CHARACTERIZATION, PROPAGATION AND MANAGEMENT OF JOJOBA (*SIMMONDSIA CHINENSIS* L.) IN SEMI-ARID AREAS OF VOI, KENYA

SHADRACK KINYUA INOTI

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ABSTRACT

Jojoba is a dioecious desert shrub which produces oil of high quality equivalent to that of sperm whale. It is used mainly in the cosmetic and lubrication industry. Currently, there is low production of jojoba globally mainly due to high male to female ratio in the plantations since they are mainly established from seed. To overcome this problem, five experiments were set up with the aim of characterization, propagation and management of jojoba in semi arid areas of Kenya. The experiments included molecular characterization of mature bushes, sex determination in young jojoba seedlings using morphological traits, identifying the most appropriate plant growth regulators and management regimes of mother plants for propagation as well as field established seedlings. Randomized complete block design was used and the treatments were replicated three times. Analysis of variance was carried out using SAS statistical package whereas the differing means were separated using the LSD and DMRT. The experiments were carried out between 2012 and 2014. The results showed that the mature jojoba bushes had a low genetic diversity which was shown by PIC range of 0.2583-0.3748. Single leaf area morphological trait for male seedlings (4.4 cm^2) was significantly higher ($p < 0.05$) compared with the females (3.2 cm^2). Anatone gave superior rooting percent of 24.2% for cuttings compared with the other plant growth regulators, IBA, Roothom and the control which were 21%, 14.8% and 11.5% respectively. Consequently, the male genotypes, M2 and M1 showed significantly higher ($p < 0.01$) rooting percent of 37.6% and 24.2%, respectively compared with the females, F2 and F1 which were 7.6% and 2.2%, respectively. Management regimes performance of the mother plants did not have any significant effect on the rooting of cuttings in the polythene sheet tunnel. However, field established seedlings at 10 months showed that a combination of manure, irrigation and micro catchment was the best management regime since it gave the highest root collar diameter of 17.1 mm which was

significantly higher ($p < 0.05$) compared with the micro catchment (12.2 mm) alone which was the least. On the other hand mcrocatchment and irrigation combination showed the highest height (86.4 cm) which was significantly higher ($p < 0.05$) compared with the control (61.5 cm) and all the single management regimes. Generally, different management regime combinations gave better growth compared with single management regimes. Due to the low genetic diversity, it is, therefore, recommended that superior genotypes be imported to increase the genetic diversity of jojoba in Kenya. Single leaf area morphological trait should be used for sexing in young seedlings in order to attain the right ratios of male to female of 1:10 respectively during field planting for improved productivity per unit area. On the other hand, Anatone is recommended for propagation of cuttings in a polythene sheet tunnel since it is also cheap and readily available from agri-veterinary shops in urban centres. The male genotypes M2 and M1 are recommended for use in future propagation of cuttings although more screening is needed to identify a wide range of genotypes especially the females which are more valued due to their seed production. The ideal management regimes for field planted seedlings are combinations of manure, irrigation and micro catchment as opposed to single management regimes.

DECLARATION

I, Shadrack Kinyua Inoti, do hereby declare to the Senate of Sokoine University of Agriculture that this thesis is my own original work and that it has neither been submitted nor being concurrently submitted in any other institution.

_____ _____

Shadrack Kinyua Inoti **Date**

PhD Candidate

The above declaration is confirmed by supervisors:

_____ _____

Professor S. A. O. Chamshama **Date**

_____ _____

Professor L. L. L. Lulandala **Date**

_____ _____

Doctor W. M. Thagana **Date**

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I wish to dedicate this thesis to my late father, Philip Inoti Bagine, who passed away during the period of my thesis write up. He showed a lot of interest in my advancement in studies and may the almighty God rest his soul in peace.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percent
˚C	Degrees centigrade
µl	Micromole
ABA	Abscisic Acid
AFLP	Amplified Fragment Length Polymorphism
Al	Aluminium
ANOVA	Analysis of Variance
ASALs	Arid and Semi-Arid Lands
B	Boron
bp	Base pairs
Ca	Calcium
CBDP	CAAT Box-Derived Polymorphism
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CJP	Centre for Jatropha Promotion and Biodiesel
cm	Centimetres
CO_2	Carbon dioxide
CTAB	Cetyl Trimethylammonium Bromide
CV	Coefficient of Variation
DMRT	Duncan·s Multiple Range Test
DNA	Deoxyribose Nucleoic Acid
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organization of the United Nations
Fe	Iron
Fig.	Figure

FYM	Farmyard Manure
g	Grammes
H_2O	Water
ha	Hectares
IAA	Indole Acetic Acid
IBA	Indole Butyric Acid
ISSR	Inter-Simple Sequence Repeat
K	Potassium
KALRO	Kenya Agricultural and Livestock Research Organization
KARI	Kenya Agricultural Research Institute
$Kgha^{-1}$	Kilogrammes per hectare
Km	Kilometres
LSD	Least Significant Difference
m^2	Square metre
m^3	Cubic metre
mA	Milliampheres
Mg	Magnesium
mgL^{-1}	Milligrammes per litre
ml	Millilitres
mm	Millimetres
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
NAA	α-Naphthalene Acetic Acid
NAD	Nicotamide Adenine Dinucleotide
NAS	National Academy of Sciences

NEMA	National Environment Management Authority
NRC	National Research Council
NVSC	National Vision Steering Committee
OC	Organic Carbon
OM	Organic Matter
P	Phosphorus
PCR	Polymerase Chain Reaction
PGRs	Plant Growth Regulators
pH	Measure of acidity and basicity of an aqueous solution
PIC	Polymorphic Information Content
ppm	Parts per million
RAPD	Random Amplified Polymorphic DNA
RCBD	Randomized Complete Block Design
RFLP	Restriction Fragment Length Polymorphism
rpm	Revolutions per minute
SAS	Statistical Analysis System
SE	Standard Error
SNP	Single Nucleotide Polymorphism
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
SSR	Simple Sequence Repeats
Std Dev	Standard Deviation
t	Tonne
TBE	Tris-Borate EDTA
TTDP	Taita Taveta District Project
UNMD	United Nations Millenium Declaration

US$	United States Dollar
USA	United States of America
USAID	United States Agency for International Development
UV	Ultra violet
yr	Year
Zn	Zinc

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Over 80% of Kenya is composed of arid and semi arid lands (ASALs) (KARI, 2009) with only a few crops being grown mainly for subsistence purposes. These areas lack cash crops which are drought tolerant and irrigation systems are poorly developed. They experience frequent drought leading to crop failure hence overdependence on food relief (Barrow, 1996).

These areas have few natural plant resources that can be relied upon on a continuous basis (Dakshini, 1985). People in ASALs depend directly on the available natural resources such as firewood and charcoal for their survival and this makes the environment to be more arid resulting into greater poverty, poor dietary intake and misery among the residents. Resources are always at the mercy of severe climatic changes and some species survive only precariously. Furthermore, some species have been over exploited to the point of becoming endangered or at least vulnerable to the pressure of man (Ayensu, 1985).

Serious mitigation programmes are needed to combat global climate change which is anticipated to have far reaching effects in many parts of the world including Kenya as outlined by Ottichilo *et al*. (1991). Priority is being given to high yielding genotypes which can survive unexpected environmental changes, particularly in regions of water deficits (Akinci and Losel, 2012). In recent years, there has been considerable interest in using ASALs more productively by promoting crops which can tolerate these conditions such as *Jatropha curcas* (Ngethe, 2007) and jojoba (*Simmondsia chinensis)* (Thagana

et al., 2004). These are multipurpose crops, and have a potential use for rehabilitation as well as provision of income to the poor communities.

Jojoba has been used to combat and prevent desertification in the Thar desert in India (Alsharhan *et al*., 2003) and Negev desert in Israel (Benzioni, 1997). Jojoba needs little water for survival (a third or less of the moisture required by crops like citrus or cotton); however, economic consideration dictates that irrigation is essential for a healthy and profitable crop in many dry areas (CJP, 2007).

Jojoba is a high value shrub growing in ASALs (Hogan, 1979; NAS, 1984; Ahmad, 2001) and is a promising cash crop for the ASALs throughout the world. It is the sole species of the family *Simmondsiaceae* and is a native shrub of Sonoran desert of Arizona, southern California and north western Mexico. Jojoba is grown in many other countries including Argentina, Brazil, India, Israel, Egypt, Saudi Arabia, Australia, South Africa, Peru, Chile and Iran (Muthana, 1981; Undersander *et al*., 1990).

It is dioecious with male and female plants in the ratio 1:1 in the field when raised from seed. A jojoba stand can be in production for 100-200 years depending on management (Martin, 1983) and has a deep rooting habit (Forster and Wright, 2002). Jojoba produces nuts with 45-55% of its weight as oil. The oil from jojoba is similar to that obtained from sperm whale (Hogan and Bemis, 1983), a species threatened with extinction (CITES, 2004). It is used in cosmetics, lubricant industry, pharmaceuticals (Muthana, 1981; Hogan and Bemis, 1983; Amarger and Mercier, 1996; Ward, 2003), electronics and computer industries (Undersander *et al*., 1990). According to Martin (1988), jojoba is highly valued in the international market where it sells at US$ 10-50 per litre (l) of oil. The seed production per bush is 2-3 kg per year (yr) which translates to 2 to 3 metric tonnes (t) per

hectare (ha). The amount of jojoba oil produced per ha is equated with the quantity produced by 124 whales (Ward, 2003). The seed yield can reach 4-5 kg $bush^{-1}$ with improved selection and management (CJP, 2007).

The world jojoba oil production is estimated at 3 500 t yr^{-1}, from the cultivated area of 8 500 ha whereas the demand is 64 000 - 200 000 t yr^{-1} (Forster and Wright, 2002) (Table 1).

Table 1: Global jojoba cultivation and seed production for the year 2000

Country	Ha under cultivation	Seed production (Metric t)	Productivity ($Kgha^{-1}$)
Argentina	4800	950	198
USA	2000	1455	728
Israel	700	1000	1429
Mexico	470	90	191
Australia	400	8	20
Peru	300	75	250
Egypt	140	15	107
Total	**8810**	**3593**	**2923**

Source: Thagana *et al*. (2003)

Clonal propagation of elite individuals of known sexuality is necessary to ensure that the plants in commercial plots will be highly productive (Chaturvedi and Sharma, 1989). This will ensure uniform stands of known sex, placement and parentage. For vegetatively propagated plants, the ability to root is affected by several factors ranging from individual plant (Bonga and Aderkas, 1992; Bashir *et al*., 2008; Inoti *et al*., 2015) to the management (Foster *et al*., 1984; Ozel *et al*., 2006). Marino (1982) stated that rooting of cuttings does better when taken during the dormant stage. This is the period when they have high carbohydrate to nitrogen (N) ratio and a general rule of thumb is the ability of the cutting

to break when bent (Berl and Trigiano, 2011). The advantages of using asexual propagules in commercial jojoba plantations are that they provide uniform and predictable plant growth and yield (Lee, 1988) and can be sexed earlier before flowering.

Rooting hormones, also known as plant growth regulators (PGRs) wholly cause a greater percentage of cuttings to root, hasten the formation of roots, induce more roots of cuttings and increase root uniformity (Godfrey *et al*., 1996; Amri, 2009). Two synthetic auxins namely Indole Butyric Acid (IBA) and α-Naphthalene Acetic Acid (NAA) are mostly used, either singly or in a combination (Berl and Trigiano, 2011; Kumlay, 2014). The most naturally occurring auxin in plants is Indole Acetic Acid (IAA), but breaks down easily, hence less effective (Amri *et al*., 2010). Vegetative propagation would overcome the problem of high male to female ratio in the field since fewer male are required compared to females.

Jojoba has come at a time when there are dwindling natural resources and increased concern for the environment (Tremper, 1996) hence meeting the aims of Kenya Vision 2030 on rehabilitation of ASALs (NEMA, 2011; NVSC, 2006) and Millennium Development Goals 2015 on ensuring environmental sustainability (UNMD, 2000).

1.2 Problem Statement and Justification

Dioecious plants have high genetic diversity within populations due to out-crossing (Hamrick and Godt, 1996). However, domestication and breeding has resulted to large scale cultivation of genetically uniform cultivars. This, in turn has led to an increasingly narrow genetic base for the crops, leading to genetic vulnerability (Rao and Hodgkin, 2002). Vegetative propagation of elite lines/clones is mainly used to establish large jojoba plantations in order to overcome high male to female ratio and increase production.

Genetic diversity of field established plantations is necessary for breeding and management purposes.

There is limited work on genetic characterization of jojoba using modern molecular marker techniques (Sharma *et al*., 2009a; Bhardwaj *et al*., 2010) and there is only one known Kenyan plantation which has not yet been reported to have been characterized and, therefore, forming the objective of the present study. DNA markers offer unlimited potential to uncover differences at the DNA level and hence are an ideal tool to differentiate individuals and genotypes (Graner and Wenzel, 1992; Tonukari *et al*., 1997; Sivaprakash *et al*., 2004; Sharma *et al*., 2009a). Simple Sequence Repeats (SSR) molecular markers were chosen in this study since they are widely used for marker assisted breeding, genetic mapping and diversity studies (Wang *et al*., 1994; Gupta *et al*., 1996; Gupta and Varshney, 2000; Masumba, 2006; Dominick, 2008). The microsatellites (SSR) are hyper-variable than most other markers hence able to distinguish among closely related plant cultivars (Davila *et al*., 1998). The study will provide a data base for the amount of variation among the existing jojoba bushes in Kenya.

Being dioecious, a seeded plantation of jojoba has genetic heterogeneity (Inoti *et al*., 2015) and low average yields (Benzioni, 1997). Seed raised seedlings give a ratio of 1:1 or even upto 5:1 (male to female) in the field leading to low production (Gentry, 1958). The recommended ratio is 1:10 (male to female) in order to obtain maximum yields ha^{-1} (Undersander *et al*., 1990; ARJP, 2001). Other authors have recommended a ratio of 1:5 (Harsh *et al*., 1987). However, there is no existing morphological trait method for distinguishing sex at an early age in jojoba (Ince and Karaca, 2011). Data is available on mature stands of dioecious plants showing that females allocate higher resources to reproduction than the males leading to lower vegetative growth in the former (Nicotra

et al., 2003; Zunzunegui *et al*., 2006; Barrett and Hough, 2013). However, Wheelwright *et al*. (2012) reported that females of *Ocotea tenera* compensated for higher costs of reproduction and diminished photosynthetic capacity by producing larger leaves. Kohorn (1995) did field assessment of mature plantations and found that female jojoba plants had larger leaves and more branches whilst males were taller than females but these parameters were also influenced by the environment.

Use of morphological traits for identifying sex at the juvenile stage is economical and more practicable for field workers hence more preferred than the molecular marker technique which is more expensive and restricted to large scale plantations and research centres. El-Baz *et al*. (2009) has reported wide diversity in several morphological traits of mature female jojoba bushes. Clonal propagation of elite individuals of known sexuality is necessary to ensure that the plants in commercial plots will be highly productive (Chaturvedi and Sharma, 1989).

Currently, there is low productivity of Jojoba seed (2-3 kg plant^{-1} yr^{-1}) mainly due to high male to female ratio in the existing plantations globally since most of them were raised from seed. The potential for jojoba yield is 4-5 kg plant^{-1} yr^{-1} in improved and well managed plantations. To overcome this problem, there is need to vegetatively propagate jojoba through selection of superior genotypes which have high yielding ability, but differ widely in their rooting ability depending on genotype and cultural factors (Foster *et al*., 1984; Bashir *et al*., 2008). A lot of work on vegetative propagation has been done especially in temperate environments (Lee, 1988; Benzioni, 1997; Zhou, 2002; Bashir *et al*., 2007; 2008; 2013), but a lot more is needed in tropical areas in order to determine the optimum requirements for jojoba cuttings in the region. Clones are expected to out-produce shrubs raised from seed by 300 to 500% (CJP, 2007) and also produce bushes

which bear seed 1-2 years earlier than the seedlings which start seeding at 3-4 years (Chaturvedi and Sharma, 1989).

Jojoba is a difficult- to- root plant and several rooting hormones have been tried with varying success (Bashir *et al*., 2013). There is a wide range of PGRs in the market and are available for use by commercial nurseries and in horticultural farms. However, they vary greatly in their composition of active ingredients, performance and price (Gitonga *et al*., 2010; Ngeno *et al*., 2013; Kumlay, 2014). Yet little work has been done on jojoba using non-conventional PGRs. It is, therefore, of necessity to test different PGRs in order to recommend the best for root initiation in jojoba cuttings in tropical environment. This will provide a cheaper and more accessible method for vegetative propagation of jojoba. Propagation of Jojoba cuttings will increase high quality oil supply to the market, provide income to the local people and also help to rehabilitate the arid environment. Increased jojoba plantations will also contribute to conserve the sperm whale (Hogan and Bemis, 1983) by supplying alternative oil. As noted previously, 1 ha of jojoba plantation can produce oil equivalent to that obtained from 124 sperm whales (Ward, 2003).

