD	Full cladodes with spines cured for 7days	FPSB
Full cladodes without spines cured for 10days	FPLD	Full cladodes without spines cured for 7days	FPLB
Inverted cladodes with spines cured for 10 days	OSD	Inverted cladodes with spines cured for 7 days	OSB
Inverted cladodes without spines cured for 10 days	OLD	Inverted cladodes without spines cured for 7 days	OLB
Mid sections of cladodes with spines cured for	MSD	Mid sections of cladodes with spines cured	MSB

10 days		for 7 days	
Mid sections of cladodes without spines cured for 10 days	MLD	Mid sections of cladodes without spines cured for 7days	MLB
Top sections of cladodes with spines cured for 10 days	TSD	Top sections of cladodes with spines cured for 7 days	TSB
Top sections of cladodes without spines cured for 10 days	TLD	Top sections of cladodes without spines cured for 7 days	TLB
Fruits with glochids cured for 0days	FSC	Fruits with glochids cured for 2 days	FSA
Fruits without glochids cured for 0days	FLC	Fruits without glochids cured for 2 days	FLA
Full cladodes with spines cured for 0days	FPSC	Full cladodes with spines cured for 2 days	FPSA
Full cladodes without spines cured for 0days	FPLC	Full cladodes without spines cured for 2 days	FPLA
Inverted cladodes with spines cured for 0 days	OSC	Inverted cladodes with spines cured for 2 days	OSA
Inverted cladodes without spines cured for 0 days	OLC	Inverted cladodes without spines cured for 2 days	OLA
Mid sections of cladodes with spines cured for 0 days	MSC	Mid sections of cladodes with spines cured for 2 days	MSA
Mid sections of cladodes without spines cured for 0 days	MLC	Mid sections of cladodes without spines cured for 2days	MLA
Top sections of cladodes with spines cured for 10 days	TSC	Top sections of cladodes with spines cured for 7 days	TSA
Top sections of cladodes without spines cured for 10 days	TLC	Top sections of cladodes without spines cured for 2 days	TLA

Length of time for curing of propagules was varied as follows; ten, seven and two days respectively from harvest to planting date. The propagules were cured by covering them with dry grass in the greenhouse and sprinkled them with water to prevent drying. Propagules that were harvested and planted the same day were used as control. Polythene pots (12in x 6in x 4in) were used to raise the propagules in garden soil medium and sand in equal volumes (1:1 ratio).

5.2.4 Experimental design

Split plot design was used in which the number of days for curing the propagules formed the main plot factor while the different propagules were the sub plot factors. The treatments were replicated four times.

Minimum and maximum daily temperatures were recorded. Watering was done when necessary, sanitation maintained and there was no fertilizer application during the entire experiment.

5.2.5 Data collection

Data was recorded on the following;

- The survival of propagules by counting the number of each type of propagule that produced growth.
- Number of buds that emerged from every propagule planted within the study period.
- Growth rates (length and width of cladodes) every 14 days.
- Number and length of roots formed was also recorded on the sixteenth week.

The parameters on which data was recorded include;

- Days to first budding; the number of days from the date of planting to the day when the plant produced the first bud.

- Cladode lengths; the lengths(cm) of the cladodes were measured from their base to the tip of the cladode using a ruler every two weeks from planting and the final lengths recorded on the sixteenth week.
- Cladode widths; the widths (cm) of the cladodes were measured by recording the widest section of the cladode using a ruler every two weeks from planting and the final widths recorded on the sixteenth week.
- Number of buds; the number of buds produced by each propagule was recorded every two weeks and the final number of buds recorded on the sixteenth week.
- Number and length of roots; on the sixteenth week the plants were uprooted and the number of roots recorded. The lengths (cm) of the longest root for each propagule was also measured and recorded.

5.2.6 Data analysis

Descriptive statistics for the recorded data of the above named parameters was analyzed using SAS V.9.1.3 (SAS Institute Inc., 2007)

5.3 Results

5.3.1 Days to first budding

Full cladodes with and those without spines survived and produced buds. All immature fruits with glochids and those without survived and produced buds. Over 93% of the mid sections of the cladodes survived and produced buds while 3 percent of the inverted bottom pieces did not survive.

The propagule and curing levels showed highly significant ($p < 0.0001$) variations in the number of days to first bud formation, while the interaction was significantly different ($p = 0.0174$). Majority of the full cladodes produced their first buds in a mean less than 31 days while most of the bottom sections that were planted in an inverted orientation took on average more than 61 days to produce buds. The rest of the propagules budded in an average mean below 51 days. TSD, TLD, FPLB and FPSD were among the fastest to produce buds with mean less than 30 days while the inverted pieces OSC, OSA and OLA were among the slowest to produce buds in means of more than 91 days.

There was no significant difference in the number of days for budding of the first buds for full cladodes, immature fruits and cladode pieces with or without spines except for those cladode pieces that were planted in an inverted orientation. Inverted pieces without spines took longer to produce buds and took significantly lower number of days to produce buds compared to those with spines.

Table 5.2: Evaluation of variation in number of days to first bud formation of propagules that were subjected to different curing periods

	Curing (0 days)	Curing (2 days)	Curing (7 days)	Curing (10 days)
Propagule	Mean±SE	Mean±SE	Mean±SE	Mean±SE
Fruits with spines	40.25±3.1^{cd}	37.25±4.1^{cde}	36.00±3.0^{bc}	35.75±1.6^{cd}
Fruits without spines	34.00±2.0^{d}	32.00±0.4^{de}	30.50±1.0^{c}	42.25±6.0^{c}
Full cladodes with spines	43.50±1.2^{cd}	30.75±0.6^{de}	30.25±0.8^{c}	23.75±1.9^{d}
Full cladodes without spines	34.00±1.4^{d}	30.50±1.3^{e}	30.00±0.8^{c}	29.75±2.5^{cd}

Inverted cladodes with spines	89.75±5.5[a]	93.75±4.3[a]	74.25±6.5[b]	80.25±10.7[a]
Inverted cladodes without spines	76.25±4.0[b]	89.75±9.0[a]	64.00±5.7[a]	61.25±9.0[b]
Mid cladode sections with spines	51.50±7.2[c]	49.75±6.3[b]	38.25±3.0[bc]	41.00±0.7[c]
Mid cladode sections without spines	43.25±2.4[cd]	49.25±3.0[bc]	33.25±1.7[bc]	35.75±3.7[cd]
Top cladode pieces with spines	44.00±5.6[cd]	42.75±1.5[bcd]	41.75±5.4[b]	29.75±3.3[cd]
Top cladode pieces without spines	40.50±3.7[cd]	45.00±3.0[bc]	32.25±3.0[cb]	29.75±1.4[cd]

Means followed by the same letters are not significantly different at P≤ 0.05 by LSD

Generally, full cladodes produced buds earlier in all cases than the inverted cladode pieces irrespective of the length of days used in curing (Figure 5.2).