Poor management of the existing jojoba bushes has led to low production of seeds (0.5-2 kg $bush^{-1}$) through severe abortion of flowers and pods. This is mainly through competition for nutrients, water and space (Weber and Stoney, 1986; Rowland, 1993). Agronomical requirements of jojoba are still under experimentation especially in the tropical environment where little has been done (NRC, 2002). Jojoba is drought tolerant and offers promise for agriculture in harsh environments where many conventional crops cannot survive, yet it requires water for the first 2 to 3 years for proper root establishment (Forster and Wright, 2002). Similarly, for sustained high yields, watering is essential in areas with low rainfall, especially in desert areas with less than 350 mm annually.

Irrigation is quite advanced in jojoba production especially in Israel, America and India (NRC, 2002; Pinoyfarmer, 2007) making the cost of production very high. A lot has been done on irrigation (NRC, 2002; CJP, 2007) as a management tool which is quite an expensive option but its use for jojoba is justified by the high cash returns.

However, there is a great need to explore possibilities of other cheaper management alternatives in order to improve the overall performance of jojoba plantations for resource poor farmers who are the majority in ASALs. There is enormous potential of harnessing excessive runoff cheaply through creation of micro-catchments (Critchley and Siegert, 1991; Rowland, 1993; Mati, 2005; Itabari *et al*., 2011) for use by the crop for a longer period. There is, also, easy availability of manure from the cattle bomas which are in close proximity, since livestock is the major enterprise in ASALs.

According to Harris and Yusuf (2001), inorganic fertilizers have low adoption in ASALs of sub saharan Africa, hence manures are the most effective ways of improving soil fertility and maintaining soil structure (Kihanda *et al*., 2006). The results of the research findings will be beneficial to the local farmers in ASALs, researchers, central government agricultural extension workers, policy makers, county governments, non-governmental organizations, community based organizations as well as private companies and industries.

1.3 Objectives and Hypotheses

1.3.1 Objectives

1.3.1.1 Overall objective

The overall objective of this study was to characterize, propagate and manage jojoba bushes in semi-arid areas of Voi, Kenya in order to determine their genetic diversity, best multiplication methods and management techniques for improved yields.

1.3.1.2 Specific objectives

i. Determine genetic diversity of jojoba plants using Simple Sequence Repeats (SSR) molecular markers.
ii. Identify sex of jojoba nursery seedlings by using morphological traits.
iii. Determine the effects of mother plant management regimes on the performance of jojoba stem cuttings raised in a polythene sheet tunnel.
iv. Evaluate the performance of different Plant Growth Regulators (PGRs), genotypes and their interactions in macro-propagation of jojoba cuttings in a polythene sheet tunnel.
v. Assess the effects of propagule type and different management regimes on field planted jojoba cuttings and seedlings.

1.3.2 Null hypotheses

The null hypotheses for this study were:

i. Genetic diversity among the jojoba plants growing in Voi, Kenya cannot be successfully determined using SSR molecular markers.
ii. Sex of jojoba nursery seedlings cannot be identified sufficiently by using morphological traits.
iii. Management of jojoba mother plants has no effect on vegetative propagation using stem cuttings.
iv. There are no differences between the performances of various PGRs, genotypes and their interactions in macro-propagation of jojoba cuttings.
v. Propagule type and management regimes have no effect on the performance of field planted jojoba cuttings and seedlings.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin, Distribution and Genetics of Jojoba

2.1.1 Origin

Jojoba (pronounced as Ho-ho-ba) is a native of Sonoran desert of California, Arizona and New Mexico. The Red Indians of the United States of America (USA) have a long history of the traditional use of jojoba as food and medical remedy. Seeds were harvested and used for making necklaces for trade or roasted and eaten as nuts especially during the period of severe droughts. On the other hand, oil extracted from the seed was used for cosmetics, skin afflictions, as well as healing of wounds, kidney and other urine disorders (Gentry, 1958; Tremper, 1996).

Jojoba has been assigned the name *Simmondsia chinensis* by the botanical world. The name, however, comes about as an error. Link, a botanist, travelled around the world collecting seeds and plants to catalog and describe. By mistake he got the seeds of the jojoba plant mixed up with seeds that he had collected in China, hence the name chinensis. International rules of nomenclature state that a plant once given a name is stuck with it. Jojoba did not occur in China naturally (Tremper, 1996).

The exploitation of jojoba was not conceived until the banning of sperm whale in 1971, a species threatened by extinction. During this period scientists were seriously looking for an alternative source of high quality oil to substitute that from the sperm whale. It was then discovered that jojoba seeds had a similar oil quality hence a complete replacement and also a conservation strategy of the endangered sperm whale (Undersander *et al*., 1990).

2.1.2 Distribution

Jojoba is widely distributed in the Sonoran desert within the Islands of the Gulf of California and the coastal Sonoran. In Arizona, it is localized in the mountains around Tucson, south and east Phoenix, Catalina, Rincon, Santa Rita, Cerro Colorado, Babquivari and Ajo ranges (Higgins, 1949). In California, it inhabits the mountains around the Salton sea basin of the Colorado desert and southern portion of San Diego county. It also grows in New Mexico (Higgins, 1949).

Jojoba is grown in many countries including USA, Mexico, Argentina, Brazil, India, Israel, Egypt, Saudi Arabia, Australia, South Africa, Peru, Chile and Iran with Israel as the largest producers followed by USA and Mexico (Muthana, 1981; Undersander *et al*., 1990). USA and Israel were among the earliest countries which started planting jojoba commercially in the late 1970s and about 700 ha were planted during the 1990-1993 period in Israel alone. Most of the earlier stands were established from seed which resulted in low yielding stands. However, all new plantations are from vegetatively propagated plants originating as cuttings from selected superior clones (Benzioni, 1997).

2.1.3 Genetics

Gentry (1965) reported the chromosome number of *Simmondsia chinensis* to be n=100. Also Raven *et al*. (1965) reported 2n=26 (erroneously cited as n=26 by Gentry, 1965) and have suggested that plants or populations with n=52 should be sought in the field. The presence of polyploidy could provide large potential for breeding varieties for agricultural use (Gentry, 1965).

2.2 Environmental Requirements: Climate, Soils and Topography

2.2.1 Climate

Jojoba naturally grows in marginal areas with rainfall ranging between 220-400 mm yr^{-1}. However, for it to produce reasonable seed yields, it requires adequate water supply of 400-700 mm yr^{-1}. Jojoba is planted when soil temperatures are 20°C and irrigation is maintained for the first 3 years at a rate of 1 litre $plant^{-1}$ day^{-1} until sufficient root growth is established (CJP, 2007). Jojoba tolerates high temperatures ranging from 0-54°C with an optimum between 27-33°C (Yermanos, 1979). However, it requires at least, one month at 15-20°C to break flower dormancy. Frosts below -3°C especially during flowering can damage the plant, although mature bushes are able to withstand cold better than the seedlings (Borlaug, 1985; Stephens, 1994; Farmnote, 2005).

2.2.2 Soils

Jojoba grows on coarse, light and medium textured well drained sandy or gravelly soils and marginal fertility with a pH of 5-8.5 (Undersander *et al*., 1990; Forster and Wright, 2002). It tolerates saline environments and a pH below 5 leads to aluminium (Al) toxicity which is unsuitable for jojoba. It prefers very sandy soils with low organic matter (OM) (1.07%) (El-Baz *et al*., 2009). Drainage is critical, hence, water-logged clay sites should be avoided since jojoba will die if flooded for a short period. Deep-rootedness nature in jojoba helps it to tap water from deep aquifers for its survival in arid environments and also to return the leached nutrients to the soil surface.

2.2.3 Topography

Jojoba grows at an altitudinal range of 0-1600 metres (m) above the sea level in gently sloping areas which are well drained (CJP, 2007). It also grows in rugged rocky hills

which have discontinuous bedrock to allow deep rooting. Direction of rows depends on wind direction and the topography (to prevent soil erosion).

2.3 Jojoba Description, Requirements, Cultivation and Uses

2.3.1 Description

Jojoba is a long-lived desert shrub and in its natural habitat tolerates high temperatures, soil salinity, soil alkalinity and it is classified as a true xerophyte. Jojoba is a dioecious, evergreen, woody shrub that has several main stems which are brittle and easily broken. Jojoba produces a very deep root system with several main roots and few surface feeding roots that develop under natural conditions (Forster and Wright, 2002). It attains a height of 1-6 m whereas the roots can reach up to 15-25 m depth since they are five times the height. Leaves are xerophic with simple leathery thick and opposite pubescent containing a thick cuticle and sunken stomata. The leaves also contain a special tissue with a high concentration of phenol compounds.

Flowers are apetalous, the female ones are solitary developed at alternate nodes on newly matured growth. Female flowers are also light green with long pedicles and normally bloom single auxiliary on each alternate node. However, flowers may also develop singly at every node, and double or multiple flowers may occur occasionally at alternate nodes or every node. Pedicles are curved towards the soil, causing drooping of flowers (Kureel *et al*., 2008). Female flowers have no petals or scent to attract insects. Therefore the plant depends almost entirely on wind pollination (Ayanoglu, 2000). The male flowers are clustered at alternate nodes (Hogan *et al*., 1980). Male flowers are, also, borne in clusters of 7-36 flowers per cluster. Flower buds form in the axils of the leaves solely on the new vegetative shoots occurring during the warm season under favourable temperature and water regime in late summer or early fall after the previous crop of mature seeds. New

flower buds are mainly dormant and do not open without exposure to cool period with enough cold units for the fulfillment of their chilling requirements (Dunstone, 1980; Hogan *et al.*, 1980).

At anthesis, pistillate flowers are grey-green, urn-shaped, and quite inconspicuous. The clusters of staminate flowers at anthesis are very conspicuous due to the large quantities of pollen produced. Pollination usually occurs about March, while the fruits develop and mature by July and August in temperate countries. Each ovary initially contains 3 ovules but only a single seed usually develops in most genotypes. The fruits are capsules which turn gradually from green to tan or brown colour on maturation. Seeds differ in shape, size, colour and weight. They are also elongated, slightly spherical and pointed (Kureel *et al*., 2008). Upon drying, the fruit may or may not split to release the seeds, and contains about 50% liquid wax which can be extracted using standard pressure or solvent techniques (Benzioni, 1995; Benzioni and Ventura, 1998; Botti *et al*., 1998).

2.3.2 Cultivation

Jojoba is the Sonoran desert's second most economically valuable plant after Washington's Palms used in horticulture (Phillips and Comus, 2000). A jojoba stand can be in production for 100-200 years depending on management (Martin, 1983). High levels of amino acid build up were observed under canopies of Jojoba bushes (Burman *et al*., 2002), making it more suitable for companion crops under parkland agroforestry system. In India, farmers intercrop jojoba with leguminous crops such as green grams under drip irrigation system during the first 4 years of growth and this helps to maximize production per unit area (ARJP, 2001). Planting along the contours is always given priority. Rows are planted perpendicular to the wind direction to enhance wind pollination. According to El-Baz *et al*. (2009), Jojoba is planted at 1 or 2 m within rows and 3 or 4 m between the rows.

Irrigation is applied once in 2-3 weeks during summer. Weeds are a major problem in the first 2 years after establishment. Weeding is done twice during the rainy season by use of mechanical or hand weeding methods. Similar techniques are used during site preparation before planting. Drip irrigation system together with other management practices such as weeding, pest control and fertilization are observed as standard procedures.

Soils with low water holding capacity are essential if water and fertilizer are applied liberally. It is recommended to apply 2 kg of compost manure twice yr^{-1} $plant^{-1}$ in order to maintain high levels of phosphorus (P) which is essential for ideal growth (CJP, 2007). Fertigation is applied in poor soils where irrigation is applied through drip lines. However, little amounts of fertilizers of N, P and potassium (K) are recommended since jojoba requires low levels of nutrients (CJP, 2007). Pests in young plants can be controlled by use of Dieldrin chemical. Further research is also needed in the area of weed control, nutrition, varietal selection of improved jojoba types (Osman and Hassan, 2000) and harvesting equipment (Benzioni, 1997).

Seed pretreatment is done by soaking in warm water for 8-10 hours. One kg contains 800-1200 seeds hence 5-6 kg can be planted in one ha for direct seeding method. However, according to Inoti *et al*. (2015), jojoba seed number ranges from 955 for large seeds up to 3 003 for small seeds kg^{-1}. Jojoba raised from seed takes about 3 years to flower and up to 4-5 years to bear seed in the tropical environment. This period varies from place to place, for example, in USA and Mexico, it takes 7 years while in the Mediterranean region, it takes even 10 years (Muthana, 1981; Martin, 1983). During 1999-2001, Israel produced one third of the world production. Average yield for Israel is 3.5 t ha^{-1} (potential yield is 4.5 t ha^{-1}) and this is relatively high compared with production potential in Argentina and USA. According to CJP (2007), Rajasthan, India, has 500 ha (comprising 90% of all the

jojoba in India). The demand is far much higher than the supply such that in India even the jojoba seed cake is used as a skin cleanser by women.

Jojoba is currently not sufficiently domesticated for commercial production in Europe. Research is being carried out on plant exploration and evaluation along with crop improvement and breeding techniques. Selective breeding is developing plants that produce more beans with higher wax content, as well as other characteristics that will facilitate harvesting. Jojoba is relatively expensive to establish but it is cheap to maintain. Leaves are also succulent and leathery in nature which reduces water loss. According to Undersander *et al*. (1990), it takes just a quarter of the amount of water taken by olive plants.

Long term production of jojoba depends on improved yields and strong market since it is an alternative industrial oil with multiple applications. The utilization of marginal lands can become a major asset to global agricultural economy hence jojoba is being examined for its potential as a commercial crop in many countries around the world with climate and soils similar to those of its original habitat (Undersander *et al*., 1990).

Jojoba cultivation is suitable in wastelands such as ASALs and may lead to economic uplifting of families, employment for generations in rural areas and putting these areas to economic use (Hassan, 2003) as well as enhancing green cover in accordance with Millennium Development Goals of 2015 (UNMD, 2000).

2.3.3 Uses

Jojoba produces oil referred to as wax which is nongreasy, odourless and can withstand very high temperatures up to 320°C. It has been used by the native Indians from time

immemorial for food (Ward, 2003) and contains no cholesterol or triglycerides hence can be used as low calorie edible oil for cooking (Maratos, 1997). It is mainly used in cosmetics (lotions, shampoos, moisturizers, hair oils, conditioners), lubricant industry (automobiles and very fast moving machines) and pharmaceuticals (anti-bacteria) (Muthana, 1981; Hogan and Bemis, 1983; Amarger and Mercier, 1996; Ward, 2003; Mills *et al*., 2004; Reddy and Chikara, 2010). Other uses include: transformer coolants, plasticizers, detergents, fire retardants, candles, polishing wax, antifoam agent in antibiotic production, electronics and computer industries (Undersander *et al.*, 1990).

Jojoba oil is a natural high temperature and high pressure lubricant (Yermanos, 1979). It can also be used as a lubricant in high pressure machinery and other industrial purposes (Sardana and Batra, 1998). Jojoba oil is rare in that it is extremely long (C36-C46) straight-chain wax ester and not a triglyceride, making jojoba and its derivatives, jojoba esters to have more similar molecular structure to human skin sebum and whale oil than to traditional vegetable oil (Tremper, 1996; Phillips and Comus, 2000). This makes it superior base carrier for cosmetics, skin care products, hair care products and aromatherapy blends (Passerini and Lombardo, 2000).

The oil is soothing and stops multitudinous skin problems such as dandruffs and prevents premature aging and wrinkling due to exposure to ultra violet radiation. The latter is usually a common problem with albinism. Jojoba oil is used for medical treatments of ailments such as eczema, acute acne, skin cancer, psoriases, sores, wounds, poison Ivy, cold and kidney malfunctioning (Naqyi and Ting, 1990; Benzioni, 1997). Jojoba cake, a by-product of oil extraction, can be used as animal feed (Reddy and Chikara, 2010), since it contains 30% crude protein although with a lot of caution since it contains simmondsin, which is toxic (Boven *et al*., 1993). Simmondsin acts as a food intake inhibitor, depressing

appetite and may serve as a dieting supplement (Benzioni, 2006) hence maintenance of body physique through slimming.

Jojoba meal has been incorporated in chicken feed (broilers) in Belgium, and it is thought that 4% of Jojoba meal in the feed can restrict the feed intake level to give a favourable muscle to fat ratio hence eliminating over fat birds in the market (Phillips and comus, 2000). This strategy is being tried in other animals in an attempt to achieve similar results hence reducing the cholesterol in humans. On the other hand, jojoba leaves contain alpha-tocopherol, which is a major liposoluble antioxidant used in stabilization of food products and the oil does not grow rancid and hence can become suitable for vegetable oil (Mallet *et al*., 1994). About 80% of jojoba oil is utilized by the cosmetic industry and it is exported to Japan and Europe while lubricants provide a market for about 100 t yr^{-1}. Since there are only a few natural products that would directly compete with jojoba oils, the future of the product appears to be bright.

2.4 Plant Improvement Through Selection

Genetic resources have a long history with the local farmers selecting the best crop for their continual use (Bunders *et al*., 1996). Minor crops have great potential to contribute to agricultural production especially in marginal areas. This will draw attention of researchers in the formal sectors in bringing new resources to utilization and improvement. In classical genetic improvement programmes, selection is carried out based on observable phenotypes of the candidates for selection but without knowing which genes are actually selected. However, molecular markers introduced in the late 1970s overcame this limitation (FAO, 2007a). The ultimate objective of tree improvement programme is to obtain high quality seed to meet operational planting needs. With time, the best trees are used as seedlings seed sources and the very best trees are selected to

establish clonal orchards to improve the genetic quality of the seed being used (Longman, 1993). Clones have the potential to produce exact copies of the selected donor trees (Ortets). Genetic gains through reforestation with superior clones can be realized sooner than through recurrent selection and seedling production (Greenwood *et al*., 1991).

Trees with desirable, inherited characters can be selected and used directly to produce improved planting stock through vegetative propagation. The direct selection captures more of the desirable characters than is possible by seed (Longman, 1993). With each selected clone existing as many plants, it is easier to check which characters are strongly inherited. Increased use of line breeding will provide accelerated improvement, and the use of marker selection techniques increase the efficiency of selection. The ability to select the trees within families with superior performance helps further to increase the rate of improvement (Sederoff, 1999).

Genetic base can be broadened by increasing the number of phenotypically superior trees for selection hence increasing the selection intensity (Balocchi, 1990). The results obtained from across-site analyses have shown a significant genotype-by-environment interaction at the family level (Balocchi, 1990). This means that the best families selected for one environment are not necessarily the best families for a different environment.

Direct selection based on identifying potential promising genotypes and their testing after vegetative propagation has enabled faster crop improvement. Selection has been done for jojoba clones with high yields and early production in several countries (Benzioni, 1997). Benzioni (2006) reviewed the problems and possible solutions of jojoba cultivation in Israel and stated that there is need to hybridize high yielding female plants with lots of flower inflorescence and pollen from superior males.

2.5 Characterization of Plants Using Molecular Marker Techniques

A central theme in agriculture is the exploitation of natural genetic variability to improve the crop varieties in order to feed the rapidly growing population. Genetic diversity can be measured in terms of gene diversity which is the proportion of the polymorphic loci across the genome, heterozygosity or the mean number of individuals with polymorphic loci and alleles per locus (McGinley and Duffy, 2008).

According to Lukonge *et al*. (2007), studies of molecular diversity also help in the elucidation of phylogeny and provide the basic knowledge for understanding taxonomy, domestication and evolution. Since the 1970s, several advanced techniques have been developed to aid the identification and transfer of desirable traits. In crops, these include tissue culture, genetic fingerprinting and the use of genetic markers (Broerse and Visser, 1996). Identification of genetic diversity in a population is required in any breeding programme to develop cultivars with a broad genetic base.

Data from 25 developing countries indicate that marker assisted selection is the second most utilized biotechnology tool applied after tissue culture, implying that emphasis should be given to the development of molecular markers to make selection more feasible through improving the efficiency of plant breeding and germplasm characterization (FAO, 2007b). The use of genetic markers may be regarded as a useful step in applying advanced techniques to the characterization and improvement of local crop varieties. This technique demands substantial investment in skill and facilities hence limiting its usage in developing countries (Broerse and Visser, 1996; Yanchuk, 2002). However, its usage is inevitable in this present time, due to its ability to differentiate among species and to unravel the desirable genes in different genotypes without necessarily using the tedious and sometimes unreliable morphological markers.

The genetic background of a plant can be identified at the molecular level by making fingerprints of its DNA using DNA fragments, without the need to trace the genes responsible for specific traits. This technique allows samples of unknown origin to be characterized and compared, and genetic distances and relationships between different varieties to be assessed (Broerse and Visser, 1996; Baldwin *et al*., 2012). Scientists use molecular markers to analyze genetic variability or diversity as well as assess the relationship within and between populations. Markers are also used in mapping of genomes for specific traits applied in identifying genotypes (Wambugu, 2001; FAO, 2007b).

The genetic material is organized into sets of chromosomes and the entire set is called the genome. There are identifiable DNA sequences found at specific locations of the genome, and transmitted by the standard laws of inheritance from one generation to the next. They rely on a DNA assay, in contrast to morphological markers that are based on visible traits, while biochemical markers are based on proteins produced by genes (FAO, 2007b). However, Alves *et al*. (2013) proposed that SSR markers commonly used in molecular diversity studies may sample diverse coding regions of the genome and may therefore have limited use in predicting phenotypic diversity of individuals especially in complex traits.

Genetic diversity determination is necessary in order to characterize jojoba plantations using molecular markers for improved production. First generation of markers became available in the 1970s which included Restriction Fragment Length Polymorphism (RFLP) which are relatively slow and cumbersome to use (Wambugu, 2001). Second generation came up in the 1990s where Polymerase Chain Reaction (PCR) technique gave rise to

Random Amplified Polymorphic DNA (RAPDs), Amplified Fragment Length Polymorphism (AFLP) and microsatellites (SSR). These are more powerful and accurate since they allow the precise identification of individual genes. In the late 1990s, third generation of even more accurate markers called DNA expression arrays became available. These reveal whether or not a gene is expressed or switched on at a given moment in the development of an organism. More recent studies by Heikrujam *et al.* (2015) using CAAT Box-Derived Polymorphism (CBDP) markers have been able to classify male and female jojoba separately by use of dendrograms.

A number of molecular markers have been used on jojoba mainly for sex determination in mature stands such as RAPDs (Agrawal *et al*., 2008; Mohasseb *et al*., 2009a; Hosseini *et al*., 2011; Yadav *et al*., 2012), AFLP (Agarwal *et al*., 2011) and Inter-Simple Sequence Repeat (ISSR) (Sharma *et al*., 2008; Heikrujam *et al*., 2014). Genetic diversity and association mapping relies on the availability of high numbers of markers distributed throughout the entire genome. Diversity studies have commonly been done with SSR markers which have a high Polymorphic Information Content (PIC) and several authors have used SSR markers to characterize various crops including cassava (Dominick, 2008) and jatropha (Haek *et al*., 2011).

Similarly, Zhou *et al*. (2012) and Katoch *et al*. (2013) used SSR for genetic diversity in *Saruma henryi* Oliv and *Picrorhiza kurrooa*, respectively. Gene diversity was termed as the probability of two randomly chosen alleles being different from a population (Weir, 1996) while PIC was defined as the measure to calculate the discrimination power of markers (Botstein *et al*., 1980). PIC = $2P_iQ_i$; where P_i = the frequency of presence, while Q_i = the frequency of absence of a particular amplicon obtained from a primer/primer pair. Discriminatory power (D_j) is an extension of PIC which provides an estimate of the

probability that two randomly chosen individuals show different banding patterns. SSR have a high mutational rate and new alleles can easily be created during DNA replication process.

Microsatellites or SSR regions of DNA are composed of short (<6bp) sequences repeated in tandem (Gardner *et al*., 2011). They are usually co-dominant and a very high number of alleles can be detected at same loci (Echt *et al*., 1996). Since the assay is easy to perform and produces polymorphic markers, microsatellites, are a highly attractive tool for genetic studies at population level (Morgante and Olieveri, 1993; Plaschke *et al*., 1995). Different types of molecular markers exist, but differ in a variety of ways as follows: their technical requirements, amount of time and labour needed, number of genetic markers that can be detected throughout the genome and also the amount of variation found at each marker in a given population (Broerse and Visser, 1996; FAO, 2007b).

RFLP is the oldest technique and very reliable but it requires the use of restriction enzymes and elaborate facilities, including hybridization equipment, X-ray film and cold storage. On the other hand, RAPD and AFLP have been applied in breeding programmes quite frequently, as they are quicker (Waugh and Powell, 1992). They also require fine tuned equipments and procedures such as PCR technique and gel electrophoresis to visualize the results. The information provided to the breeder by the marker varies depending on the type of marker system used and each one has its own merits and demerits. Molecular marker system allows high density DNA marker maps to be constructed for a range of economically important agricultural species, thus providing the framework needed for eventual applications of marker assisted selection (FAO, 2007b).

RAPD technique uses short primers of arbitrary sequence to amplify at random from a few anonymous genomic sequences (William *et al*., 1990). The method is versatile and has

been used in many plant population biology studies. However, only dominant markers are generated and reliability and reproducibility are sometimes difficult to reach (Lu *et al*., 1997). AFLP offers the advantages of RAPD technique but generates more markers per assay and is more reliable (Breyne *et al*., 1997).

Microsatellites have also been identified in chloroplast DNA according to Powell *et al*. (1995), which creates a lot of interest as the average level of sequence variation in chloroplast DNA is low (Clegg and Zurawski, 1992). Determining the nucleotide sequence of a DNA fragment is reliably applied to questions at population and taxonomic levels and provides the ultimate resolution for detecting genetic variation (Nei, 1987). Several authors have used microsatellite (SSR) markers to characterize various crops including cassava (Dominick, 2008) and jatropha (Haek *et al*., 2011).

Dodds (1991) reported that the transition from primitive to advanced cultivars has had the effect of narrowing the genetic base. This has happened in two distinct ways which include: selection for relative uniformity, resulting in pure lines, multilines, single or double lines as well as selection of closely defined objectives. These processes here have led to a marked reduction in genetic variation. Since no two seedling trees have the same genotype, man selects those with favourable genotypes as the ones to produce the most offsprings. Thus, over a period of time, the average genetic constitution of the population changes (Wright, 1976). Since selection is only for one parent (the female) genetic gain is half as large as when selection is for both parents (Fins *et al*., 1992). A study on jojoba involving five primers showed highly polymorphic nature of the studied plants in the natural habitat of Egypt, an indication that they belong to different genotypes (Gaber *et al*., 2007). Al-Soqeer (2010) investigated 8 jojoba genotypes and clones in central region of Saudi Arabia and reported high genetic variability, whereas Osman and AboHassan

(2013) in western and northern Saudi Arabia showed moderate genetic variability among jojoba clones. This could permit improvement by selection and breeding for commercial plantation establishment.

Studies on oil palm (Ting *et al*., 2010) showed high PIC range. This is expected because this crop is established through seeds and have not been much exploited, thus have a wide gene pool. PIC more than 0.5 are efficient in discriminating genotype (Sharma *et al*., 2009b). Other studies on papaya cultivars in Thailand by Ratchadaporn *et al*. (2007) reported a PIC range of 0.35 to 0.40 and concluded that all the cultivars were genetically closely related to each other.

Similar studies on jojoba by Hosseini *et al*. (2011) and Heikrujam *et al*. (2015) reported a PIC range of 0.32-0.89 and 0.38-0.43, respectively and concluded that all the cultivars were genetically closely related to each other. This is indicative of the possibility of similar pedigree of the genotypes in the studied plantation. Other studies by Ince and Karaca (2011) reported a PIC of 0.08-0.29 while Naek *et al*. (2011) revealed a PIC of 0.379 for *Jatropa curcas* in Brazil and concluded that few differences were found among the plant cultivars. However, Bhardwaj *et al*. (2010) reported average PIC of 0.16 and 0.18 using RAPD and ISSR, respectively in jojoba germplasm which were low indicating use of clonal cuttings in jojoba propagation. A high degree of relatedness was demonstrated by the low PIC range found in jojoba, which is the only species in Simmondsiaceae family. However, PIC more than 0.5 are efficient in discriminating genotypes (Sharma *et al*., 2009b; Ganapathy *et al*., 2012; Sharifova *et al*., 2013). Studies by Stewart (2004) and Halkett *et al*. (2005) reported that clonal plants generally have low genetic diversity similar to that found in self-pollinated plants.

2.6 Characterization of Plants Using Morphological Characters

Morphological characters have long been the means of studying genetic variability in population and species of plants (Parfitt and Arulsekar, 1987). Even though they represent a fraction of the genome, taxonomists have used them extensively for classification and to study the degree of relatedness among population and species of plants. These characters include: growth habit, branching type, leaf shape and size, floral characters, fruit and seed morphologies and fertility traits. Genetic variability in morphology, anatomy and physiology within the species is very large and enable selection of clones for high yield and other agricultural attributes (Benzioni, 1997).

Earlier morphological studies of jojoba by Gentry (1965) found that variations occur randomly across various populations such that there is no distinct subspecies recognized. However, variations reflect the diversity of habitats in which jojoba grows and its geographical distribution in the Sonorant desert. Several aspects of this genetic variability are of interest in the selection of strains of jojoba for cultivation. It was clear that coastal forms differ significantly from desert forms in habit, with the former plants growing closer to the ground. Leaves vary on different plants in size, shape, colour, thickness and amount of pubescence. In Arizona populations, female flower buds remain dormant at every other node whilst in California, flowering occurs at every node. A capsule can contain 1-3 seeds but one is common. This can be due to genetic control modified by environmental conditions or even differential success in pollination (Gail, 1964).

Asymmetry between sexes in the morphology of reproductively more successful individuals points to a potential for a resource-based evolutionary origin of sexual dimorphism in jojoba, arising from different morphological optima for each sex (Kohorn, 1994; 1995).

Both wild and most of cultivated jojoba plantations are propagated by seeds, composed of multiple genotypes that are represented in a wide range of phenotypes or a combination of characters such as plant shape, leaf size, growth rate, duration of flowering and seed productivity. Systematic germplasm collection, clonal evaluation, variety trials and cultivar selection are the keys of successful jojoba production (Purcell and Purcell, 1988).

Many authors found significant variability in different morphological and traits of productive characteristics, such as node density, branching, leaf shape and area, seed size and weight and wax content per seed. They concluded that selection of large seed size and high oil content may be important for developing high-yielding jojoba cultivars (Yermanos and Duncan, 1976; Hogan *et al*., 1980; Nagvi *et al*., 1990; Gaber *et al*., 2007; Inoti *et al*., 2015). Studies by Osman and Hassan (2000), indicated that plant height, crown diameter and internode length in jojoba were negatively correlated to one another at (P=0.01). Coefficient of determination (R^2=0.111) indicated a low contribution of these traits in the total variability associated with seed yield, indicating the importance of directly selecting high yielding plants in yield improvement programmes. Morphological studies on 24 female jojoba genotypes by El-Baz *et al*. (2009) found considerable variability among all parameters studied ranging from seed, leaves, node, shoot, flowers and plant height. The natural degree of variability was found to be enormous, and this gives a plant scientist a huge range of possible gene combinations for future selection and improvement of jojoba as a new-industrial and commercial crop. Similar results were reported by Tobares *et al*. (2004) who used agronomic and chemical traits as descriptors for selection of jojoba clones.

El-Baz *et al*. (2009) noted that variations observed in the morphological parameters were principally due to genotype differences. The existence of apparent differences among

multiple genotypes resulted in a wide range of phenotypes or a combination in almost all of the studied parameters. A large source of genetic variation in jojoba may be useful for clonal improvement which may offer excellent production prospects and good adaptation to areas marginal for traditional agricultural purpose hence offering an interesting alternative for development of the zone.

Most studies of dioecious species have focused on mature individuals (Gehring, 1990) and very little work is reported on cuttings at the nursery stage. Dudley (2006) stated that comparisons of short term studies can be misleading because correlations of fitness and growth to physiological traits vary widely among plant species, environmental conditions and life stages. Hence long term growth studies rather than short term physiological studies are recommended. However, similar work by Nicotra (1999) reported studies on *Siparuna grandiflora*, a dioecious shrub on the vegetative traits for sex-based differences in both cuttings and mature plants.