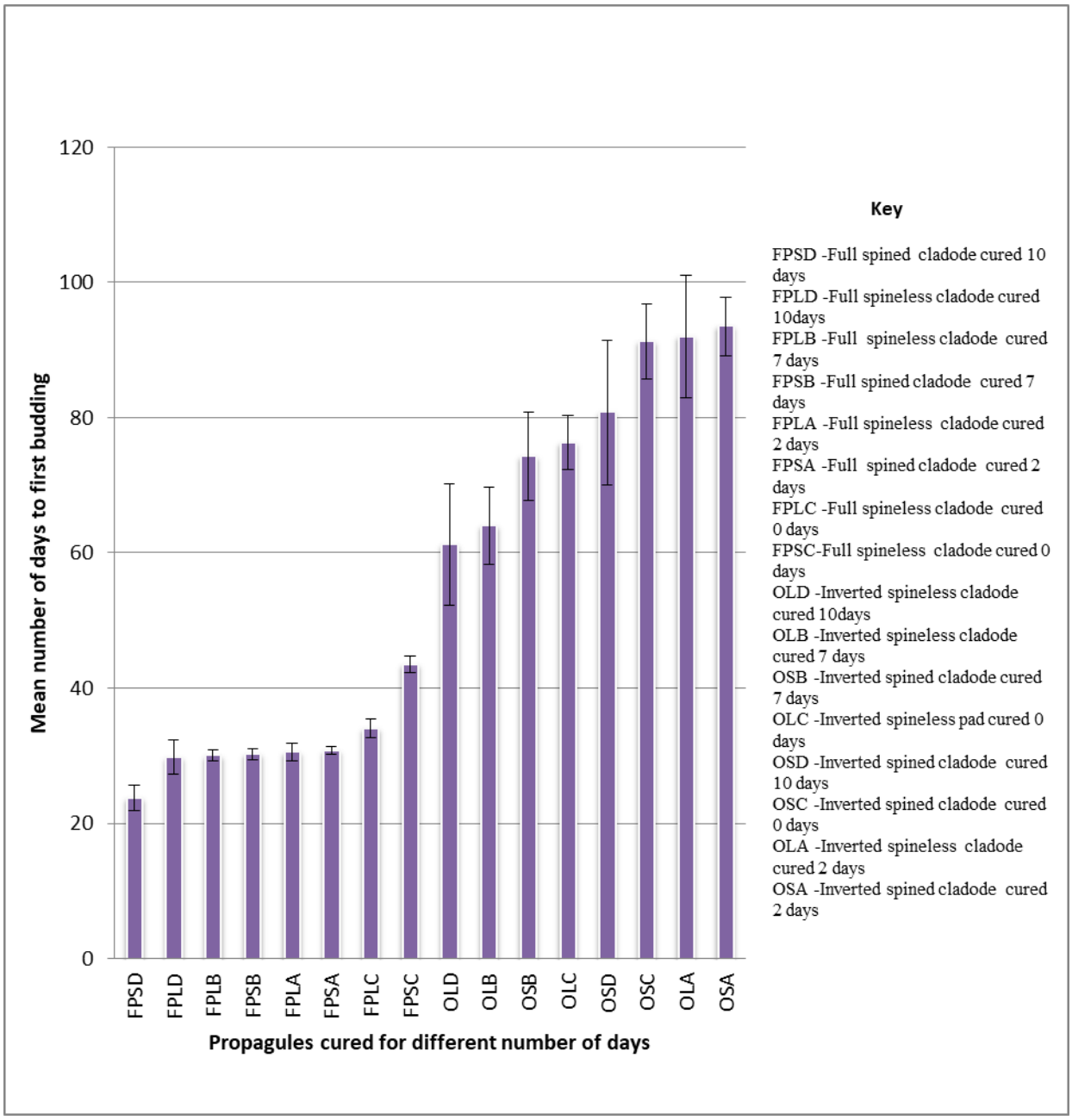

Figure 5.2: The mean number of days to first budding for full cladodes and inverted pieces of cladodes that were cured for different number of days

5.3.2 Cladode lengths

The propagules and curing levels showed highly significant ($p < 0.0001$) variations in the length of cladodes (Table 4.2), but no interaction. Mid sections of cladodes with spines that were cured for seven days (MSB) produced the longest mean length cladodes (28.25cm) followed closely by FPLD with 27.05cm mean length of cladodes. The inverted pieces of cladodes with spines cured for 0 and 2 days had the least average lengths of 2.95cm and 1.3 respectively.

There was no significant difference in the length of cladodes of full cladodes without spines, immature fruits and cladode pieces with or without spines except for those cladode pieces that were planted in an inverted orientation. Inverted pieces without spines produced mean of cladode lengths (9.53cm) significantly longer compared to those with spines (4.15cm).

Table 5.3: Evaluation of variation in length of newly generated cladodes for propagules that were subjected to different curing periods

	Curing (0 days)	Curing (2 days)	Curing (7 days)	Curing (10 days)
Propagule	Mean±SE	Mean±SE	Mean±SE	Mean±SE
Fruits with spines	12.33±4.0^{cd}	17.38±0.2^{b}	20.83±0.6^{bc}	15.63±1.9^{c}
Fruits without spines	22.00±0.8^{ab}	19.85±0.7^{ab}	19.25±0.9^{bc}	16.68±2.9^{c}
Full cladodes with spines	20.43±1.2^{ab}	23.4±0.5^{a}	23.65±2.4^{abc}	23.73±1.1^{ab}
Full cladodes without spines	23.25±1.7^{a}	22.43±0.4^{ab}	24.25±1.6^{ab}	27.05±2.1^{a}
Inverted cladodes with spines	2.95±1.1^{e}	1.30±0.6^{c}	7.50±3.1^{e}	4.88±3.4^{d}
Inverted cladodes without spines	7.08±1.9^{de}	6.30±3.4^{c}	12.25±2.6^{de}	12.50±3.9^{c}
Mid cladode sections with spines	18.83±2.5^{ab}	19.5±2.8^{ab}	28.25±4.1^{a}	25.13±1.0^{ab}
Mid cladode sections without spines	20.00±0.4^{ab}	19.85±1.9^{ab}	24.63±1.1^{ab}	23.68±2.5^{ab}
Top cladode pieces with spines	17.20±2.3^{bc}	21.88±2.3^{ab}	17.90±1.0^{cd}	24.98±1.6^{ab}

Top cladode pieces without spines	16.50±0.3^{bc}	18.75±1.8^{ab}	24.38±1.0^{ab}	18.98±0.6^{bc}

Means followed by the same letters are not significantly at $P \leq 0.05$ by LSD

5.3.3 Width of cladodes

The type of propagule and curing levels showed highly significant ($p < 0.0001$) variations in the width of cladodes, but no interaction. Full cladodes with and without spines produced wide cladodes that were not significantly different. However, these were significantly higher than mean widths produced by immature fruits and cladode pieces that were not inverted at planting. There was significant a difference in mean width between spined (3.6cm) and spineless (2cm) pieces of cladode planted in an inverted orientation. OSA had the least mean width (0.9cm) while FPLD produced the widest mean width of 12.9cm.

Table 5.4: Evaluation of variation in width of newly generated cladodes for propagules that were subjected to different curing periods

	Curing (0 days)	Curing (2 days)	Curing (7 days)	Curing (10 days)
Propagule	Mean±SE	Mean±SE	Mean±SE	Mean±SE
Fruits with spines	5.98±2.0^{c}	8.15±0.2^{bcd}	10.83±0.9^{a}	7.55±1.0^{c}
Fruits without spines	8.50±0.3^{abc}	9.01±0.1^{abc}	9.38±0.2^{ab}	7.63±1.2^{c}
Full cladodes with spines	8.65±0.6^{ab}	10.70±0.4^{a}	10.65±1.4^{a}	10.50±0.2^{ab}
Full cladodes without spines	10.73±0.9^{a}	9.25±0.3^{ab}	10.28±0.1^{ab}	12.93±0.9^{a}
Inverted cladodes with spines	1.38±0.6^{d}	0.88±0.2^{e}	4.05±1.8^{c}	1.73±0.9^{e}
Inverted cladodes without spines	2.95±0.6^{d}	2.70±1.3^{e}	4.25±0.9^{c}	4.50±1.2^{d}
Mid cladode sections with spines	7.65±1.0^{bc}	6.20±1.3^{d}	10.13±1.0^{ab}	9.25±0.3^{bc}
Mid cladode sections without spines	8.43±0.2^{abc}	7.00±0.1^{cd}	9.98±0.8^{ab}	9.00±0.8^{bc}
Top cladode pieces with spines	7.50±0.9^{bc}	8.35±0.6^{bc}	7.50±0.7^{b}	10.4±0.8^{b}
Top cladode pieces without spines	7.48±0.2^{bc}	7.63±1.0^{bcd}	10.33±1.0^{ab}	9.00±0.5^{bc}

Means followed by the same letters are not significantly at $P \leq 0.05$ by LSD

5.3.4 Number of buds

The type of propagules showed highly significant ($p < 0.0001$) variations in the number of buds they produced (Table 5.5). Full spineless cladodes produced the highest average number of buds (≥ 7) as compared to the rest of the propagules evaluated (Table 5.5). The least average number of buds produced was one. This minimum average was recorded for the following propagules; bottom inverted sections with spines that were cured for 2 days and for 10 days.