Results on pre-reproductive males showed larger leaves and leaf areas than females while mature females allocated less biomass per unit stem length than males (Nicotra, 1999). Females allocate more biomass to reproduction and the latter has negative effect on the growth of females but not in males. Other authors have identified a wide range of dioecious species with patterns of growth equivalent despite reproductive allocation (Sakai and Burris, 1985; Gehring and Linhart, 1993; Ramadan *et al*., 1994).

Various authors have reported morphological trait studies in mature plants (Krischik and Denno, 1990; Kohorn, 1994; Kohorn *et al*., 1994; Culley *et al*., 2005) and also genotype/clonal differentiation within similar sex ((Benzioni, 1997; Tobares *et al*., 2004; El-Baz *et al*., 2009; Nderitu *et al*., 2014). However, a few studies have been reported by

some authors to characterize crops such as vanilla (Mantengu *et al*., 2007) and papaya (Reddy *et al*., 2012) before the flowering stage. Leaf morphology has been used to identify apaya sex at seedling stage where male leaves are 3-lobed while females are 5-lobed (Reddy *et al*., 2012). The males were slower growing compared to females in papaya.

Several studies have reported different male trees/shrubs species having larger size and faster growth rate (*Fragaria chiloensis*) and greater biomass (*Laretia acauli*, *Lithrea caustic* and *Peumus boldus* than females (Hoffman and Alliende, 1984). Studies on *Baccharis halimifolia*, a dioecious shrub by Krischik and Denno (1990) showed that males possessed longer shoots and more tender leaves, grew faster and flowered and senesced earlier than female plants. The tenderness of leaves made males to be herbivore targets by beetles than females hence resulting to a female bias in the field of 59%. Studies by Kohorn (1994) on jojoba in California stated that females were smaller than the males. Kohorn (1994) also reported that females had larger leaves than males. However, where sexes are dimorphic in terms of mean values of these morphological parameters, there is substantial overlap between sexes and considerable within population variation within each sex (El-Baz *et al*., 2009).

Generally the male plants outnumber the female plants when raised from seeds (Harsh *et al*., 1987). Gentry (1958) attributed this ratio to environmental rather than genetic factors. Apparently, a greater proportion of males survive the stress of seedling establishment probably due to high proline levels in males which are associated with plant stress tolerance (Ketchum *et al*., 1991). Proline protects membranes and proteins against high concentrations of inorganic ions and temperature extremes (Santarius, 1992) as well as development of pollen grains in males.

Husseini *et al*. (2013) reported that increasing the irrigation interval of jojoba increased the amount of proline soluble carbohydrates, chlorophyll a+b, as well as the value of succulence and osmotic potential but decreased all the studied growth characters. Freeman *et al*. (1976) investigating on a range of dioecious plants concluded that for all the species, males were more abundant in xeric microsites while females were over represented in the moister parts.

Jojoba can display some level of phenotypic plasticity which is the ability to develop different phenotypes in response to environmental conditions (Winn, 1996), is heritable and plays an important role in species evolutionary strategy (Agrawal *et al*., 2008). In xeric sites, males have smaller leaves and more compact canopies than females. In more mesic sites, populations of jojoba do not differ in vegetative morphology (Kohorn *et al*., 1994).

Allocation differences in dioecious species have been found in species that are markedly dimorphic, such as *Simmmondsia chinensis* (Kohorn *et al*., 1994; Wallace and Rundell, 1979) as well as in *Siparuna grandiflora* that is less obviously dimorphic (Oyama and Dirzo, 1988). Several studies have demonstrated that if there are no mechanisms to compensate for resource allocation to reproduction, males achieve greater growth than females (Lloyd and Webb, 1977; Popp and Reinartz, 1988; Garcia and Antor, 1995 Forero-Montana and Zimmerman, 2010; Zhao *et al*., 2012; Barrett and Hough, 2013). Studies on growth characteristics between males and females of dioecious plants have shown that females are smaller than the males (Hoffman and Alliende, 1984; Vasiliauskas and Aarsseen, 1992) and the females grow more slowly (Jing and Coley, 1990; Cipollini and Whigham, 1994). Studies on *Schiedea salicaria* showed evidence of sexual

dimorphism where males had higher mass based photosynthetic rate and specific leaf area than females (Culley *et al*., 2005), although this is in contrast to that predicted if females have higher reproductive costs. Females of *Ocotea tenera* compensated for higher costs of reproduction and diminished photosynthetic capacity by producing larger leaves (Wheelwright *et al*., 2012). Jing *et al*. (2008) reported that net photosynthetic rate of female ginkgo was significantly higher than males.

Work by Lambers *et al*. (1998) noted that females had thicker and denser leaves leading to lower specific leaf area, which could potentially slow their growth rate. Studies on *Silene latifolia* by Laporte and Delph (1996) noted that higher leaf size and leaf area lead to higher photosynthesis and more carbon accumulation in males than females of dioecious plants. These differences are thought to exist because females allocate more resources to reproduction than males and therefore should have fewer resources for vegetative growth (Wilson, 1983).

In general, it has been found that male plants of perennial species are either larger, grow faster, have more ramets or have higher biomass than female plants though the growth rate could be habitat dependent (Jing and Coley, 1998). Gao *et al*. (2010) reported that climatic sensitivity in male and female trees of dioecious species is different, yet this difference is not stable through time. Work by Cepedo-Cornejo and Dirzo (2010) on Neotropical palms reported that asymmetrical allocation to reproduction by females may lead to reproduction-growth tradeoff, where female plants grow less than male plants, but invest more in defense and thus experience lower herbivory than male plants. On the other hand, work by Li *et al*. (2007) on *Hippophae rhamnoides*, a deciduous shrub in south west China showed that females had a higher specific leaf area than males along an altitudinal gradient. Studies in *Corema album*, a dioecious plant in Iberian Peninsula, showed that

reproductive effort was 3 times higher in females than in males or hermaphrodite plants (Zunzunegui *et al*., 2006).

Studies by Dawson and Bliss (1989) on *Salix arctica* in different sites, reported that males showed faster growth rate in dry site while females were faster in wet site. This may change the sex ratio in the field depending on the adaptive ability (Hultline *et al*., 2008), since males are more adapted to warmer climates (Zhao *et al*., 2012). Hence a climate change phenomenon can lead to more male bias. Dioecious plants are vulnerable to change in population size and structure, thus sensitive to habitat fragmentation through human or livestock encroachment (Yu and Lu, 2011).

2.7 Plant Macro-Propagation Through Cuttings

Jojoba plantations are established through seeds, seedlings, rooted cuttings, or plantlets from tissue culture. Propagation through cuttings has been seen to be relatively the most successful in jojoba and is usually cheap. Cuttings shorten the time period taken for plants to reach production. It is used where seed is limiting or where germination is a problem. This method has advantage of shape and sex of the plant. Plantations from cuttings are uniform, have higher survival and yield more as well as stable in yield potential (Hogan and Palzkill, 1983).

Vegetative propagation can be achieved by rooting semi-hardwood cuttings, but the maximum number of possible propagules is limited by plant size and time of planting (Low and Hackett, 1981). Vegetative propagation enables the establishment of plantations with the desired proportion of male to female plants from pre-selected superior clones (Benzioni, 1997). It also creates uniformity, high yields, early bearing and reduced cost of cultural and harvesting operations (Hogan and Palzkill, 1983). Consequently, several

investigators have attempted clonal propagation of jojoba shrub (Llorente and Apostolo, 1998; Roussos, *et al*., 1999; Tyagi and Prakash, 2004; Singh *et al*., 2008; Mills *et al*., 2009; Bashir *et al.,* 2013). Great variation in jojoba clones was noted by Llorente and Apostolo (1998) with some exhibiting 75% root formation at 60 days after transplanting into an Agrosoil media while others displaying little success. Attempts have been made to propagate jojoba vegetatively through air-layering (Reddy, 2003), grafting (Shah and Bashir, 2000), stem cuttings (Bashir *et al*., 2007) and tissue culture (Bashir *et al*., 2008). Although jojoba is a difficult to root plant, yet propagation through cuttings is the most commonly used asexual method although with limited success (Palzkill and Feldman, 1993).

The relevance of mother plant status in propagation has often received little attention (Da Costa *et al*., 2013), yet it is a key determinant in rooting propensity of cutting derived from it. Da Costa *et al*. (2013) reported that adventitious rooting in cuttings is a multifactorial response leading to new roots at the base and establishment of an autonomous plant. It occurs in two phases: a) induction, with a requirement of high auxin and occurs within 96 hours after harvesting the cutting, b) formation, inhibited by high auxin and in which anatomical changes take place. The combined increased accumulation of basipetally transported auxins from the shoot apex to the cutting base is sufficient for adventitious rooting in easy - to - root species.

Hence, control of environmental variables around the stock plant is quite relevant for clonal propagation process. Ability to root is also affected by the physiological condition of the stock plant (Low and Hackett, 1981), cultural factors (Foster *et al*., 1984) and maturation (Ozel *et al*., 2006). Cultural practices such as pruning, fertilizing and watering during the dry season can encourage sprouting of shoots for cuttings (Longman, 1993).

The presence of auxins, enzymes and phenolic compounds in the mother plant affected rooting in cuttings, whereas the physiological and biochemical quality could limit rooting in cuttings. Physiological condition is affected by environmental conditions such as light, temperature, water and nutrients (Moe and Andersen, 1988). Endogenous auxin, carbohydrate content, mineral nutrients and other biochemical components such as phenolics which act as auxin transport modulators may be affected by environmental factors (Da Costa *et al*., 2013).

Management techniques applied to the mother plants can exert positive effects on the rooting potential of cuttings (Loreti and Morini, 1983). The ability to root also depends on the endogenous balance of carbohydrates, auxins and rooting co-factors according to Sadhu (1989) who reported that endogenous auxin accumulation helps to initiate rooting and this varies with species and season. Season of harvesting the cuttings has been ranked higher than variation within jojoba plants in initiating rooting (Low and Hackett, 1981). Carbohydrate allocation distribution within the cutting could be more important than the content itself (Druege, 2009; Ruedell *et al*., 2013). Light and current photosynthesis of cuttings is vital in this scenario. Rooting hormone such as IBA promotes rooting during periods of high rooting potential but can have no effect or become slightly inhibitory during periods of low rooting potential (Low and Hackett, 1981).

The events leading to adventitious rooting strongly depend on the mother plant nutritional status, both in terms of minerals and carbohydrates, as well as sink establishment at the cutting base (Da Costa *et al*., 2013). High levels of amino acid build up were observed under canopies of jojoba bushes (Burman *et al*., 2002), due to nutrient recycling making it more suitable for companion crops under parkland agroforestry system. Rooting of cuttings depends on the age of the plant, the condition of the cutting, the time of

collection, the rooting medium and special management treatments (Low and Hackett, 1981; Sadhu, 1989). Several authors have reported that ability to root is affected by the genotype (Bonga and Aderkas, 1992; Bashir *et al*., 2008; Kesari *et al*., 2010; Hassanpour and Ali Shiri, 2014). Similarly, other studies have documented that clones display great variation in rooting ability (Prat *et al*., 1998; Dick *et al*., 1999).

Work by Ozel *et al*. (2006) on rooting of soft woods reported that juvenile cuttings rooted better than from mature plants. Rooting of cuttings range from 15-95% depending on clone and season (Van Buijtenen *et al*., 1975; Greenwood *et al*., 1980; Sadhu, 1989), but season has been seen to have the greatest effect (Bashir, *et al*., 2013). Large differences occur in growth parameters of cuttings due to mother plants (Thomson, 1982) and clones (Lee and Palzkill, 1984). Danthu *et al*. (2008) also reported that rooting success for cuttings from natural forest trees depended on the season of cutting (high in the hot season and low in the cold season). Low and Hackett (1981) also reported similar results in temperate countries by recommending harvesting of cuttings during summer and spring.

Nutritional factors affect the rooting of cuttings. It is generally observed that high carbohydrate to N ratio in stock plants favour rooting in cuttings (Sadhu, 1989). Mineral nutrients such as P and trace elements namely, boron (B), zinc (Zn) and molybdenum (Mo) have been reported to stimulate rooting (Weiser and Blaney, 1960). The ability to root also depends on the endogenous balance of carbohydrates, auxins and rooting co-factors. Paton *et al*. (1970) stated that the ability of cuttings to form roots tends to decrease with the age of the mother plant due to increase in rooting inhibitors.

Work by Zhou (2002) also related the rooting rates of jojoba cutting to age of the mother plant, temperature and humidity as the rooting rates of cuttings from young and old female Jojoba shoots were 60-64% and 3-4%, respectively, at a temperature of 19-23°C and

relative humidity of 80-85%. The dormant stage after rains is the best period for cuttings according to Hartmann and Kester (1975) and Marino (1982). Studies by Sadhu (1989) recommended shoots of desirable maturity where rapid growth has ceased (with low to medium N) and carbohydrates have accumulated for use by cuttings.

The rooting potential of a cutting is influenced by the position within a stem from which the cuttings originates (Aderkas and Bonga, 1988; Leakey and Coutts, 1989; Dick *et al*., 1991). Several workers have reported that central and basal parts of the mother plant generally make the best cuttings (Aminah, 1990; Tchoundjeu and Leakey, 1996; Palzer, 2002). This is explained by Sadhu (1989) as the portions having high levels of carbohydrate and low amounts of N as compared to the shoot tips.

At the same time, root promoting substance such as growth regulators and stored assimilates move from the leaves and buds polarly from the tip to the base (Dick *et al*., 1999). At least 2 nodes (but preferably 4-6 nodes) must be included in each cutting. The basal cut is made just below the node while the upper is 1-3 cm above the node. The portions of the shoot tip can be discarded because they are usually low in stored food (Palzer, 2002).

The presence of leaves in the cutting is also essential for rooting in many tropical plants (Hartmann and Kester, 1975; Newton *et al*., 1992; Tchoundjeu and Leakey, 1996) since it helps to accumulate more carbohydrate through photosynthesis. Leaf area significantly affected rooting in *Prunus africana* leafy cuttings where rooting ability increased proportionately with leaf area up to 20 cm^2 (Tchoundjeu *et al*., 2002). Sadhu (1989) reported that endogenous auxin accumulation helps to initiate rooting and this varies with species and season. Many environmental effects such as cultural factors or positions

within the crown of the tree can influence cutting behaviour. Geneticists use “C effect” to describe the extent to which these considerations alter the clonal mean for a character of interest (Foster *et al*., 1984). The performance of a clone in height or rooting may be increased or decreased by C- effects. The dormant stage after rains is the best period for cuttings (Hartmann and Kester, 1975; Marino, 1982).

Vegetative propagation allows new trees to be raised any time and can help to speed up domestication to allow urgently needed tree planting to be carried out with mixtures of superior selections (Longman, 1993). Combined with initial high temperature growing conditions which add further protection against viral diseases, tissue culture has been applied to many crop varieties including ornamentals and tree crops. However, problems of tissue culture include: maintenance of sterile conditions and narrowing the genetic base according to Dodds (1991) and Broerse and Visser (1996). Zhou (2002) reported that the rooting time for cuttings ranged from 4 to 9 months although even shorter period between 2 weeks to 2 months under ideal conditions has been documented by Benzioni (1997) for jojoba. Environmental factors such as shade and high humidity inside the polysheet can even be more important than the auxin concentration (Bashir *et al*., 2008). Water misting in plastic tunnels gave the best results in rooting and vegetative growth followed by cuttings without misting.

Consequently, increasing the period of rooted cuttings in the greenhouse was reported by Osburn *et al*. (2014) to increase the chances of diseases infection due to misting which weakens the cuttings. Benzioni (1997) reported that *Alternalia* fungus species causes a disease which is common during the propagation of rooted cutting and can lead to low survival and rooting of the cuttings if not well managed. Similarly, Cother *et al*. (2004) reported a new bacterial disease in jojoba caused by *Burkholderia andropogonis* and it is

favoured by heavy rains, low temperature, shading and high humidity. It interferes with the growth of young seedlings leading to shedding of leaves from the severely affected ones which also leads to death and abortion of capsules in the mature plants.

Many long-lived trees do not produce seed until a certain age. This aspect coupled with out-breeding reproductive behaviour and long juvenile phase of most horticultural crops and woody tree species pose serious problems for multiplying their propagation material (Chopra and Narasimhulu, 1990), hence must be propagated vegetatively when it is desired to produce the parental genotype (Dodds, 1991). Preferential cloning of mature trees is desired when establishing plantations of dioecious species. In this case, the female trees have economic value and since sex is unknown until sexual maturity, they are cloned preferentially. These examples include *Carica* sp. (Jordan *et al*., 1983) and jojoba (Rost and Hinchee, 1980). Abscisic acid (ABA) accumulates under water stress conditions and is a known inhibitor of cell cycle progression (Wolters and Jurgens, 2009).

Hence the level of water stress is a relevant factor for establishment of cutting that should be minimized in order to avoid losses and slow establishment of plants. Husseini *et al.* (2013) reported that increasing the irrigation interval of jojoba increased the amount of proline soluble carbohydrates, chlorophyll a+b, as well as the value of succulence and osmotic potential but decreased all the growth characters. Leaves are also succulent and leathery in nature which reduces water loss. It takes about one quarter of the amount of water taken by olive plants according to Undersander *et al*. (1990). Several authors have emphasized the importance of mineral nutrients such as P and trace elements namely, B, Zn and Mo in stimulating rooting (Weiser and Blaney, 1960; Feldman *et al*., 1982). Feldman *et al*. (1982) further reported that jojoba leaf N and K content was positively correlated to root and shoot growth in spring and to shoot growth in summer. Studies by

(Sadhu, 1989) reported high carbohydrate to N ratio in stock plants favouring rooting in cuttings. High accumulation of carbohydrate and starch at the rooting zone was associated with improved rooting in *Eucalyptus globulus* (Ruedell *et al*., 2013) and *Tectona grandis* cuttings (Husen and Pal, 2007).

Studies on Peach by Blazich (1988) also reported significant effect on rooting of cuttings when mother plants were supplied with nutrients such as N, P, K and Ca. Mineral nutrition of the stock plant is important in determining rooting capacity. The auxin precursor requires Zn (Blazich, 1988; Marschner, 1995; Tromas *et al*., 2010). Manganese (Mn) and Iron (Fe) are components of peroxidases which is vital in rooting according to Fang and Kao (2000). High N supply to stock plant has been seen to promote adventitious rooting in herbaceous cuttings (Zerche and Druege, 2009).

2.8 Role of Plant Growth Regulators (PGRs) in Macro Propagation

During root regeneration, auxins, both naturally occurring (endogenous) and externally applied (exogenous) and environmental factors such as mist, light, temperature, are of particular importance (Van Buijtenen *et al*., 1975; Greenwood *et al*., 1980). Seasonal effects on ortet and rooting chamber environment affects rooting (Marino, 1982). Rooting environment includes: carefully controlled mist, supplemental carbon dioxide (CO_2), bottom heat and photoperiod extension (Marino, 1982). These environmental factors are controlled in a glass or green house under which the propagation experiments are conducted.

Auxins are used for root development. They are transported at the base of the plant (polar transportation) even if the plant is inverted for long periods (Sheldrake, 1974). Inhibiting polar transport inhibits root development. A rooting powder for root development

sometimes can be quite effective since it could have other additional substances compared with auxin alone. Its effectiveness varies with species, time of the year cuttings are taken and concentration of solution (Greenwood *et al*., 1980; Foster *et al*., 1984).

Rooting hormones in plants stimulate the formation of new root tips in stem cuttings. Depending on the level of endogenous growth regulating substances, exogenous application of auxins may be promotive, ineffective or even inhibitory for rooting of cuttings. High auxin concentration has been reported to be inhibitory to root growth in several tropical tree species such as *Dalbergia sissoo* (Leakey and Coutts, 1989) and *Pingamia pinnata* (Kesari *et al*., 2010). The ability of cuttings to form roots tends to decrease with the age of the mother plant (Paton *et al*., 1970; Zhou, 2002; Mourao *et al*., 2009; Awang *et al*., 2011) due to increase in rooting inhibitors such as essential oils and phenolic compounds (Kibbler *et al*., 2002). This can be reversed by de-topping of the mature plant to encourage new sprouts near the base assuming juvenile characteristics (Sadhu, 1989). Several authors have documented that clones display great variation in rooting ability (Prat *et al*., 1998; Dick *et al*., 1999). Successful rooting of jojoba cuttings can be achieved by the use of different auxins but their performance varies greatly among them.

However, (Benzioni, 1997) reported that cuttings of jojoba start to root within 2-8 weeks but this period varies depending on many factors (Bashir *et al*., 2008). The rooting ratio of semi-hardwood cuttings was increased by IBA at 1 000 mgL^{-1} and the rooting ratio of young individuals was higher than the mature ones (Cao and Gao, 2003). Bashir *et al.* (2008) reported that the jojoba cuttings of PKJ-3 strain were the most responsive to the various levels of auxins, followed by those of PKJ-6. Hence, both strains are the most suitable for multiplication through cuttings. IAA significantly promoted shoot growth, and

IBA significantly enhanced the root growth and increased survival of cuttings. A more recent work by Eed and Burgoyne (2015) using jojoba cuttings treated with IBA, NAA and NAA at 4 000 parts per million (ppm) and cultured under plastic tunnel conditions showed that IBA had the highest rooting percentage (37.3%) in the media containing sand and peat compared with the other PGRs. These results were considered good when no modern tools such as intermittent mist propagation system are available in some countries.

Studies by Hasanuzzaman *et al*. (2007) using *Capsicum annum* in Bangladesh recommended use of higher doses of the hormones in future research since the findings exhibited a pattern of higher growth in the variables relative to the control but the difference was not significant. Kebede *et al*. (2013) reported propagation of leafy stem cuttings of *Prunus africana* and *Syzygium guineense* in Ethiopia using both IBA and NAA at 0.0, 0.2 and 0.4% concentrations in a non-mist poly-propagator. Humidity was 85% inside the poly-propagator while temperature ranged from 7-22.5℃. The results showed that auxins had no significant effect on rooting percentage of both species.

However, the auxin treatment on root number, root and shoot length was significant at $p < 0.01$. Work by Kebede *et al*. (2013) also indicates that *Prunus africana* and *Syzygium guineense* rooted successfully without hormone application in the control treatment. Successful rooting without auxin application has been reported in a number of tropical tree species such as *Nauclea* and *Vochysia* (Leakey and Coutts, 1989). Rooting hormones or plant regulators wholly cause a greater percentage of cuttings to root, hasten the formation of roots, induce more roots of cutting and increase root uniformity (Godfrey *et al*., 1996; Tchoundjeu *et al*., 2002). IBA intensifies the root formation process which influences polysaccharide hydrolysis resulting to increased content of physiologically active sugar

needed to provide energy for meristematic tissues and later for root primordia and roots formed as observed in *Dalbergia melanoxylon* by Amri (2010).

Other findings have reported IBA use for rooting of cuttings to be superior to NAA (Ahmad *et al*., 1998; Tchoundjeu *et al*., 2002; Kesari *et al*., 2008; Ngeno *et al*., 2013). The use of Anatone was reported by Gitonga *et al*. (2010) to be comparable to NAA in initiating the rooting of *in vitro* bananas but recommended that further research should be conducted to analyze the active ingredients of Anatone and other non conventional PGRs.

Several studies have reported varying rooting percentage depending on auxin type and concentration, humidity and temperature. Creating an atmosphere of 100% humidity contributed to high degree of rooting (Brown and Campbell, 1985). Thomson (1982) reported 30-70% rooting with 4000 mgL^{-1} of IBA under intermittent misting after every 4 minutes. IBA and NAA have been found to initiate rooting in jojoba of 56% and 26%, respectively (Arce and Jordon, 1988). A temperature of 30^0C induced rooting in jojoba within 3-5 weeks according to Benzioni (1997).

Bashir *et al*. (2001) reported 56% and 61% rooting in jojoba by using 1 500 and 4 000 mgL^{-1} of IBA, respectively in polyethylene sheet having 90-95% humidity and temperature of 15-30°C. The use of polyethylene sheet tunnel for jojoba cuttings propagation is a successful cheaper technique in lieu of costly greenhouse or mist propagation chambers (Garrity, 2004; Bashir *et al*., 2008; Amri, 2010). Very high rooting rates of cuttings treated with IBA, NAA and IAA (100 mgL^{-1} each) were 82, 80 and 76%, respectively were reported by Zhou (2002). However, young semi-lignified shoots of jojoba were used with appropriate temperature and humidity control for rooting. According to Singh *et al*. (2003), a concentration of 5 000 mgL^{-1} of IBA with an addition

of 31 mgL^{-1} boric acid was effective for rooting. Successful rooting of jojoba cuttings can be achieved by the use of different auxins but their performance varies greatly among them. The rooting ratio of semi-hardwood cuttings was increased by IBA at 1 000 mgL^{-1} and the rooting ratio of young individuals was higher than the mature ones (Cao and Gao, 2003). Work by Ozel *et al*. (2006) on rooting of soft woods reported similar findings that juvenile cuttings rooted better than from mature plants.

Other studies using nonconventional rooting hormones such as 150 mgL^{-1} of Seradix 2 which was reported by Ngeno *et al*. (2013) show significantly higher mean rooting percent in *Strychnos heeingsii* cuttings compared with NAA and IAA but was similar to IBA. Similarly, Araya (2005) also reported Seradix 2 to increase root numbers in *Athrixia phylicoides* (Bush tea) in South Africa. Seradix 2 was reported to have 0.3% IBA, NAD carrier and Thiram fungicide which could have contributed to its good rooting performance (Ngeno *et al*., 2013). Semi-hardwood cutting require 0.1-0.5% of rooting hormone (Amri, 2009). Auxins, being root promoters had no direct impact on sprouting percentage of buds in guava (Luqman *et al*., 2004) since bud sprouting is attributed to the stored carbohydrate in the cutting (Mabood *et al*., 1996; Wahab, 1999).

Early rooting in the cuttings was caused by IBA and this could have improved the performance of other root parameters as compared to NAA which requires maximum time to root. Further work by Bashir *et al*. (2008) noted that buds sprouted earlier at the highest auxin concentration sparing more time to increase length of shoot and number of leaves. A rooting powder for root development sometimes can be quite effective since it could have other additional substances compared with auxin alone. Its effectiveness varies with species, time of the year cuttings are taken and concentration of solution (Greenwood *et al*., 1980; Foster *et al*., 1984). According to Amri (2010), IBA can be applied to plant

using powder or liquid form as carrier. However, much powder at the base can sometimes stop outgrowth of the new roots. Similarly use of water to dilute IBA is more effective than alcohol since the latter can dehydrate or injure the basal stems.

Work by Hartmann *et al*. (2002) reported IBA, NAA and IAA to be reliable synthetic chemicals in promotion of rooting, whereas IAA significantly promoted shoot growth, and IBA significantly enhanced the root growth and increased survival of cuttings (Bashir *et al*., 2009). The highest level of each auxin was the most effective. Therefore, 10 000 mgL^{-1} of IBA could be applied to Jojoba cuttings for mass multiplication from a selected strain/clone/plant. El-Deen *et al*. (2014) reported studies on carob propagation and noted that IBA at 8 000 mgL^{-1} + NAA at 200 mgL^{-1} gave the highest values of the parameters (shoot length, number of lateral shoots, number of leaves, root length, number of roots and root dry weight) followed by IBA at 6 000 mgL^{-1} + NAA at 200 mgL^{-1} in both April and September seasons.

Apart from the expensive laboratory hormones used in tissue culture and rooted cuttings propagation, there are a wide variety of cheaper and effective forms of PGRs commonly sold in agro-chemical shops in major towns. These bear various brand names such as: Anatone, Roothom, Seradix, Miracle-Grow, Bonide, Hormex, Root booster, Clonex and Garden safe just to mention a few. Work by Bashir *et al*. (2008) reported effect of jojoba strain × auxin interaction to be significant for all the root parameters as well as for number of leaves, length and diameter of primary shoot. Strain x auxin concentration was also significantly different for diameter of primary root, number of leaves and shoot length. Bashir *et al*. (2007) reported significant effect by interactions of jojoba strains and growth regulator combinations on number of shoots and primary root length *in vitro*. Other studies by Owais (2010) reported that rootability of pomegranate is influenced by the interactive

effect of cutting age, IBA concentration and variety. Studies by Rogalski *et al*. (2003) reported significant interaction between genotype and IBA concentration in *Prunus* rootstocks for survival.

Significant effect between genotypes and synthetic hormones (Milstim and litosen) interaction in *Capsicum annum* for number of leaves were reported by Hasanuzzaman *et al*. (2007). However, they found that height and number of branches were not significant. Work by Kesari *et al*. (2010) stated that interaction among auxins, genotypes and month of collection had no significant effect on root induction and differentiation in *Pongamia pinnata*. Further work by Ansari (2013) and Sarrou *et al*. (2014) reported significant interaction effect between time of cutting collection, media, auxin and cutting thickness on rooting characteristics in pomegranate. Sarrou *et al*. (2014) observed that melatonin can be substituted for IBA to produce rooting. Khattab *et al*. (2014) showed significant effect on rooting due to interaction between auxin, cutting date and wounding in jojoba cuttings which was consistent with a study reported by Hegazi *et al*. (2010) on olive cultivars.

Further research by Bashir *et al*. (2013) reported significant differences between jojoba genotypes when combined with IBA. Some bacteria such as those belonging to the genus *Agrobacterium* and rhizobia release auxin and can have positive effect on rooting of cuttings (Sezai *et al*., 2003). Dodd *et al*. (2010) reported interaction between bacteria isolates and apple rootstock genotype which resulted in elongation of roots. Similar results were reported by Gosal *et al*. (2010).

2.9 Management Techniques of Jojoba in ASALs

Increasing production has made the marginal rainfall areas more vulnerable to drought. This is because more land is bare of cover due to intensive human activities and higher

livestock stocking. More trees are cut for fuel and building purposes leaving the soil exposed to soil and wind erosion. This has resulted to low fertility and less water retention ability leading to decline in crop adaptation and diversity (Rowland, 1993). Selection from the existing traditional and improved crop cultivars can lead to development of varieties that are more yield stable over a wide range of environments. Bhandari (1993) recommended the development of water harvesting techniques as well as the need to breed and select water efficient and drought resistant plants to reclaim the ASALs.

However, besides breeding, there are many agronomical measures that can be used to enable crops to produce more in ASALs. A relatively small amount of extra soil water available during a crucial growth period can have highly beneficial effects on yield (Rowland, 1993). These techniques include water harvesting, preservation of runoff, maintenance of good soil structure and surface condition, good weed control and sound husbandry. Rapid seedling establishment is an important requirement for successful crop farming in ASALs.

The development of green revolution technology was associated with use of high-inputs. However, in recent years there has been a shift to low level inputs which is in line with resource poor farmers according to DeBoef *et al*. (1996). About a quarter of the world population depends on low input agriculture. In many parts of the tropics, the physical environment, commercial infrastructure, and/or price ratio between external inputs and farm outputs do not allow the use of large quantities of purchased inputs especially agrochemicals (Wolf, 1986). ASALs have enormous potential which are little exploited and these include irrigation, honey production, reseeding with appropriate grass species, planting of drought tolerant crops and trees, indigenous knowledge and proper marketing

of livestock. A more favourable environment may be restored when harmony is re-established between nature and human activities (Goor and Barney, 1976). Failure of forest establishment in ASALs is due to poor species selection and establishment techniques. Forests in ASALs improve and protect the environmental values and provision for economic benefits hence raising the level of the local economy.

Water harvesting can be practiced in a micro-scale level using small catchments referred to as micro-catchments. These are built around an individual tree or shrub, forcing rainfall run-off from a larger than normal area to a plant, where water infiltrates into the soil and percolates to sufficient depth to keep the tree/shrub alive (NAS, 1974). Micro-catchments have been used successfully in Near east, Africa and America and especially in the Negev desert in Israel. This technique is more widely applicable than irrigation and may have useful environmental benefits as regards erosion control. It offers more hope for increasing production in ASALs for which irrigation is not a viable option due to capital limitations (Critchley and Siegert, 1991). Most tropical semi-arid regions receive 700 mm of annual rainfall with a bimodal rainfall pattern and fantastic solar radiation hence the potential for harvesting twice a year (Rockstrom, 2003). However, political upheavals and economic decline compounded by climate change has caused most of Africa to be more vulnerable to drought leading to famine even in areas which were formerly not affected. This calls for adoption of dryland farming techniques in order to counteract these threats. Human tragedy usually occurs when rainfall drops below a critical threshold. There is barely enough food to take a rural family through one year even after a good rain year in ASALs.

Rockstrom and Falkenmark (2000) showed that low crop yields are attributed to a set of management-related water deficiencies such as: short periods of water balance (high run-

off, large evaporation and drainage losses), poor soil fertility and crop management resulting in low crop water intake capacity. Securing a water source during dry spells through irrigation and/or water harvesting can be an incentive needed for investment on improved soil moisture, which in turn can result in progressively increasing yields and profits (Cooper *et al*., 1987; Rowland and Whiteman, 1993; Figueres *et al*., 2003).