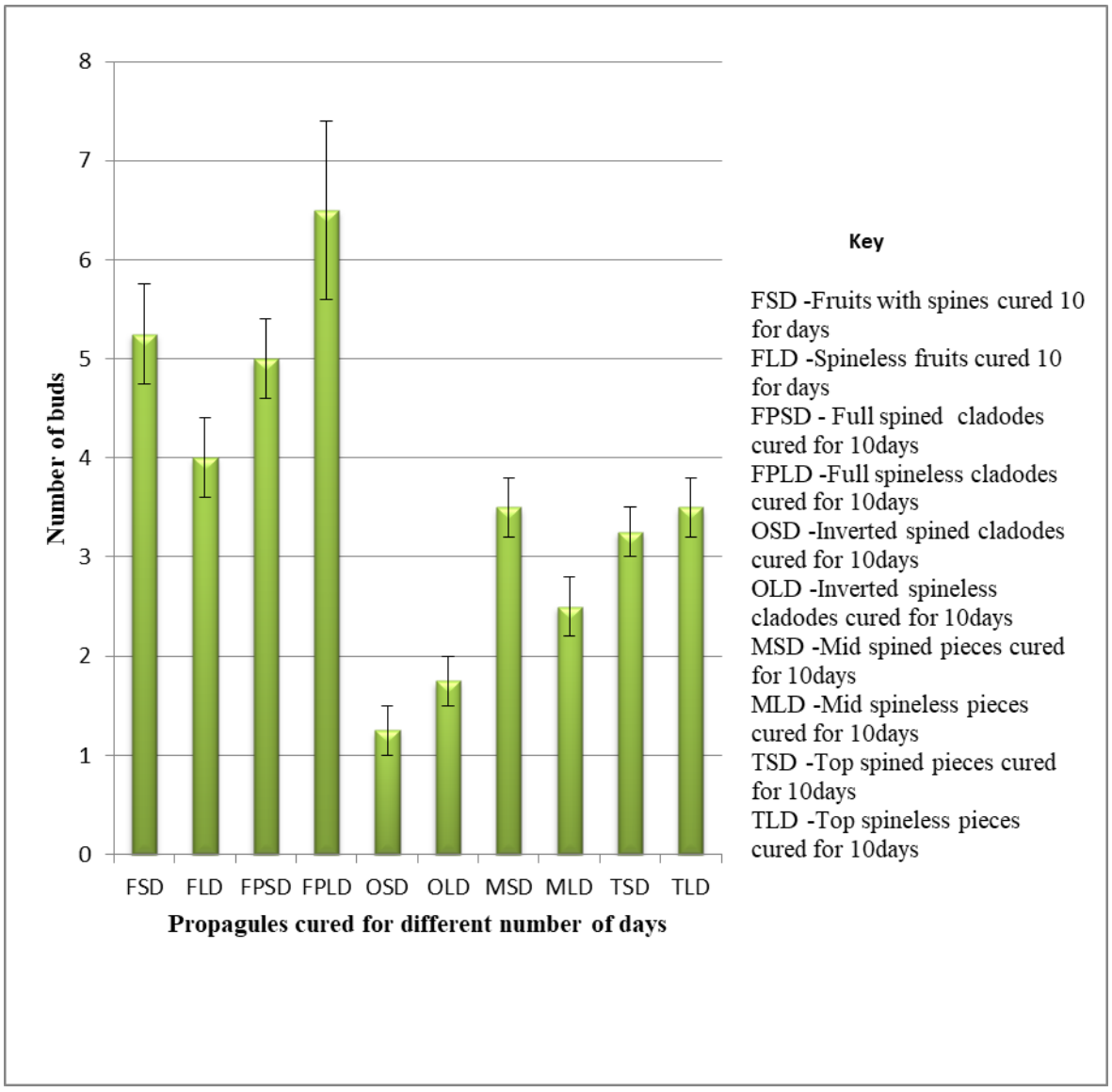

Figure 5.3: Mean number of buds produced by different propagules cured for ten days

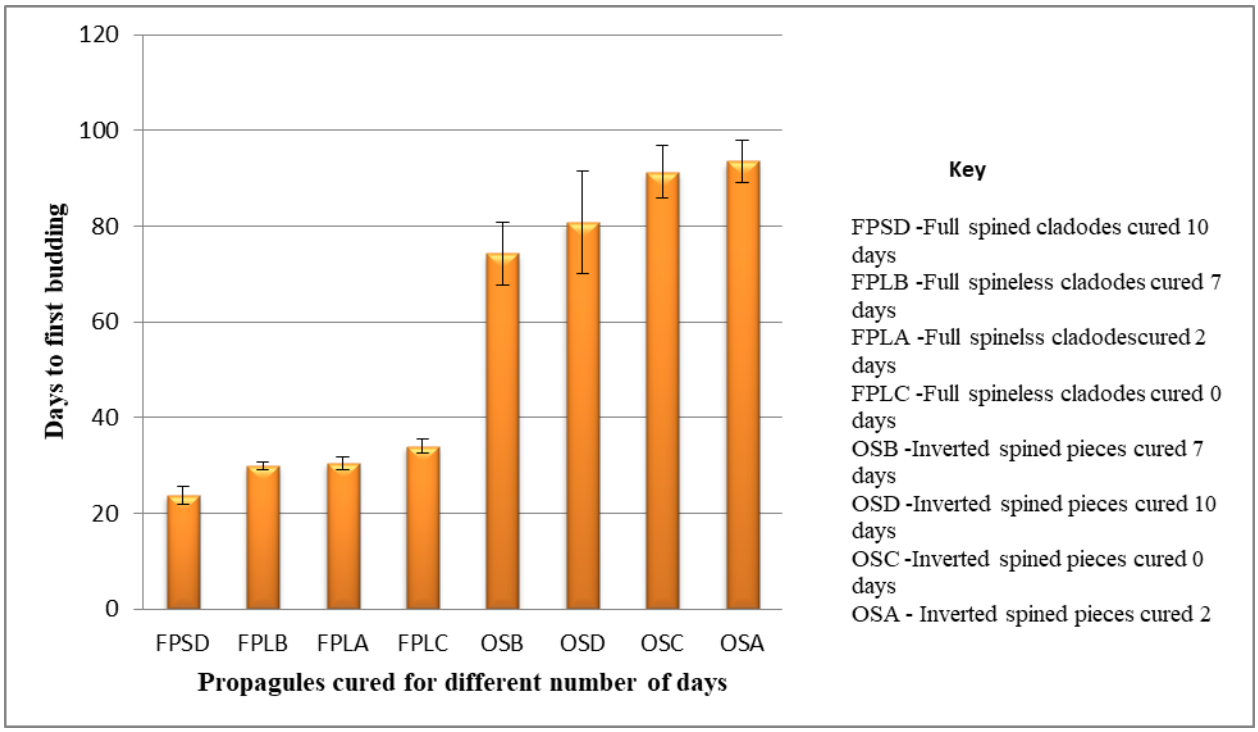

Figure 5.4: The mean number of days to first bud formation for propagules cured for different number of days

Table 5.5: Evaluation of variation in number of buds produced of propagules that were subjected to different curing periods.

	Curing (0 days)	Curing (2 days)	Curing (7 days)	Curing (10 days)
Propagule	Mean±SE	Mean±SE	Mean±SE	Mean±SE
Fruits with spines	3.00 ± 0.7^{cde}	4.50 ± 0.3^{b}	4.00 ± 0.7^{b}	5.25 ± 0.5^{b}
Fruits without spines	4.25 ± 0.5^{c}	4.25 ± 0.6^{b}	3.50 ± 0.9^{bc}	4.00 ± 0.4^{cd}
Full cladodes with spines	7.75 ± 0.5^{b}	7.00 ± 1.5^{a}	7.50 ± 0.6^{a}	5.00 ± 0.4^{bc}
Full cladodes without spines	9.50 ± 1.2^{a}	8.75 ± 1.1^{a}	6.75 ± 0.8^{a}	6.50 ± 0.9^{a}
Inverted cladodes with spines	1.50 ± 0.3^{e}	1.13 ± 0.3^{c}	2.25 ± 0.8^{cd}	1.25 ± 0.3^{g}
Inverted cladodes without spines	2.00 ± 0.4^{e}	1.25 ± 0.3^{c}	1.50 ± 0.5^{d}	1.75 ± 0.3^{fg}
Mid cladode sections with spines	2.50 ± 0.3^{de}	2.25 ± 0.3^{c}	3.00 ± 0.4^{bcd}	3.50 ± 0.3^{de}
Mid cladode sections without spines	3.00 ± 0.0^{cde}	1.75 ± 0.3^{c}	3.25 ± 0.5^{bc}	2.50 ± 0.3^{ef}
Top cladode pieces with spines	2.25 ± 0.7^{e}	3.00 ± 0.4^{bc}	3.50 ± 0.3^{bc}	3.25 ± 0.3^{de}
Top cladode pieces without spines	4.00 ± 0.4^{cd}	2.75 ± 0.5^{bc}	2.50 ± 0.3^{bcd}	3.50 ± 0.3^{de}

Means followed by the same letters are not significantly at $P \leq 0.05$ by LSD

5.3.5 Number of roots

The lower mean number of roots (2 roots) produced by inverted cladode pieces was not significantly different between those with and without spines. But, these were significantly lower than number of roots produced by immature fruits and pieces of cladodes planted normally (5-7 roots) while full cladodes with and without spines produced significantly higher mean root numbers of 11 and 14 roots respectively. Full cladodes without spines had the highest number of root averages.