On-farm trials in Arusha and Arumeru districts in Tanzania by Rockstrom (1997) reported that combined water and fertility management can almost double the maize yields as opposed to either of the two. Only 15-30% of rainfall is actually used in productive food making, while 70-85% of rainfall in water-scarce farming is lost through runoff, evaporation and drainage which mean that there is a high volume of water that can be tapped for use for productive purposes (Rockstrom, 1997). There is also a direct linear relationship between biomass production and green water flow. The latter refers to the rainfall that is available for crop growth or some may be lost through evaporation. This can also be harnessed in dams for irrigation in ASALs. On the other hand, water from lakes, rivers and aquifers which is referred to as blue water is quite vital for irrigation where available. This shows great opportunities for improving livelihoods of the rural poor and environmental condition of savannah landscapes.

According to Rowland (1993), water harvesting involves treating unproductive land in such a way as to increase runoff and to direct water into a cultivated area to increase yields and also reduce soil erosion in ASALs. Work by NAS (1974) observed the beneficial effects of 32 m^2 micro-catchments for growing pasture shrubs in Negev, Israel and reported maximum response which increased the yields of shrubs 15 times those of untreated land. Water harvesting has also been equated to irrigation since it gives similar yields. Findings of Sullivan (2002) and FAO (2005) reported yield increase of 300%

compared with yields without runoff harvesting in drylands. However, the micro catchments used were 25 m^2 and 100 m^2 which yielded 8 and 12 t ha^{-1} yr^{-1} of fodder, respectively. Mortimore *et al*. (1995) reported sustainable growth in Machakos, Kenya as a result of maximizing the efficiency of water use in an ASAL environment. Water harvesting through terracing, cut off drains and pits for trees helps to control runoff and provide water storage.

Contour ridges and semi-circular bunds are essential for water harvesting and wide spacing is recommended in ASALs. After each runoff event, the soil around the tree should be dug over to maintain an adequate infiltration rate. Semi-circular bunds offer several advantages over the trapezoidal types. For the same perimeter length, a semi-circular type gives 20% more surface storage and 17% more impounded area than trapezium. Before leveling out, a semi-circular type gives a more uniform distribution of water and requires less labour. Small circles also maximize runoff collection per unit area. Similarly, retained bunds are useful in rangeland rehabilitation especially where trees are planted on the lowest end of the impounded area (FAO, 2005).

Weber and Stoney (1986) mentioned the need to have wide spacing in marginal areas in order to have sufficient area for micro-catchment. These have established trees successfully in northern Kenya, west of Lake Turkana. Plants such as Prosopis tree species, sorghum and grasses have benefited from this technique in marginal areas (Koohafkan and Stewart, 2008). Agroforestry in ASALs can ameliorate the crop environment and also use the land more effectively. Trees can provide shelter from winds or rain, shade for vulnerable plants, benefits to soil fertility and structure and also provide fuelwood and building materials. Inter-cropping with legumes and incorporation of animal manure are necessary to combat decline in soil fertility and structure (Rowland, 1993).

Manure or compost application can improve N and P levels as well as soil structure and moisture retention leading to increased crop production for one year and influenced soil properties for several years (Mugwira, 1979; Mortimore *et al*., 1995; Eghball *et al*., 2002; Eghball *et al*., 2004). Feldman (1982) reported that early growth of transplanted jojoba seedlings in the field was significantly greater for fertilized plants in the nursery.

Benzioni and Nerd (1985) observed the increase in jojoba growth and yield due to irrigation as well as large additional effect through fertilizer application. However, the latter has limited impact on jojoba productivity although foliar application of K and Zn help the plants to tolerate drought leading to improved growth (Hussein *et al*., 2013). The low requirement for nutrients especially for mature bushes can partially be due to deep rooted nature of jojoba which enables it to bring back to the surface the leached soil nutrients through senescent leaves and prunings. Manure is necessary to provide nutrients and improve soil structure for fast growing jojoba seedlings, since the pots used in the nursery are not large enough to provide further nutrients for growth in the field considering the fast growth of roots at 2.5 cm day^{-1} according to CJP (2007).

Manure or compost application can improve N and P levels as well as soil structure and moisture retention leading to increased crop production for one year (Murwira *et al*., 1995; Nelson, 2001) and influenced soil properties for several years (Mugwira, 1979; Mortimore *et al*., 1995; Eghball *et al*., 2002; Eghball *et al*., 2004; Kihanda *et al*., 2006). Increase of OM by 1% has been reported by Sullivan (2002) to raise water storage by 120 m^3 ha^{-1}. This reduces the severity of drought and also the need for irrigation. Feldman (1982) reported that early growth of transplanted jojoba seedlings in the field was significantly greater for inorganic fertilized plants in the nursery. Benzioni and Nerd (1985) observed the increase in jojoba growth and yield due to irrigation as well as large additional effect

through NPK fertilizer application. CJP (2007) recommended application of NPK to Jojoba at a rate of 75, 37.5 and 75 kg for NPK ha^{-1} yr^{-1}, respectively, whereas Pinoyfarmer (2007) reported response of jojoba to N and Zn and but recommended little fertilization.

According to Undersander *et al.* (2009) and Hussein *et al*. (2013), fertilization has limited impact on jojoba productivity although foliar application of K and Zn help the plants to tolerate drought through accumulation of ABA (Ferriere *et al*., 1989) leading to stomatal closure. The low requirement for nutrients especially for mature bushes can partially be due to the deep rooted nature of jojoba (Osman and AboHassan, 2013). Heterogeneous nature of jojoba population contributes to lack of positive continuous response to NPK fertilizer application (Nerd and Benzioni, 1988; Al-Soqeer, 2010).

Soil surface disturbance through tillage causes moisture loss. Every soil disturbance on the top 5 cm depth causes soil moisture loss of 6 mm although this varies with texture, percent OM and amount of surface residue (Al-Kaisi *et al*., 2000). When moisture is very limited, soils tend to have massive structure and any disturbance could damage newly formed root systems. Under dry conditions, it is recommended to scrap small weeds on the surface without disturbing the soil too deeply (Al-Kaisi *et al*., 2000).

According to Ramos and Martinez-Casasnovas (2007), highly disturbed soils had lower soil moisture than low disturbed soils and accompanied by less infiltration and more sealing. After a long dry spell, highly disturbed soils dry faster. However, Schiffner (2012) stated that soil moisture storage efficiencies of 40-60% in ASALs are achieved when tillage is minimized or eliminated. Benites and Castellanos (2003) reported that minimal soil disturbance leads to increase in soil moisture resulting to increased yields if nutrients

are available. Albedo or soil spectral reflectance decreases with increase in soil organic matter (SOM), surface roughness (Matthias *et al*., 2000) and soil moisture (Vinogradov, 1983). Studies by Roxy *et al*. (2010) reported that albedo of smooth surfaces was 2 times higher that of rough surfaces. The range of albedo for dark coloured, wet rough soils was 0.05-0.15 while that of light coloured, dry and smooth surfaces was 0.35-0.4. Roots absorb more water when soil temperature increases up to a certain maximum which depends on the crop. High temperatures can also restrict water absorption. High temperatures adversely affect seedling establishment, crop growth and also microbial population development (McGarry *et al*., 2007). Increase in OM raises the water holding capacity by increasing the number of micropores and macropores resulting to less water needed for irrigation of the same crop.

In ASALs, most crops will require watering in areas with rainfall below 250 mm yr^{-1}. Jojoba plants failed to flower below 109 mm of rain yr^{-1}. In the natural range, jojoba can grow in areas with rainfall between 80-460 mm yr^{-1} (Gentry, 1958; Pinoyfarmer, 2007). However, supplementary irrigation can maximize and sustain production in jojoba where annual rainfall is less than 640 mm (Pinoyfarmer, 2007). According to El-Bassam (2010), jojoba requires 750 mm yr^{-1} for adequate growth which is critical for early stages, flowering and seeding. Securing a water source during dry spells through irrigation can be an incentive for investment on improved soil moisture, which in turn can result in progressively increasing yields and profits (Cooper *et al*., 1987; Rowland and Whiteman, 1993; Figueres *et al*., 2003).

Irrigation ensures good crop establishment, shortens time to maturity, doubles the number of roots, increases number of buds, allows more dense plantings and increases time of photosynthesis (Pinoyfarmer, 2007). Over 80% of the lateral roots of jojoba are found

within the top 1 m soil depth (Pinoyfarmer, 2007). Average annual irrigation in USA is 2 000- 4 000 litres plant^{-1} in plantations. Irrigation is applied daily in the first month and once a week in sandy soils and then once in 2-3 weeks during summer. Water for irrigation is 0.35 of evaporation and NPK fertilizers are added in the water (Gentry, 1958). In drylands, the quantity of manure applied is restricted due to burning of the crop when insufficient moisture is available at the time of application (FAO, 2004). Farmers wait until onset of rains to apply manure in ASALs. Organic carbon (OC) was 50% greater in the top 4 cm of soil of no-tillage compared with ploughing (FAO, 2004).

Incorporation of animal manure is necessary to combat decline in soil fertility and structure (Rowland, 1993; Kihanda and Gichuru, 1999) and the recommended rate is 5-10 t ha^{-1} (Kihanda, 1996). According to Andrews and Foster (2007), manures release 40-70% nutrients within 4 - 8 weeks after application. However, in drylands, the quantity of manure applied is restricted due to burning of the crop when insufficient moisture is available at the time of application (FAO, 2004). Farmers wait until onset of rains to apply manure in ASALs.

There was also accelerated decomposition of OC resulting from higher soil moisture maintained throughout the growing season. Depletion of SOM was reported by Du Preez *et al*. (2010) to be relatively higher in irrigated compared to non-irrigated in drylands. OM can increase water storage by 120 m^3 ha^{-1} for each 1% OM (Sullivan, 2002). This reduces the severity of drought and also the need for irrigation.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Location

The research was conducted at Rukinga wildlife works ltd, Maungu, Voi, where jojoba bushes have been established. It is located 20 km east of Voi urban centre, Taita Taveta county, Voi district, coast province of Kenya. It is located 140 km (near Buchuma) north west of Mombasa port along the Mombasa-Nairobi highway, at an altitude of 892 m above the sea level. It lies between latitudes 3° 23' 60" to 3° 24' 26" S and longitudes 37° 40' 60" to 38° 35' 25" E on the western side of Taru desert, south west of Tsavo east National Park and surrounded by Sagalla and Taita hills (Fig. 1) (TTDP, 2008).

3.1.2 Description

The study site lies in the semi arid savannah which receives an average annual rainfall of 458 mm with a bimodal pattern of distribution (Table 2 and Fig. 2). Long rains are received between March and may while the short rains are received between November and December. Temperatures range from 16-37°C with an average of 25°C with moderate relative humidity of 59% and annual number of rainy days being 42.7 (Table 3) (TTDP, 2008). Soils are moderately fertile with sandy loam and gravel texture and pH of 5-7 (Jaetzold and Schmidt, 1983; Thagana *et al*., 2003). Sandy loam soil was characterized by Njeru *et al*. (2011) in a semi arid environment of Mbeere south district, Kenya to comprise of 60% sand, 23% silt and 17% clay and fertility of 0.15% n, 130.5 and 410 ppm (P and K respectively) and 0.93% carbon (C).

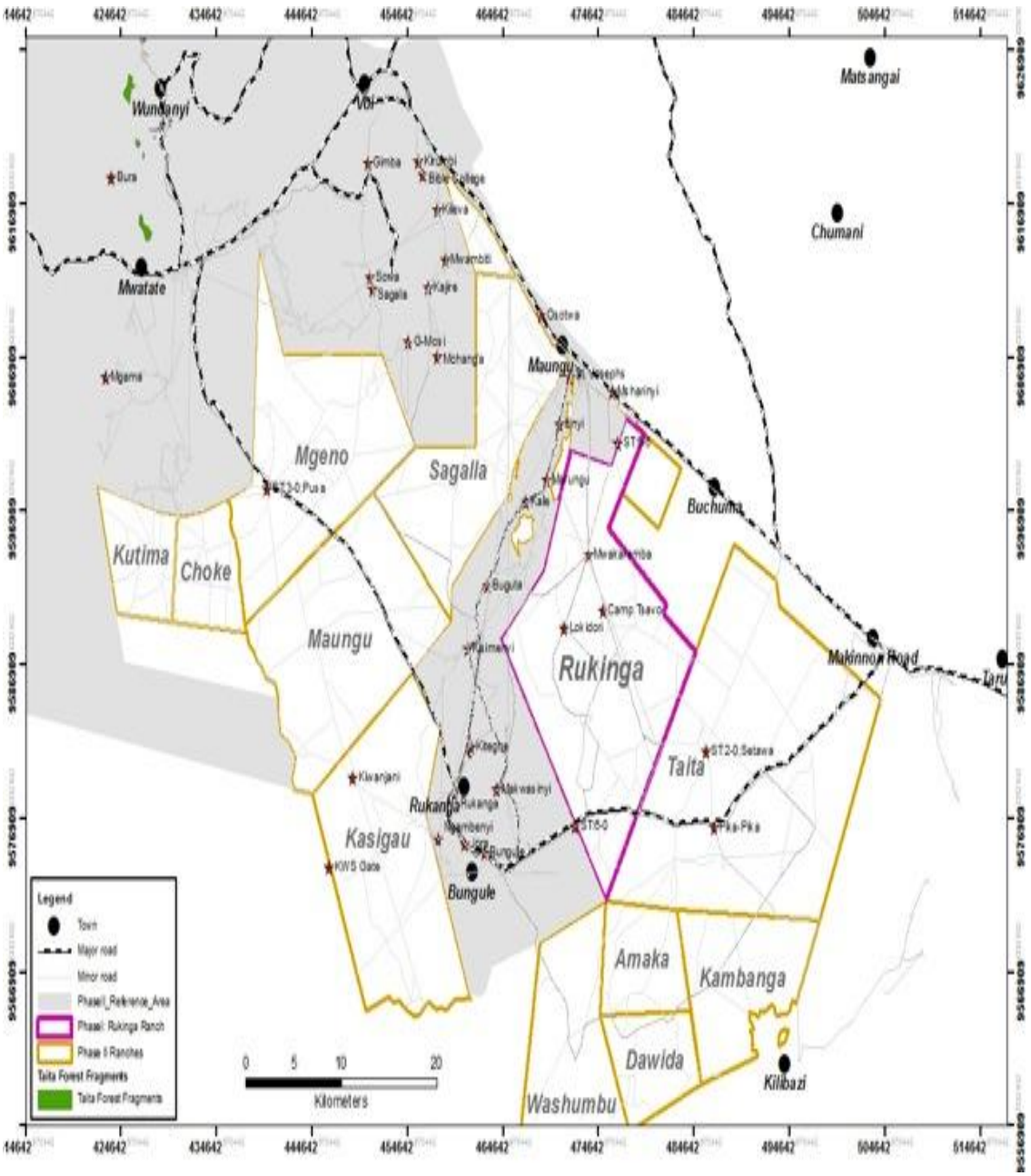

Figure 1: Map showing the location of the study site

Table 2: Rainfall data for 8 years for the study site

Year/Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
2006	0	23.5	103	37	24	6	0	10	80	33	157.5	190	663.5
2007	44	4	89	0	69.5	32	0	26	15	4	107.5	158.5	549.5
2008	48	35	89.5	62	2	22	0	0	4	26	66	16	370
2009	8	16	0	53	40	25	0	0	0	48	84.5	93	367
2010	102	0	28	112	39	5	0	0	13	20	71	26.5	416
2011	0	33	22	118	51	1	2	0	27	79	160	36	529
2012	0	5	41	11	0	0	0	33	12	13	116	69	300
2013	17	0	110	72	21	0	7	0	0	0	109.5	130.5	467
Total	**218**	**116**	**482.5**	**465**	**247**	**91**	**9**	**69**	**151**	**223**	**587.2**	**719.5**	**3662**
Mean	**27**	**14.6**	**60.3**	**58**	**30.8**	**11.4**	**1.1**	**9**	**18.9**	**28**	**109**	**89.9**	**458**

Source: Wildlife Works Station, Maungu

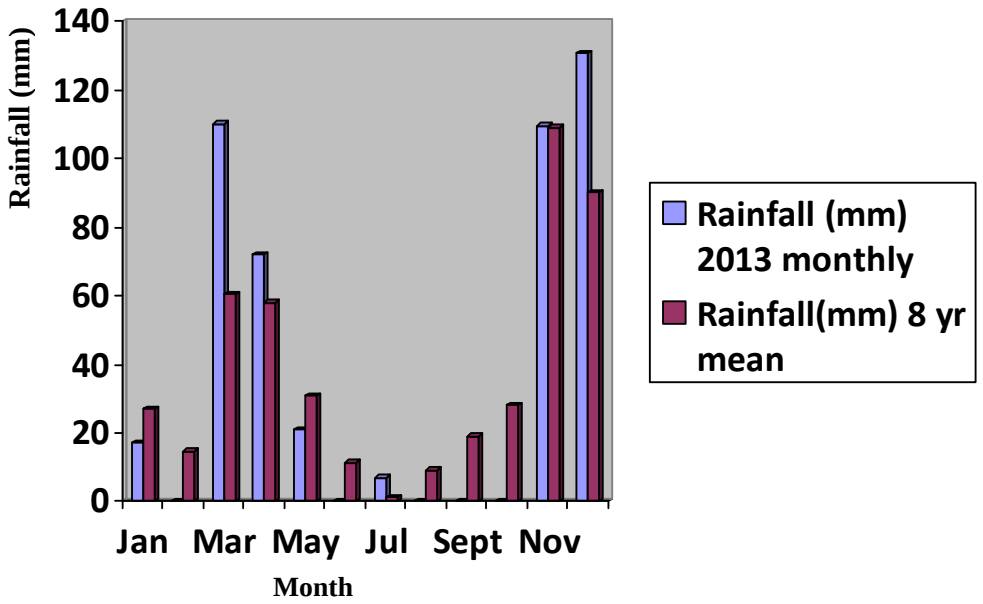

Figure 2: Monthly rainfall distribution during the study period (Year 2013) and monthly mean for 8 years

Table 3: Monthly temperature, humidity and rainy days for 8 years for the study site

Climatic parameter/ Months	Jan	Feb	Mar	Apr	May	June	Jul	Aug	Sep	Oct	Nov	Dec	Annual
No. of rainy days	2.9	2.7	6	7.1	3.1	0.7	0.3	0.7	1.1	1.9	6.6	9.6	**42.7**
Temperature (°C)	26	27	27	26	24	23	22	22	23	25	26	26	**25**
Rel. humidity (%)	60	56	58	62	63	57	57	58	57	55	60	63	**59**

Source: TTDP (2008)

Taita Taveta county covers an area of 16 975 km^2 of which the bulk (62%) is within Tsavo National Park. The remaining area is occupied by ranches, sisal estates and subsistence farming as well as water bodies such as Mzima springs which supplies Mombasa port with water. The county has a human population of 246 671 with Voi district having 54 562 people. The area has about 25 ranches making it the major land use enterprise but this also integrates wildlife and tourism (TTDP, 2008).

The vegetation is characterized by wooded savannah with scattered trees, shrubs and grasses. It supports unique forest remnants at the Taita Hill tops which form good catchment areas for water. However, the area is characterized by frequent drought leading to famine relief food programmes due to climate change, hence lack of sustainable food reserves (TTDP, 2008). The main crops grown in the area include maize, beans, green gram, cowpeas and pigeon peas. Wildlife damage is a major bottleneck for any agricultural activity in the region. However, jojoba bushes are less palatable to most of the herbivores except in times of extreme drought when there is hardly any available desirable forage. The laboratory work was conducted between June 2013 and June 2014 at the Kenya Agricultural and Livestock Research Organization (KALRO), Njoro Centre Molecular Laboratory, which is located 540 km northwest of the study site.

3.2 Methods

The research involved raising seedlings in the open nursery, cuttings in a polythene sheet tunnel and field planting of the raised seedlings. All the nursery and field planting operations were carried out between January 2012 and December 2013. Samples for laboratory analysis were collected from the mature jojoba bushes and then transported to KALRO, Njoro laboratory in June 2012. They were dried and then stored until the period of the experiment which was completed in June 2014. All these required different sets of experimental designs and sampling procedures as well as data collection and data analysis. The details of each are given in the subsections below for each specific objective.

3.2.1 Experimental designs and sampling procedures

3.2.1.1 Genetic diversity using SSR molecular marker technique

The experimental design was an 8x8 Latin Square Design with 8 treatments replicated 8 times (Gomez and Gomez, 1984) (Fig. 3).

Column/ row	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8
Row 1	Ml	Mm	Ms	Mh	Fl	Fm	Fs	Fh
Row 2	Mm	Ms	Mh	Fl	Fm	Fs	Fh	Ml
Row 3	Ms	Mh	Fl	Fm	Fs	Fh	Ml	Mm
Row 4	Mh	Fl	Fm	Fs	Fh	Ml	Mm	Ms
Row 5	Fl	Fm	Fs	Fh	Ml	Mm	Ms	Mh
Row 6	Fm	Fs	Fh	Ml	Mm	Ms	Mh	Fl
Row 7	Fs	Fh	Ml	Mm	Ms	Mh	Fl	Fm
Row 8	Fh	Ml	Mm	Ms	Mh	Fl	Fm	Fs

Figure 3: Field experimental layout representing the jojoba bushes in Voi, Kenya Key: Ml= Male large, Mm= Male medium, Ms= Male small and Mh= Male horizontal

The same order is repeated for female bushes represented by F

Statistical model for Latin square design was stated as follows:

$Y_{ijk} = \mu + \rho_i + \beta_j + \tau_k + \varepsilon_{ijk}$……..(1)

Where; μ= population mean; ρ_i = t (treatment levels); β_j = columns; τ_k = rows; ε_{ijk} = error term. Treatments = rows = columns. Each treatment is represented in each row and column.

The experiment consisted of 8 blocks where the first 4 blocks were established earlier in 1981 using materials from Israel and USA. Later in 1986, blocks 5, 6, 7 and 8 were planted using both seed and cuttings selected from the earlier established blocks. The existing mature jojoba bushes (Appendix 1) occupy an approximated area of 20 ha within Wildlife Works Ltd, Maungu, Voi. Eight jojoba bushes block^{-1} were sampled from 8 blocks consisting of different genotypes each. Stratified random sampling procedure was used to select 8 bushes, which consisted of 4 males and 4 females. Among the 4 bushes of each sex, the first was large, second medium, third small (based on height and crown diameter) while the fourth had horizontal (plagiotropic) growth habit.

Five to six fresh succulent leaves equivalent to 2 g in weight were harvested per bush and placed in polythene bags where 10 g of silica gel crystals were added to absorb the moisture before transporting to the laboratory. The trial was carried out between June 2012 and June 2014.

3.2.1.2 Identification of sex using morphological traits in jojoba seedlings and cuttings

The experimental design was a Randomized Complete Block Design (RCBD) consisting of 8 treatments replicated 3 times (Gomez and Gomez, 1984) (Fig. 4).

REP 1	REP 2	REP 3
M3 M4 F1 M1 F2 F4 M2 F3	F1 M2 M1 F4 F1 M4 F2 M3	F1 M4 M1 F2 M3 F4 F3 M2
M3 M4 F1 M1 F2 F4 M2 F3	F1 M2 M1 F4 F1 M4 F2 M3	F1 M4 M1 F2 M3 F4 F3 M2
M3 M4 F1 M1 F2 F4 M2 F3	F1 M2 M1 F4 F1 M4 F2 M3	F1 M4 M1 F2 M3 F4 F3 M2
M3 M4 F1 M1 F2 F4 M2 F3	F1 M2 M1 F4 F1 M4 F2 M3	F1 M4 M1 F2 M3 F4 F3 M2
M3 M4 F1 M1 F2 F4 M2 F3	F1 M2 M1 F4 F1 M4 F2 M3	F1 M4 M1 F2 M3 F4 F3 M2
M3 M4 F1 M1 F2 F4 M2 F3	F1 M2 M1 F4 F1 M4 F2 M3	F1 M4 M1 F2 M3 F4 F3 M2
M3 M4 F1 M1 F2 F4 M2 F3	F1 M2 M1 F4 F1 M4 F2 M3	F1 M4 M1 F2 M3 F4 F3 M2
M3 M4 F1 M1 F2 F4 M2 F3	F1 M2 M1 F4 F1 M4 F2 M3	F1 M4 M1 F2 M3 F4 F3 M2
M3 M4 F1 M1 F2 F4 M2 F3	F1 M2 M1 F4 F1 M4 F2 M3	F1 M4 M1 F2 M3 F4 F3 M2
M3 M4 F1 M1 F2 F4 M2 F3	F1 M2 M1 F4 F1 M4 F2 M3	F1 M4 M1 F2 M3 F4 F3 M2

Figure 4: Experimental layout of propagated jojoba cuttings for sex determination using morphological traits
Key: M = Male (M1, M2, M3, M4), F = Female (FI, F2, F3, F4)

Statistical model for RCBD was as follows:

$Y_{ij} = \mu + \alpha_i + \beta_j + \chi_{ij}$……………………………………………………………………(2)

Where; μ is the overall mean; α_i is the effect due to the i^{th} treatment (t)

β_j is the effect due to the j^{th} block (b); ε_{ij} is the error term (random errors)

3.2.1.2.1 Identification of sex using morphological traits in jojoba seedlings

Seedlings were raised for a period of 7 months (Plate 1). The potting media used consisted of sand and boma manure in the ratio of 2:1 respectively. The large seeds (18.4 mm length) were selected and directly sown in potted polythene bags measuring 12.5 cm width by 20 cm length. A few seedlings started flowering at 6 months due to water stress (with male to female ratio of 5:1) and this enabled selection and isolation of the males and females for the experiment. A total of 4 males and 4 females were randomly selected to constitute each replicate.

Plate 1: A fully grown jojoba seedling raised in an open nursery

3.2.1.2.2 Identification of sex using morphological traits in jojoba seedlings and cuttings

The treatments consisted of 4 males and 4 females per replicate. Stratified random sampling was used to select 4 female as well as 4 male bushes from which 40 stem cuttings were collected for each sex per replicate from the clean weeded bushes. Each treatment constituted a row of 10 potted plants per replicate. Therefore, there were 8 rows per replicate which were made up of 4 males and 4 females (Fig. 4). The treatments were independently and randomly allocated in each replicate.

For cuttings, the experiment was carried out in a polythene sheet tunnel which was 1 m wide and 50 cm high (Plate 2), while the seedlings were raised in an open nursery. Stem cuttings consisting of 5 nodes each were harvested from the middle portion of the crown using a sharp secateur sterilized by use of 70% methylated spirit. These were collected at the dormant stage according to recommendations by Benzioni (1997), 4-5 weeks after onset of rains. The cuttings were collected in the morning or late in the afternoon. After

harvesting, they were placed inside a polythene bag and misted before transporting them to the polythene sheet tunnel.

Plate 2: Polythene sheet tunnels for propagation of jojoba cuttings under *Delonix elata* tree for shade

Cuttings were propagated in the polythene sheet tunnel for a period of 5 months for rooting and sprouting. The potting media used was sterilized sand. A rooting hormone, IBA+ boric acid was used. A volume of 5 000 mgL^{-1} IBA+ 15.5 mgL^{-1} boric acid was prepared and placed in a container according to recommendations by Singh *et al*. (2003). Freshly harvested twigs were then quickly dipped in the PGR for 10 seconds and then planted immediately into the potted polythene bags. These containers were then left in the polythene sheet tunnel where humidity (80-95%), temperature (23-28°C) and watering (at 4 day interval) were regulated. Routine procedures involved watering, misting (3 times per day) and weeding. Humidity was regulated by misting and slightly opening the ends of the polythene sheet tunnel. These cuttings were raised between April 2012 and August 2012.

3.2.1.3 Determining the effect of management regimes on macro-propagation of stem cuttings

The experiment was laid down in a RCBD with 8 treatments replicated 3 times. The statistical model is as stated in 3.2.1.2. The 8 treatments were as follows: manure; micro-catchment; irrigation; manure and micro-catchment; manure and irrigation; micro-catchment and irrigation; manure, micro-catchment and irrigation; control (Fig. 5).

REP 1

M2	Control	MI+M2	I	M1+I	M1+M2+I	M1	M2+I

REP 2

M1+I	I	M1+M2+I	M1	M2+I	Control	M2	MI+M2

REP 3

I	M2	M1+I	Control	M1	MI+M2	M1+M2+I	M2+I

Figure 5: Experimental layout for the effect of management regime on macro-propagation of jojoba stem cuttings
Key: Management regimes abbreviated M1= manure, M2= micro-catchment, I= irrigation and their combinations

These treatments were applied in mature jojoba bushes for 3 years before the cuttings were harvested. Water was applied at a rate of 6 litres fortnightly bush^{-1} while manure was 2.5 kg bush^{-1} yr^{-1}. Micro catchment size was 1 m width by 3 m length. Irrigation was only applied during the dry season which occurs during the months of January to February as well as June to October. Stem cuttings were obtained from the middle portion of the crown from different management regimes of female bushes found in block 8. This block was selected for management because it had continuous rows and a more uniform stand for easier comparison. Females were chosen because of the grain yield component used in assessment for an ongoing long term trial. This experiment was carried out from early September 2012 to January 2013. A total of 12 stem cuttings were randomly harvested from each treatment per replicate, which consisted of 4 bushes each. The stem cuttings

were harvested at the dormant stage and each twig consisted of 5 nodes. The cuttings were raised in a polythene sheet tunnel for 5 months to allow rooting and sprouting. The rate of IBA used was 5000 mgL^{-1} + 15.5 boric acid which were placed in a container. Freshly harvested twigs were then quickly dipped in the PGR as in 3.2.1.2.2. These containers were then left in the polythene sheet tunnel where humidity, temperature and watering were regulated as outlined in 3.2.1.2.2.

3.2.1.4 Macro-propagation of jojoba cuttings using different Plant Growth Regulators (PGRs)

The experiment was a 4^2 factorial laid down in a RCBD with 16 treatments replicated 3 times (Gomez and Gomez, 1984) (Fig. 6). The treatments comprised of 2 factors (PGRs and genotypes) at 4 levels each.

REP 1	REP 2	REP 3
M21	F1A	F1R
M1A	M2A	F2R
F1R	F1C	M2R
F2C	M2R	M1A
F2I	M21	F1A
M1I	F2I	F1R
F1A	M2C	F1C
M2C	M1C	M2C
F1I	F1R	F2A
M2A	M1R	M21
F1C	F1R	F1I
M1R	F2R	M1C
F2A	M1A	M1I
F2R	F1I	F2I
M1C	F2A	M2A
M2R	M1I	M1R

Figure 6: Experimental layout on the effect of PGRs on macro-propagation of jojoba cuttings Key: M1- male 1 genotype, M2 - male 2 genotype, F1- female 1 genotype, F2- female 2 genotype. On the other hand, I, R, A, C refer to: IBA, Roothom, Anatone and Control, respectively

Statistical model for Factorial experiment laid down in RCBD was as follows:

Response = factor A (PGR) + factor B (Genotype) + factor A: factor B + error.

$Y_i = \mu + \alpha_j + \beta_k + \gamma_{jk} + \varepsilon_i$……..……(3)

Where; μ is the overall population mean; α_j is the mean effect of A (PGRs)

β_k is the mean effect B (Genotypes); γ_{jk} is the interactive effect of A and B; for each PGR level j and for each genotype level k; ε_i is the error term.

Model for 2-way ANOVA with interaction is given as:

$X_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \varepsilon_{ijk}$……..……(4)

Where; μ is the overall mean; α_i is the effect due to the i^{th} treatment (t)

β_j is the effect due to the j^{th} block (b); γ_{ij} is the interaction between levels i and j of factors A and B and ε_{ijk} is the error terms which are normally distributed with mean zero and unknown standard deviation.

The levels of PGRs were: IBA, Roothom, Anatone and the Control for PGRs while those of genotypes were: male 1, male 2, female 1, and female 2. Roothom and Anatone are non-conventional synthetic PGRs. Their cost is approximated to be 5 to 10 times lower compared with that of IBA. Since jojoba is dioecious and reproduces by means of out crossing, each individual plant is genetically different from each other when raised from seed, hence the basis of selection of the genotypes used in this study.

A total of 40 stem cuttings were randomly harvested from each genotype and their combination with the PGRs constituted a replicate. The treatment combinations per replicate were as follows: M1I, M1R, M1A, M1C, M2I, M2R, M2A, M2C, F1I, F1R,

F1A, F1C, F2I, F2R, F2A and F2C. The treatment combinations were independently and randomly allocated to each replicate. Each treatment consisted of 10 potted plants. This experiment was carried out from April to August 2013.

The stem cuttings were harvested and treated with IBA at a rate of 5 000 mgL^{-1} + 15.5 boric acid as outlined in 3.2.1.2.2. Roothom (with 0.6% IBA) was applied in powder form which involved dipping the basal freshly cut portion into the powder followed by planting. On the other hand, Anatone was applied at a rate of 1 000 mgL^{-1} of IBA. This was placed in a container where the freshly cut twigs were dipped for a period of 5 minutes and then planted in a polythene sheet tunnel. The cuttings were left to grow for five months (Plate 3).