5.3.6 Length of roots

Inverted sections of the cladodes produced the shortest roots (10.6cm) while the top sections with spines had the longest roots (63.4cm). Only inverted pieces of cladodes with and without spines produced mean length of roots significantly shorter than the rest of the propagules. There was no significant difference between them. The shortest and longest mean of root lengths were 10.6cm for OSB and 69.4cm for TSB (Fig. 11). The differences in length of days of curing of the propagules was not statistically different.

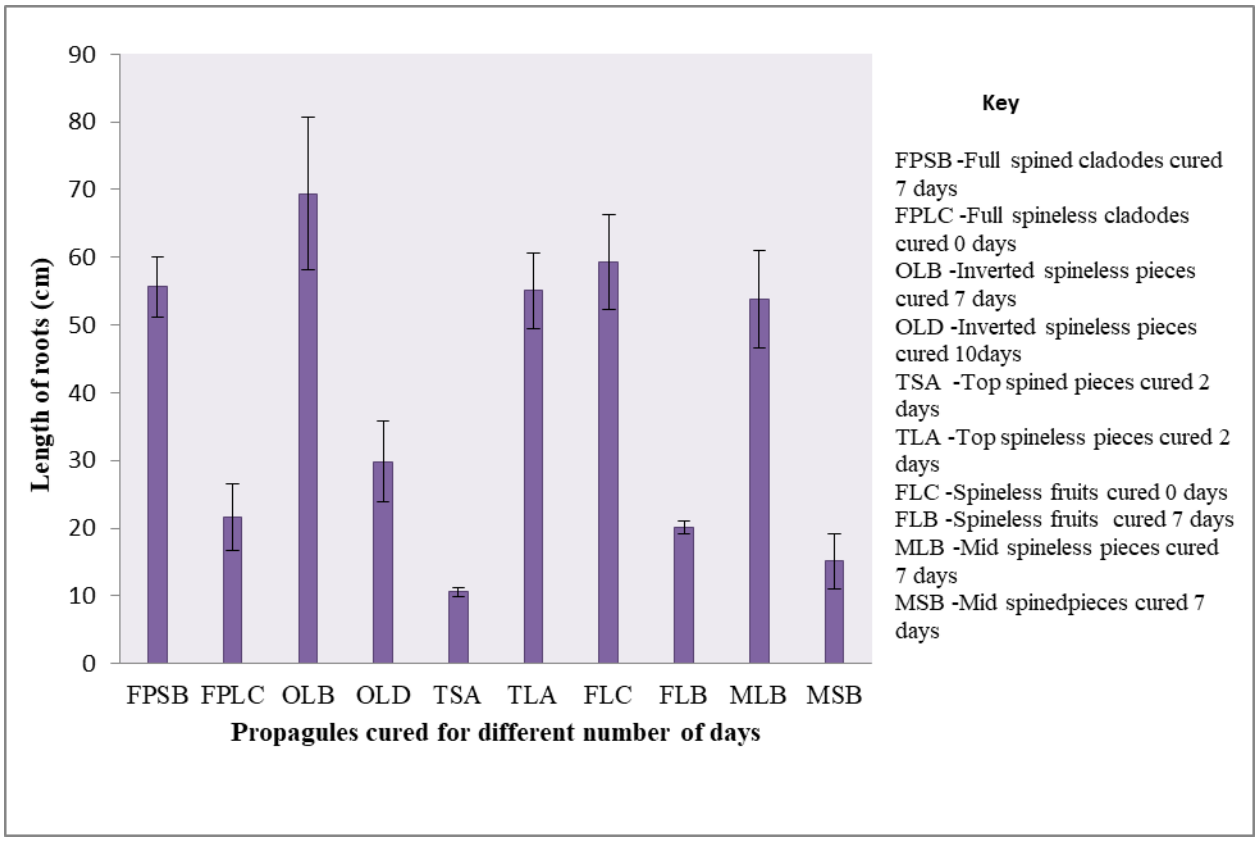

Figure 5.5: Mean length of roots produced by different propagules that were cured for different number of days

Figure 5.6: The new cladodes of *Opuntia monacantha* that were generated from pieces of cladodes that were sown in an inverted orientation

5.4 Discussion

The *Opuntia* monacantha propagules were capable of generating new cladodes with or without any form of curing irrespective of the orientation in which they were planted. Asexual reproduction of *Opuntia* is normally enhanced by the fact that each areole has the ability to produce shoots or roots (Böhm, 2008). Thus, vegetative parts namely; full cladodes, cladode pieces (Rojas-Aréchiga & Vázquez-Yanes, 2000) and immature fruits that have areoles successfully produced shoots and roots. The success of these vegetative propagules could be due to greater water and carbon reserves (Holthe & Szarek, 1985).

The high rate of survival of the *Opuntia monacantha* vegetative propagules confirms that vegetative propagation is highly viable and convenient method that can be utilized by both commercial and domestic farms in growing these plants. Vegetative propagules reduce the challenges of using seeds that require treatment with hormones such as auxins or *sodium nitrofenolate* (L. Mihalte *et al.,* 2011) to overcome their inability to germinate. This is also because the efficiency of establishing detached stem joints (vegetative parts) is deemed to be greater than that of establishing seedlings (Holthe & Szarek, 1985).

However, use of full cladodes or immature fruits is likely to give a higher survival rate as compared to use of cladode cuttings. Full cladodes are ideal for the early

establishment of the crop since they produce buds earlier than sliced cladodes and immature fruits. However, the planting of cladodes in an inverted orientation does significantly delay the development of buds and negatively affects number of buds produced.

Similarly, full cladodes, cladode pieces and immature fruits form the ideal candidates for establishment of *Opuntia* as a vegetable or as fodder for animals because they produced cladodes with big sizes compared to those that were inverted at planting. The larger sizes could be attributed to the fact that the buds formed much earlier compared to those that were inverted.

The overall number of days to first budding can potentially be reduced by curing the cladodes for a week or more days as opposed to no curing at all. This enhances formation of calluses at the wounds of the cutting and prevents them from rotting and eventual death of the propagules when planted. In establishing the crop, there are chances of delaying production if the propagules are planted in an inverted orientation since these propagules take longer to produce buds.

Full cladodes may also be ideal for the use in production of *Opuntia* as a vegetable due to the higher number of buds they yield compared to cladode pieces and immature fruits. This large number of vegetative structures leads to a greater harvest of fruits due to accumulation of the necessary reserves to reach minimum fruiting weight. Thus, the morphological traits are necessary in indicating the productivity of the identified species. The number of buds produced is important in the *Opuntia* plants given that it is an

indicator of the size of the plant produced (Felker *et al.,* 2003). This in turn determines the number of fruits initiated by the plant (Bowers, 1996) and the eventual fruit production of the plant.

Planting *Opuntia* cladodes in an upright and horizontal orientation has demonstrated positive results (Pereira, 2013), but no study has been done to demonstrated that *Opuntia* cladodes could grow if planted in an inverted orientation. The current study has confirmed that viability of cladodes planted in an inverted orientation (Figure 5.6) is not lost but may only lead to delayed bud formation.

In general, the inverted pieces of cladodes may produce less number of roots compared to full cladodes, cladode pieces and immature fruits. This is as a result of the fact that roots are produced from the areoles. Hence, the lower number of roots produced may be due to the fewer number of areoles capable of producing roots and the possibility of the inverted orientation reducing the viability of some areoles. The high rooting ability of full cladodes, cladode pieces and immature fruits demonstrated by the several numbers of roots that were relatively long suggests the ability of the species to survive and proliferate suitable spaces in the ASALs of Kenya. This high rooting ability is among the reasons *Opuntias* may be widely useful for vegetative reproduction (Bobich & Nobel, 2001).

5.5 Conclusion and Recommendations

The vegetative propagules of *Opuntia monacantha* are capable of generating new plants with or without any form of curing. The orientation with which the cladode pieces are

planted does not prevent them from establishing growths. However, cladodes should not be planted in an inverted orientation in order to avoid delays of the plant establishment. Vegetative propagation is a highly viable and convenient method that can be utilized by both commercial and domestic farms in growing these plants.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

This is the first study on the assessment of the Cacti species growing in the ASALs of Kenya. The following *Opuntia* species are present in Kenya; *Opuntia monacatha* in Baringo and Makueni counties, *Opuntia exaltata* in Nakuru, Laikipia and Machkos counties, *Opuntia ficus-indica* in Nakuru and Laikipia counties, *Opuntia stricta* in Makueni County and other species in Machakos County, *Thrixanthocereus blossfeldiorum* in Machakos county, *Euphorbia abyssinica* in Baringo, Laikipia, Machakos and Makueni Counties. *Cereus peruvianus* and *Euphorbia ingens* in Nakuru and Baringo.

The identified Cacti in the ASALs of Kenya are morphologically distinct in their growth forms, shape of their stems, presence or absence of cladodes, spines characteristics and fruit characteristics. These were as follows; *Opuntia ficus-indica* is arborescent with elliptic cladodes, orange corolla and orange fruits, *Opuntia exaltata* is shrubby with cylindrical cladodes, green fruits and pink corolla, *Opuntia monacantha* is arborescent with oval to elliptic cladodes, purple fruits and yellow corolla with purple strips, *Opuntia stricta* that has oval cladodes, bright yellow corolla and purple fruits, *Thrixanthocereus blossfeldiorum* which is columnar with, red and single-seeded fruits,

Cereus peruvianus that grows arborescent with ribbed stems and branches, red-violet fruits and brown spines, *Euphorbia ingens* that is round-shaped arborescent with some drooping branches and ovoid purple fruits and, *Euphorbia abyssinica* which is arborescent with black short spines, globuse and purple fruits.

All the *Opuntia* species bear cladodes, which are important in characterization (Peña-Valdivia, *et al,.* 2008) while the other genera such as *Cereus* and *Thrixanthocereus* do not bear cladodes. Cladode characteristics for the *Opuntias* were also useful in distinguishing species into cylindrical (*Opuntia exaltata*), ovate (*Opuntia stricta,* or elliptic (*Opuntia monacatha* and *Opuntia ficus-indica* (Chalak *et al.,* 2014; Peña-Valdivia *et al.,* 2008)).

Cacti species in Kenya are mainly utilized as fencing/border material in farms (*Opuntia monacantha, Opuntia exaltata* and *Opuntia ficus-indica*) and to smaller extent as ornaments (*Cereus peruvianus, Thrixanthocereus blossfeldiorum and Euphobia abyssinica*). The rest are dispersed intermittently in uncultivated lands (*Cereus peruvianus, Euphorbia abyssinica, Euphorbia ingens* and *Opuntia stricta*).

The marginal differences in the diversity among populations in different counties confirm that the same species have been exchanged across these borders. The eleven SSR markers used were not polymorphic and did not sufficiently distinguish the 39 *Opuntia* species investigated. In order to establish further details of the molecular differences of the cactus species in Kenya, there is need for identification and/or development of more informative and specific markers for the same. The low genetic

distances for some of the populations between counties call for further investigation and confirmation.

Vegetative propagation through cladodes; immature fruits and stem cuttings of Cacti are effective planting material for Cacti especially *Opuntia* species. These vegetative propagules are capable of generating new plants with or without any form of curing. The use of full cladodes gives an early budding crop with sufficient numbers and good rooting system which eventually may result to highly productive plants. Cladode pieces and immature fruits can too be utilized widely given their portability in large numbers and ease of forming new growths.

The orientation with which the cladode pieces are planted does not prevent them from establishing growths. However, cladodes should not be planted in an inverted orientation in order to avoid delays of the plant establishment. Subsequently, vegetative propagation being a highly viable and convenient propagation method, it can be utilized by both commercial and domestic farms in growing these plants.

More research is needed to cover the entire country to identify other species available in these areas and their mode of distribution as well as their productivity to influence the choice species for utilization.

Cacti were spread to the rest of the world in the 16th century (Anderson, 2001; Casas & Barbera, 2002; Defelice, 2004) to Europe by Spanish explorers (Erre *et al.,* 2009), while more distribution across borders occurred from Mexico (FAO, 2013). In Ethiopia they were introduced in mid-19th century (FAO, 2013) while white settlers may have brought

it to Kenya (Kang'ara & Gitari, 2008). With increase in population which consequently exerts pressure on the available productive land resource, there is a rising interest on utilization of marginalized areas and diversify crop production from the traditional cereal-crops economy to untamed potential of crops including Cacti species. Other than the current findings, the exact identities and distribution of the species in the country have not been documented.

The spread of Cacti species in the ASALs of Kenya and beyond may increase because of conducive environmental factors that range from favorable temperatures, limited crop densities in ASALs of Kenya, annual rainfall ranges above 400mm and increasing awareness of its importance. This is also supported by Kunyanga *et al.,* (2009), who suggested that some *Opuntia* species invasions have increased due to changing land use patterns and degradation of range lands. Considerably, the wide adoption of the *Opuntia* varieties in Kenya for subsistence production and commercialization will be enhanced by identification and promotion of spineless species. Chalak *et al.,* (2014) observed a preference for foreign spineless varieties in Lebanon as opposed to the local accessions that had spines. Likewise, the spineless varieties were the choice varieties for farmers in Tunisia (Chalak *et al.,* 2014). Considerably the *Opuntia ficus-indica* identified in Nakuru and Laikipia County with minimal spines on their cladodes is potentially a candidate for fruit and fodder production Kenya's ASALs.

The distribution of Cacti species is mostly influenced by human activity in the areas of study. A similar distribution trend was also observed by Erre *et al.,* (2009); who further assert that morphological descriptors are not influenced by slope or distance from the sea

but that climate influences the morphology of *Opuntia* with the exception of fruit size. In the case of *Opuntia exaltata* for instance, distribution could be by communities sharing vegetative materials for marking borders and as fencing material.

Aged *Opuntia ficus-indica* plants produce cladodes with minimal spines which they later lose as the cladodes age. This may probably be the reason why it could be identified as spineless in some cases. The highest numbers of spines arising from a given areole occurs on the edges of cladodes in most *Opuntia* species.

Since the morphological diversity or similarity in the Cacti species is not in itself ultimate in their classification as has been spelt out by studies that indicated significant differences in the fruits of morphologically similar prickly pear ecotypes, molecular analysis of the available species and their classification was helpful in distinguishing them. The absence of amplification in the *Euphorbia* genera was probably as a result its distant relatedness to the Cacti family (Anderson, 2001)

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APPENDICES

Appendix 1: Eigen Values by Axis and Sample Eigen Vectors for *Opuntia* species

Axis No.	1	2	3	4	5	6	7	8	9	10	11
EigenValue	9.137	1.532	1.311	0.679	0.625	0.571	0.465	0.376	0.282	0.196	0.041
Opuntia monacantha	0.547	-0.328	0.063	0.014	-0.021	-0.100	-0.011	-0.043	-0.009	0.047	0.003
Opuntia ficus-indica	-0.157	-0.183	-0.091	0.355	-0.343	0.065	-0.050	0.055	-0.116	0.063	0.000
Opuntia ficus-indica	-0.416	-0.164	-0.080	-0.020	0.287	-0.083	-0.023	0.138	-0.157	-0.024	-0.071
Opuntia ficus-indica	-0.548	-0.049	-0.009	0.020	0.051	-0.023	0.035	0.001	0.033	0.022	-0.002
Opuntia monacantha	0.670	0.133	-0.049	0.071	-0.033	-0.287	0.097	0.109	-0.021	-0.056	-0.016
Opuntia monacantha	0.598	0.316	-0.085	0.201	-0.115	0.065	0.289	-0.082	0.208	0.036	0.033
Opuntia ficus-indica	0.670	0.133	-0.049	0.071	-0.033	-0.287	0.097	0.109	-0.021	-0.056	-0.016
Opuntia ficus-indica	0.547	-0.328	0.063	0.014	-0.021	-0.100	-0.011	-0.043	-0.009	0.047	0.003
Opuntia ficus-indica	0.628	-0.009	0.004	0.055	0.020	-0.141	-0.210	0.001	0.024	0.000	0.031
Opuntia ficus-indica	0.542	0.115	-0.021	0.253	0.155	0.217	-0.089	-0.167	-0.168	-0.160	-0.071
Opuntia ficus-indica	0.628	-0.009	0.004	0.055	0.020	-0.141	-0.210	0.001	0.024	0.000	0.031
Opuntia ficus-indica	0.687	0.059	-0.103	0.145	0.175	0.152	-0.075	-0.053	0.063	0.045	0.011
Opuntia exaltata	-0.326	0.297	-0.172	-0.119	0.065	-0.122	-0.142	-0.251	-0.017	-0.016	0.034
Opuntia exaltata	0.239	0.190	-0.107	-0.251	-0.277	0.049	-0.196	-0.001	0.199	0.085	-0.137
Opuntia exaltata	-0.414	-0.154	-0.167	0.043	-0.143	0.072	-0.008	0.070	0.087	-0.204	0.012
Opuntia ficus-indica	-0.132	-0.083	-0.165	-0.147	-0.348	0.139	-0.007	-0.131	-0.197	-0.017	0.031
Opuntia ficus-indica	-0.548	-0.049	-0.009	0.020	0.051	-0.023	0.035	0.001	0.033	0.022	-0.002
Opuntia ficus-indica	-0.548	-0.049	-0.009	0.020	0.051	-0.023	0.035	0.001	0.033	0.022	-0.002
Opuntia monacantha	-0.548	-0.049	-0.009	0.020	0.051	-0.023	0.035	0.001	0.033	0.022	-0.002
Opuntia monacantha	0.431	-0.255	0.093	-0.309	0.001	0.032	0.010	0.065	-0.006	-0.041	0.026
Opuntia monacantha	0.431	-0.255	0.093	-0.309	0.001	0.032	0.010	0.065	-0.006	-0.041	0.026
Opuntia ficus-indica	0.571	0.131	-0.072	-0.178	0.196	0.284	-0.054	0.055	0.065	-0.043	0.035
Opuntia ficus-indica	-0.548	-0.049	-0.009	0.020	0.051	-0.023	0.035	0.001	0.033	0.022	-0.002
Opuntia ficus-indica	-0.039	0.315	0.450	0.034	0.005	0.077	-0.124	0.083	0.015	-0.030	0.020
Opuntia exaltata	-	0.314	0.038	0.049	-	0.060	-	0.138	-	0.154	0.038

	0.326				0.087		0.185		0.135		
Opuntia exaltata	-0.120	-0.004	0.509	-0.006	-0.036	0.118	0.075	0.039	-0.018	0.017	-0.008
Opuntia exaltata	0.145	0.484	0.292	-0.129	-0.075	-0.127	0.204	-0.104	-0.113	-0.077	-0.019
Opuntia stricta	-0.548	-0.049	-0.009	0.020	0.051	-0.023	0.035	0.001	0.033	0.022	-0.002
Opuntia stricta	-0.414	-0.154	-0.167	0.043	-0.143	0.072	-0.008	0.070	0.087	-0.204	0.012
Opuntia stricta	-0.548	-0.049	-0.009	0.020	0.051	-0.023	0.035	0.001	0.033	0.022	-0.002
Opuntia ficus-indica	0.547	-0.328	0.063	0.014	-0.021	-0.100	-0.011	-0.043	-0.009	0.047	0.003
Opuntia ficus-indica	0.606	-0.261	-0.044	0.105	0.134	0.193	0.124	-0.097	0.030	0.092	-0.018
Opuntia ficus-indica	-0.406	-0.022	-0.113	-0.160	0.024	-0.081	0.057	-0.294	-0.050	0.032	0.006
Opuntia exaltata	0.327	0.273	-0.537	-0.147	0.051	0.121	0.191	0.218	-0.131	0.085	0.005
Opuntia ficus-indica	-0.467	0.270	-0.068	0.060	0.092	-0.064	-0.164	0.044	0.066	-0.025	0.027
Opuntia ficus-indica	-0.548	-0.049	-0.009	0.020	0.051	-0.023	0.035	0.001	0.033	0.022	-0.002
Opuntia ficus-indica	-0.120	-0.004	0.509	-0.006	-0.036	0.118	0.075	0.039	-0.018	0.017	-0.008
Opuntia exaltata	-0.548	-0.049	-0.009	0.020	0.051	-0.023	0.035	0.001	0.033	0.022	-0.002
Opuntia exaltata	-0.548	-0.049	-0.009	0.020	0.051	-0.023	0.035	0.001	0.033	0.022	-0.002

Appendix 2: UPOV derived Cacti descriptors

	Trait	Degree
	Plant height	a) Short(<1m) b) Medium(1-2m) c) Tall(>2m)
	Plant habit	a) Arborescent/treelike b) Shrubby c) Columnar d) Globular e) Epiphytic/Climbers
	Stem type	a) Ribbed/fluted b) Flattened c) Columnar d) Globular
	Stem length(measured from ground to the first branch of mature plants)	
	Stem diameter	
	Stem color	a) Brownish green b) Bluish green
	Angle of branches with the stem	a) Acute ($<90^0$) b) Obtuse(between 90^0 and 180^0)
	Visible Leaves	a) Present b) Absent
	Types of leaves	a) Simple b) Compound c) Both
	Color of young leaves	a) Light green b) Green c) Dark green
	Color of fully developed leaves	a) Light green b) Green c) Dark green
	Leaf blade shape	a) Cordate b) Ovate c) Elliptic d) Oblique e) Other

	Spines	a) Present b) Absent
	Spine color	a) Green b) Grey c) Black
	Spine shape	a) Straight b) Hair-like c) Hooked
	Spine size	a) Short (<1cm) b) Medium c) Long(>2cm)
	Fruit yield	Count fruit number per cladode
	Fruit set	a) On cladode edges b) On stem/body c) On areoles
	Areole shape	a) Circular b) Elongated(oval shaped) c) Separated in two parts
	Number of areoles	Count for individual plant
	Areole lengths	To be measured in cm
	Cladodes	a) Present b) Absent
	Cladodes – shape	a) Ovate b) Elliptic c) Round
	Cladodes - size	Measure of the width and length of cladodes in cm
	Cladodes - color	
	No of branches (with Cladodes)	
	Cladodes – glochids	a) Present b) Absent
	Flowering time	
	Flowering duration	
	Regularity of flowering	a) Regular b) Irregular
	Secondary/Off season flowering	a) Absent b) Rare

		c) Intermediate d) Frequent
	Corolla color	a) White b) Yellowish c) Pinkish d) Reddish e) Purplish
	Anther color	a) White b) Pale yellow c) Pinkish d) Purplish e) Violet
	Fruit shape	a) Rounded/Globose b) Pear shaped/Ovoid c) Elliptic/Ellipsoid
	Fruit pedicel	a) Absent b) Present
	Fruit – size	a) Small b) Medium c) Large
	Immature fruit color	a) Green b) Light green c) Dark green d) Purplish
	Mature fruit color	a) Green b) Yellowish c) Red d) Dark red e) Orange f) Purple
	Number of fruits per inflorescence/cladode	
	Fruit length	
	Fruit width	
	Fruit size uniformity	a) Low b) Intermediate c) High
	Fruit - peel color	a) Green

		b) Yellow c) Red d) Orange
	Fruit – glochids	a) Present b) Absent
	Fruit - pulp color:	a) Green b) Yellow c) Red d) Orange
	Fruit - pulp firmness	
	Fruit surface	a) Smooth b) Slightly rough
	Fruit – weight of ten fruits(ripe)	
	Fruit muscilage color	a) Whitish b) Orange c) Yellow d) Purple e) Other
	Seed number per fruit	
	Weight of 100 seeds	
	Seed length	
	Seed width	
	Seed hairiness	a) Absent b) Present
	Fruit – seed color	a) White b) Yellow c) Brown d) Black
	Fruit attractiveness(combined assessment of size, shape, coloration, appearance etc	a) Poor b) Average c) Good d) Excellent
	Fruit flavor	a) Acidic b) Moderate sweet c) Sweet
	Pulp juiciness	a) Slightly juicy b) Juicy c) Very juicy

	Pulp aroma	a) Mild b) Intermediate c) Strong
	Receptacular scar position	
	Receptacular scar diameter(mm)	
	Receptacular scar depth(mm)	
	Fruit peeling	a) Easy b) Intermediate c) Difficult
	Fruit skin/peel thickness(mm)	
	Fruit ripening uniformity	a) Poor b) Intermediate c) Good
	Fruit susceptibility to bruising	a) Sensitive b) Intermediate c) Resistant
	Roots	a) Taproots b) Adventitious/Fibrous
	Mode of reproduction	a) Vegetative b) Seeds c) Both
	Seasonality	a) Available only in season b) Available throughout the year
	Higher landform	a) Plain b) Valley c) Upland

Appendix 3: Plant habit, shape, spine and cladode characteristics

Species	**Population Code**	**Plant habit**	**Spine color**	**Cladodes**	**Cladode shape**	**Cladode glochids**	**Plant shape**
Opuntia monacantha	DM01	arborescent	white	present	ovate	present	round
Opuntia ficus-indica	OM01	arborescent	white	present	ovate	present	elongate
Opuntia ficus-indica	OM02	arborescent	white	present	ovate	present	elongate
Opuntia ficus-indica	OM03	arborescent	white	present	rounded	present	elongate
Opuntia monacantha	OM04	arborescent	white	present	ovate	present	elongate
Opuntia monacantha	OM05	arborescent	white	present	ovate	present	elongate
Opuntia ficus-indica	DP01	arborescent	white	present	elliptic	present	elongate
Opuntia ficus-indica	DP02	arborescent	white	present	elliptic	present	elongate
Opuntia ficus-indica	DP03	arborescent	white	present	elliptic	present	elongate
Opuntia ficus-indica	ML01	arborescent	white	present	elliptic	present	elongate
Opuntia ficus-indica	ML02	arborescent	brown	present	elliptic	present	elongate
Opuntia ficus-	ML03	arborescent	brown	present	elliptic	present	elongate

indica							
Opuntia exaltata	JL01	shrubby	white	present	cylindrical	absent	round
Opuntia exaltata	JL02	shrubby	white	present	cylindrical	absent	round
Opuntia exaltata	JL03	shrubby	white	present	cylindrical	absent	round
Opuntia ficus-indica	DF01	shrubby	grey	present	elliptic	present	elongate
Opuntia ficus-indica	DF02	shrubby	grey	present	elliptic	present	elongate
Opuntia ficus-indica	DF03	shrubby	grey	present	elliptic	present	elongate
Opuntia exaltata	NK02	columnar	grey	present	cylindrical	absent	round
Opuntia ficus-indica	VK01	arborescent	white	present	elliptic	present	round
Opuntia ficus-indica	VK02	arborescent	white	present	elliptic	present	round
Opuntia ficus-indica	VK03	arborescent	white	present	elliptic	present	round
Opuntia monacantha	UT01	arborescent	grey	present	elliptic	present	round
Opuntia monacantha	UT02	arborescent	grey	present	elliptic	present	round
Opuntia monacantha	UT03	arborescent	grey	present	elliptic	present	round

Opuntia ficus-indica	UK01	arborescent	white	present	ovate	present	round
Opuntia ficus-indica	UK02	arborescent	white	present	ovate	present	flat
Opuntia ficus-indica	UK03	arborescent	white	present	ovate	present	flat
Opuntia exaltata	AR01	shrubby	golden	present	cylindrical	absent	elongate
Opuntia exaltata	AR02	shrubby	golden	present	cylindrical	absent	elongate
Opuntia exaltata	AR03	shrubby	golden	present	cylindrical	absent	elongate
Opuntia ficus-indica	KM01	arborescent	grey	present	ovate	present	elongate
Opuntia ficus-indica	KM02	arborescent	grey	present	ovate	present	elongate
Opuntia ficus-indica	KM03	arborescent	grey	present	ovate	present	elongate
Opuntia stricta	SH01	shrubby	golden	present	ovate	present	round
Opuntia stricta	SH02	shrubby	golden	present	ovate	present	round
Opuntia stricta	SH03	shrubby	golden	present	ovate	present	round
Opuntia exaltata	NK01	shrubby	golden	present	rounded	absent	elongate
Opuntia exaltata	NK02	shrubby	golden	present	rounded	absent	elongate
Opuntia	MS01	arborescent	white	present	elliptic	present	round

monacantha							
Opuntia monacantha	MS02	arborescent	white	present	elliptic	present	round
Thrixanthocereus blossfeldiorum	LK01	columnar	white	absent	N/A	N/A	elongate
Thrixanthocereus blossfeldiorum	LK02	columnar	white	absent	N/A	N/A	elongate
Thrixanthocereus blossfeldiorum	LK03	columnar	white	absent	N/A	N/A	elongate
Euphorbia abyssinica	GP01	arborescent	grey	absent	N/A	N/A	round
Euphorbia abyssinica	GP02	arborescent	grey	absent	N/A	N/A	round
Euphorbia abyssinica	GP03	arborescent	grey	absent	N/A	N/A	round
Cereus peruvianus	KB01	arborescent	brown	absent	N/A	N/A	elongate
Cereus peruvianus	KB02	arborescent	brown	absent	N/A	N/A	elongate
Cereus peruvianus	KB03	shrubby	brown	absent	N/A	N/A	elongate
Euphorbia ingens	RB01	arborescent	grey	absent	N/A	N/A	round

Euphorbia ingens	RB02	arborescent	grey	absent	N/A	N/A	round
Euphorbia ingens	RB03	arborescent	grey	absent	N/A	N/A	round
Euphorbia abyssinica	NN01	arborescent	black	absent	N/A	N/A	round
Euphorbia abyssinica	NN02	arborescent	black	absent	N/A	N/A	round
Euphorbia abyssinica	NN03	arborescent	black	absent	N/A	N/A	round
Euphorbia abyssinica	SE01	arborescent	black	absent	N/A	N/A	round
Euphorbia abyssinica	SE02	arborescent	black	absent	N/A	N/A	round
Cereus peruvianus	PLK01	arborescent	brown	absent	N/A	N/A	elongate
Cereus peruvianus	PLK02	arborescent	brown	absent	N/A	N/A	elongate
Cereus peruvianus	PLK03	arborescent	brown	absent	N/A	N/A	elongate
Euphorbia ingens	ENN01	arborescent	grey	absent	N/A	N/A	round
Euphorbia ingens	ENN02	arborescent	grey	absent	N/A	N/A	round
Cereus peruvianus	PN01	arborescent	brown	absent	N/A	N/A	round
Cereus	PN02	arborescent	brown	absent	N/A	N/A	round

peruvianus							
Cereus peruvianus	PN03	arborescent	brown	absent	N/A	N/A	round
Thrixanthocereus blossfeldiorum	DS01	columnar	white	absent	N/A	N/A	elongate
Thrixanthocereus blossfeldiorum	DS02	columnar	white	absent	N/A	N/A	elongate
Thrixanthocereus blossfeldiorum	DS03	columnar	white	absent	N/A	N/A	elongate

Appendix 4: Corolla and fruit characteristics

Species name	Population Code	Fruit set	Corolla color	Fruit shape	Mature fruit color	Fruit glochids
Opuntia monacantha	DM01	cladode edges	purple stripped yellow	ovoid	purple	present
Opuntia ficus-indica	OM01	cladode edges	orange	ovoid	purple	present
Opuntia ficus-indica	OM02	cladode edges	orange	ovoid	purple	present
Opuntia ficus-indica	OM03	cladode edges	orange	ovoid	purple	present
Opuntia monacantha	OM04	cladode edges	orange	ovoid	purple	present
Opuntia monacantha	OM05	cladode edges	orange	ovoid	purple	present
Opuntia ficus-indica	DP01	cladode edges	orange	ovoid	orange	present
Opuntia ficus-indica	DP02	cladode edges	orange	ovoid	orange	present
Opuntia ficus-indica	DP03	cladode edges	orange	ovoid	orange	present
Opuntia ficus-indica	ML01	cladode edges	orange	ovoid	orange	present
Opuntia ficus-indica	ML02	cladode edges	orange	ovoid	orange	present
Opuntia ficus-	ML03	cladode	orange	ovoid	orange	present

indica		edges				
Opuntia exaltata	JL01	stem	pink	ovoid	green	absent
Opuntia exaltata	JL02	stem	pink	ovoid	green	absent
Opuntia exaltata	JL03	stem	pink	ovoid	green	absent
Opuntia ficus-indica	DF01	cladode edges	orange	ovoid	orange	present
Opuntia ficus-indica	DF02	cladode edges	orange	ovoid	orange	present
Opuntia ficus-indica	DF03	cladode edges	orange	ovoid	orange	present
Opuntia exaltata	NK02	stem	pink	ovoid	green	present
Opuntia ficus-indica	VK01	cladode edges	orange	ovoid	orange	present
Opuntia ficus-indica	VK02	cladode edges	orange	ovoid	orange	present
Opuntia ficus-indica	VK03	cladode edges	orange	ovoid	orange	present
Opuntia monacantha	UT01	cladode edges	purple stripped yellow	ovoid	purple	present
Opuntia monacantha	UT02	cladode edges	purple stripped yellow	ovoid	purple	present
Opuntia monacantha	UT03	cladode edges	purple stripped yellow	ovoid	purple	present
Opuntia ficus-indica	UK01	cladode edges	orange	ovoid	orange	present
Opuntia ficus-	UK02	cladode	orange	ovoid	orange	present

indica		edges				
Opuntia ficus-indica	UK03	cladode edges	orange	ovoid	orange	present
Opuntia exaltata	AR01	stem	pink	ovoid	green	absent
Opuntia exaltata	AR02	stem	pink	ovoid	green	absent
Opuntia exaltata	AR03	stem	pink	ovoid	green	absent
Opuntia ficus-indica	KM01	cladode edges	orange	ovoid	orange	present
Opuntia ficus-indica	KM02	cladode edges	orange	ovoid	orange	present
Opuntia ficus-indica	KM03	cladode edges	orange	ovoid	orange	present
Opuntia stricta	SH01	cladode edges	yellow	globuse	purple	present
Opuntia stricta	SH02	cladode edges	yellow	globuse	purple	present
Opuntia stricta	SH03	cladode edges	yellow	globuse	purple	present
Opuntia exaltata	NK01	stem	pink	ovoid	green	absent
Opuntia exaltata	NK02	stem	pink	ovoid	green	absent
Opuntia monacantha	MS01	cladode edges	purple stripped yellow	ovoid	purple	present
Opuntia monacantha	MS02	cladode edges	purple stripped yellow	ovoid	purple	present
Thrixanthocereus	LK01	stem	cream	ovoid	red	present

blossfeldiorum						
Thrixanthocereus blossfeldiorum	LK02	stem	cream	ovoid	red	present
Thrixanthocereus blossfeldiorum	LK03	stem	cream	ovoid	red	present
Euphorbia abyssinica	GP01	branches	cream	globuse	purple	absent
Euphorbia abyssinica	GP02	branches	cream	globuse	purple	absent
Euphorbia abyssinica	GP03	branches	cream	globuse	purple	absent
Cereus peruvianus	KB01	branches	white	elliptic	violet-red	absent
Cereus peruvianus	KB02	branches	white	elliptic	violet-red	absent
Cereus peruvianus	KB03	branches	white	ovoid	violet-red	absent
Euphorbia ingens	RB01	branches	cream yellow	ovoid	purple	absent
Euphorbia ingens	RB02	branches	cream yellow	ovoid	purple	absent
Euphorbia ingens	RB03	branches	cream yellow	ovoid	purple	absent
Euphorbia abyssinica	NN01	branches	cream	globuse	purple	absent
Euphorbia	NN02	branches	cream	globuse	purple	absent

abyssinica						
Euphorbia abyssinica	NN03	branches	cream	globuse	purple	absent
Euphorbia abyssinica	SE01	branches	cream	globuse	purple	absent
Euphorbia abyssinica	SE02	branches	cream	globuse	purple	absent
Cereus peruvianus	PLK01	branches	white	elliptic	violet-red	absent
Cereus peruvianus	PLK02	branches	white	elliptic	violet-red	absent
Cereus peruvianus	PLK03	branches	white	elliptic	violet-red	absent
Euphorbia ingens	ENN01	branches	cream yellow	globuse	purple	absent
Euphorbia ingens	ENN02	branches	cream yellow	globuse	purple	absent
Cereus peruvianus	PN01	branches	white	elliptic	violet-red	absent
Cereus peruvianus	PN02	branches	white	elliptic	violet-red	absent
Cereus peruvianus	PN03	branches	white	elliptic	violet-red	absent
Thrixanthocereus blossfeldiorum	DS01	stem	cream	ovoid	red	present
Thrixanthocereus	DS02	stem	cream	ovoid	red	present

blossfeldiorum						
Thrixanthocereus blossfeldiorum	DS03	stem	cream	ovoid	red	present

Appendix 5: Presence or absence of bands for *Opuntia* species in the ASALs of Kenya

Samples	Population	Opufic17	*Opuntia*21	*Opuntia*10	*Opuntia*11	*Opuntia*4	*Opuntia*8	*Opuntia*9	*Opuntia*1	*Opuntia*16	*Opuntia*12	*Opuntia*3
Opuntia monacantha	Baringo	1	1	1	1	0	1	1	1	0	0	1
Opuntia ficus-indica	Baringo	1	0	0	0	0	1	0	1	0	0	0
Opuntia ficus-indica	Baringo	0	1	0	0	0	0	0	0	0	0	0
Opuntia ficus-indica	Baringo	0	0	0	0	0	0	0	0	0	0	0
Opuntia monacantha	Baringo	1	1	1	1	1	1	1	1	1	0	1
Opuntia monacantha	Baringo	1	0	1	1	1	1	1	1	1	1	1
Opuntia ficus-indica	Laikipia	1	1	1	1	1	1	1	1	1	0	1
Opuntia ficus-indica	Laikipia	1	1	1	1	0	1	1	1	0	0	1
Opuntia ficus-indica	Laikipia	1	1	1	1	0	1	1	1	1	0	1
Opuntia ficus-indica	Laikipia	1	1	1	0	0	1	1	1	1	1	1
Opuntia ficus-indica	Laikipia	1	1	1	1	0	1	1	1	1	0	1
Opuntia ficus-indica	Laikipia	1	1	1	1	0	1	1	1	1	1	1
Opuntia exaltata	Laikipia	0	0	1	0	0	0	0	0	1	0	0
Opuntia exaltata	Laikipia	1	0	1	1	0	0	0	1	1	0	1
Opuntia exaltata	Laikipia	0	0	0	0	0	0	0	1	0	0	0
Opuntia ficus-indica	Machakos	1	0	1	0	0	0	0	1	0	0	0
Opuntia ficus-indica	Machakos	0	0	0	0	0	0	0	0	0	0	0
Opuntia ficus-indica	Machakos	0	0	0	0	0	0	0	0	0	0	0
Opuntia monacantha	Machakos	0	0	0	0	0	0	0	0	0	0	0
Opuntia monacantha	Machakos	1	1	1	1	0	0	1	1	0	0	1
Opuntia monacantha	Machakos	1	1	1	1	0	0	1	1	0	0	1
Opuntia ficus-indica	Machakos	1	1	1	1	0	0	1	1	1	1	1
Opuntia ficus-indica	Machakos	0	0	0	0	0	0	0	0	0	0	0
Opuntia ficus-indica	Machakos	1	0	0	0	0	0	1	0	1	0	1
Opuntia exaltata	Machakos	1	0	0	0	0	0	0	0	1	0	0
Opuntia exaltata	Machakos	1	0	0	0	0	0	1	0	0	0	1
Opuntia exaltata	Machakos	1	0	1	0	1	0	1	0	1	0	1
Opuntia stricta	Makueni	0	0	0	0	0	0	0	0	0	0	0
Opuntia stricta	Makueni	0	0	0	0	0	0	0	1	0	0	0
Opuntia stricta	Makueni	0	0	0	0	0	0	0	0	0	0	0
Opuntia ficus-indica	Nakuru	1	1	1	1	0	1	1	1	0	0	1
Opuntia ficus-indica	Nakuru	1	1	1	1	0	1	1	1	0	1	1
Opuntia ficus-indica	Nakuru	0	0	1	0	0	0	0	0	0	0	0
Opuntia exaltata	Nakuru	1	1	1	1	1	0	0	1	1	1	0
Opuntia ficus-indica	Nakuru	0	0	0	0	0	0	0	0	1	0	0

Opuntia ficus-indica	Nakuru	0	0	0	0	0	0	0	0	0	0	0
Opuntia ficus-indica	Nakuru	1	0	0	0	0	0	1	0	0	0	1
Opuntia exaltata	Nakuru	0	0	0	0	0	0	0	0	0	0	0
Opuntia exaltata	Nakuru	0	0	0	0	0	0	0	0	0	0	0