Plate 3: Jojoba cuttings inside a polythene sheet tunnel

Both Roothom and Anatone are brand names with varying IBA concentration and form, and were applied according to manufacturers' recommendations for propagation of semi-hardwoods where jojoba is classified.

3.2.1.5 Management regimes of young field planted jojoba cuttings and seedlings

The experiment was a Split plot laid down in a RCBD with 2 main plots formed by propagule type namely seedlings and cuttings while subplots comprised of 8 management regimes replicated 3 times (Gomez and Gomez, 1984) (Fig. 7).

REP 3

Row 6	C	M1+M2+I	M1+M2	M2+I	Control	I	MI	M1+I	M2
Row 5	S	M1+M2+I	Control	M2+I	M1+I	I	M2	MI	M1+M2

REP 2

Row 4	S	M1+I	M1+M2+I	MI	M1+M2	M2+I	M2	Control	I
Row 3	C	M2	M1+M2+I	I	M1+I	M1+M2	MI	M2+I	Control

REP 1

Row 2	S	MI	M1+M2	M1+M2+I	Control	M1+I	M2+I	I	M2
Row 1	C	M2	M1+M2+I	M1+M2	I	Control	M1+I	M2+I	MI

Figure 7: Experimental field layout of young planted jojoba cuttings and seedlings

Key: Propagule types were cuttings (C) and seedlings (S) whereas management regimes are abbreviated by M1= manure, M2= micro-catchment, I= irrigation and their combinations

Statistical model for Split plot in RCBD was as follows:

Response = factor A*factor c + whole plot (random) + error

$Y_i = \mu + \alpha_j + V_\kappa + (\alpha V)_{j\kappa} + \gamma_i + \varepsilon_i$ ……...……(5)

Where; μ: population mean across all treatments; α_j: a (fixed) main effect of A (Propagule type); V_κ : a (fixed) main effect of C (Management regime); $(\alpha V)_{j\kappa}$: a (fixed) interaction effect of A and C; for each cutting management regime and for each seedling management regime; γ_i : represents the large plot effect, nested in cutting and seedling; ε_i : error term.

The treatments were constituted by management regimes which were as follows: manure; micro-catchment; irrigation; manure and micro-catchment; manure and irrigation; micro-catchment and irrigation; manure, micro-catchment and irrigation and control. All the treatments (management regimes) were independently and randomly allocated and each treatment consisted of 4 seedlings.

The experiment was carried out in the field at Rukinga Wildlife Works, Maungu, Voi, Kenya, between mid-January and mid-November 2013. The experimental site was 0.1 ha and it was cleared and ploughed 3 months before planting. The cleared branches mainly acacias were piled along the boundary to provide protection from wildlife damage. The seedlings from both nursery and the cuttings from the polythene sheet tunnel were raised for a period of 7 and 5 months, respectively. Field spacing was 1 × 4 m (within and between rows, respectively) according to recommendations by Yermanos (1979) and CJP (2007), while the planting holes were circular with 60 × 30 cm (depth and width, respectively). There is a wide range of hole sizes depending on the crop and site, but USAID (2006) recommended 75 - 90 cm depth × 50 cm width for jojoba in Egypt.

The micro-catchments were constructed after planting and these were rectangular in shape with a length of 3 m and width of 1 m depending on spacing, giving a surface area of 3 m^2 on a gentle slope of 8%. Microcatchments are water harvesting structures which involves

modifying the ground surface by construction of bunds in order to direct the runoff to the bottom of the plant (Mati, 2005).

Irrigation water was applied only during dry season (mid-January to mid-March and June to October) at a rate of 10 litres seedling^{-1} week^{-1} (equivalent to 320 litres seedling^{-1} yr^{-1}) according to CJP (2007) for young seedlings. Irrigation water application has been reported to range between 2 000 and 4 000 litres bush^{-1} yr^{-1} for mature Jojoba plantations (CJP, 2007). However, at planting, enough water was applied equally to all seedlings at a rate of 10 litres after 2 days and was observed for the first 2 months before the onset of rains.

On the other hand, 4 kg hole^{-1} (equivalent to 10 t ha^{-1}) of cattle boma manure was applied at planting according to recommendations by Kihanda (1996) and CJP (2007). This was thoroughly mixed with the top soil. According to Mureithi *et al*. (1994) most of the boma manures are highly variable with N level ranging from 0.46-1.98%. Nutrients from manure are released within 4 to 8 weeks after application (Andrews and Foster, 2007).

3.2.2 Data collection

3.2.2.1 Genetic diversity using SSR molecular marker technique

3.2.2.1.1 DNA extraction procedure

Cetyl trimethylammonium bromide (CTAB) protocol was used in the extraction of DNA from the leaf samples according to modified method by Charles (2001). A weight of 0.1 g of each sample was transferred to a flame sterilized mortar and 1 400 µl of pre-heated CTAB extraction buffer (Appendix 2) added and crushed using a pestle and mortar. The substance was transferred to fresh labelled micro centrifuge tubes of 1.5 mls and incubated in water bath for 10 minutes at 65°C. A volume of 700 µl chloroform: isoamylalcohol

(24:1) was added to each sample. The tubes were centrifuged at 13 000 revolutions per minute (rpm) for 10 minutes.

A volume of 500 µl of aqueous layer was transferred to micro centrifuge tube and 0.7 ml of isopropanol was added (samples stored in -20°C overnight). The tubes were centrifuged at 13 000 rpm for 15 minutes. The supernatant was discarded DNA and pellets were washed with 200 µl of alcohol (70%). The DNA pellet was air dried for 30 minutes and then re-suspended in 70 mls mineral water.

3.2.2.1.2 DNA quality assessment

Gel electrophoresis was performed. A volume of 100 ml of 1X TBE was added to 0.1 g of agarose to prepare 1% agarose (Appendix 3). This was heated in a microwave for 2 minutes to dissolve the agarose. The gel was allowed to set after which 5 µl of ethidium bromide was added into it. Agarose gel visualization under ultra-violet (UV) light was used to estimate the quantity of the DNA.

3.2.2.1.3 PCR amplification and gel analysis

Polymerase chain reaction was carried out in a reaction volume of 10 µl in small reaction tubes of 0.5 mls in a thermal cycler (Appendices 4 and 5) (make GeneAmp PCR System 9700, version 3.08). Ten SSR primers, sourced from Iqba East Africa, were used according to Ince *et al*. (2010), cited by Ince and Karaca, (2011). (Appendix 6) to amplify a target sequence from the samples. These 10 SSR primers were chosen because they are useful for diversity studies and are specific for jojoba. The set gel was placed in the buffer tank of 1X TBE and 5 µl of each sample re-suspended with 2 µl of bromophenol blue in each well. The samples were left to run for 30 minutes at 100 voltage at 400 mA. Agarose gel electrophoresis was employed for size separation whereas the size of the PCR products

was determined by comparison with a DNA ladder which ranged between 100-1000 base pairs. The DNA fragments of known sizes, run on the gel alongside the PCR products and the allele scores of each fragment were scored on a data sheet.

3.2.2.2 Identification of sex using morphological traits in nursery seedlings

Morphological data was collected from both the jojoba cuttings and seedlings according to modified jojoba descriptors procedure outlined by El-Baz *et al*. (2009) (Table 4). Three seedlings were randomly sampled for each sex treatment^{-1} and the variables scored were as outlined in El-Baz *et al*. (2009) procedure.

Table 4: Morphological descriptors for sex identification modified from El-Baz *et al.*, (2009)

Morphological character	Description
1) Height (cm)	Main shoot measured from the ground level to the tip.
2) Node density	Main shoot counted from the top to a certain length in cm. Node density = number of nodes in length A divided by length of A.
3) Shoot number	Number of shoots or sprouts per plant.
4) Shoot length (cm)	Average length of all the shoots. The results were represented as an average shoot length per seedling. Shoot length = total length of shoots divided by number of shoots.
5) Number of internodes per shoot	The number of internodes per shoot.
6) Internode length (cm) and thickness (mm)	The internode length and thickness of the second internode from the shoot base were measured and expressed in cm and mm respectively by using a ruler and a veneer caliper.
7) Number of leaves per shoot	The number of leaves per shoot was counted.
8) Single leaf area (cm^2)	A representative sample of three leaves from shoots on seedlings of each replicate was chosen from the second or third leaf from the top of the shoots. These were then traced on a graph paper and the number of squares inside the leaf were counted and computed to give the area.
9) Total leaf area per shoot (cm^2)	The estimation of leaf area was done according to the following equation: Total leaf area = single leaf area x number of leaves.
10) Leaf area/0.3 m of vegetative growth (cm^2)	It was calculated using the following equation: Leaf area /0.3 m of vegetative growth = Total leaf number multiplied by leaf area divided by shoot length which is the multiplied by 100.
11) Shape index of leaf per shoot	The shape index of leaf per shoot was calculated using the following formula and second or third leaf from the top were measured: Shape index of leaf (mm) = Leaf length (mm) divided by leaf width (mm).
12) Root number	Number of roots per plant.
13) Root length (cm)	Complete root length of the main root from the root collar to the tip.
14) Fresh shoot biomass (g)	Weight of the fresh above ground mass from the root collar to the shoot tip.
15) Fresh total plant biomass (g)	Weight of both fresh root and shoot biomass.

3.2.2.3 Determining the effect of management regimes on macro-propagation of stem cuttings

Three seedlings were randomly sampled per treatment per replicate. The variables scored from cuttings were: plant height, height of new growth, number of shoots, internode length, leaf length, leaf width, number of leaves, single leaf area, total leaf area, root collar diameter, number of roots, root length and fresh total plant biomass. However, survival and rooting percentage were calculated through complete enumeration. Survival/rooting% = Number of surviving/rooted cuttings divided by the total number of cuttings multiplied by 100.

3.2.2.4 Macro-propagation of jojoba cuttings using different PGRs

Three rooted cuttings were randomly sampled per treatment for data collection as in 3.2.2.3.

3.2.2.5 Management regimes of field planted jojoba cuttings and seedlings

Three plants were randomly sampled per treatment per replicate and data was collected over time (after intervals of 3 months) at 1, 4, 7 and 10 months of the seedling growth. The data collection interval was chosen on the basis of growth rates of the seedlings and the period of the trial. The following variables were scored: plant height, root collar diameter, number of leaves and number of shoots. Data on survival was based on complete enumeration of the surviving seedlings in relation to the initial planting.

3.2.3 Data analysis

Genetic diversity data was analysed using XLSTAT software (2009) whereas diversity data was obtained by scoring shared bands between pairs of different jojoba genotypes and only the strongest bands were considered as recommended by Wu and Tanksley (1993).

The binary code (1) was used to designate the presence and (0) for absence of a band at a specific location for each genotype. Coded data of genetic diversity was computed and arranged in similarity matrix as described by Tonukari *et al.* (1997). The PIC values were used to reveal the ability of each primer to distinguish the different jojoba genotypes and their sex which was calculated for each primer used. Data was further analyzed using DARwin5 software (2011) programme and this enabled the construction of a dendrogram through neighbour-joining method which revealed the relationship among the jojoba genotypes.

Data analysis for specific objectives 2 to 5 was carried out using One-way analysis of variance (ANOVA) model. This model was used to test differences between treatment means using Statistical Analytical System package according to SAS (1996). The significantly different treatment means were separated by F ratio using Least Significance Difference (LSD) and Duncan´s Multiple Range Test (DMRT) at $p \leq 0.05$. However, interactions were analyzed using Two-way ANOVA model inorder to determine the PGR × genotype effects in specific objective 4. There was no arc-sine data transformation performed as it was assumed that the data were normally distributed except in specific objective 5 where survival percent was transformed.

Limitations of the study: The propagation of cuttings was carried out for a short period of five months which was probably not sufficient enough for rooting and growth in slow growing genotypes. The polythene sheet tunnel used for propagation was misted manually hence difficult to maintain uniform humidity. On the other hand, the field experiment was conducted over a short period of 10 months, microcatchments were small in size and the rainfall was low and poorly distributed during the year of study.

CHAPTER FOUR

4.0 RESULTS

4.1 Genetic Diversity Using SSR Molecular Marker Technique

Ten SSR markers were used and eight were able to amplify the DNA with clear bands that could be clearly scored at 200 base pairs using a ladder of 100-1000 base pairs (Fig. 8). Among the 8 blocks, block 8 had the highest number of genotypes that were polymorphic (100%) followed by intermediate polymorphism (87.2%) in blocks 2, 3 and 5, and 75% in blocks 1 and 7.

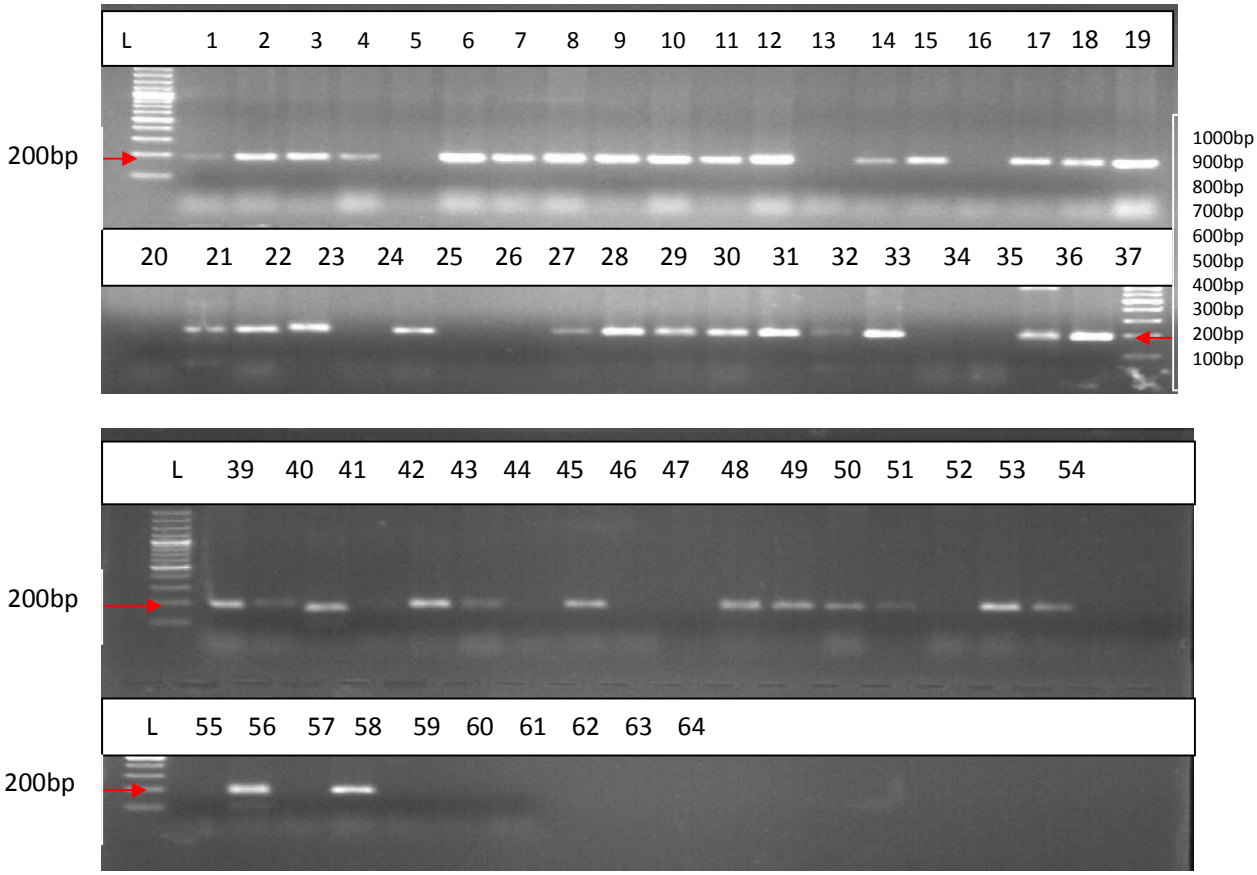

Figure 8: Polymorphic band pattern generated by JMA08 marker on jojoba genotypes. The standard ladder is 100-1000 base pairs (bp) and arrow points a polymorphic band at 200 bp

On the other hand, block 6 had the least (50%) followed by block 4 (62.5%). (Table 5).

Table 5: Morphological characteristics and genetic diversity of 64 jojoba genotypes used in the study

Sample No	Block No	Bush No	Bush sex	Ht (cm)	Crown diam (cm)	Size character	No. of loci	Genotypes showing polymorphism
7	1	1488	Male	222	310	Horizontal	4	
12	1	1501	Male	112	170	Small	7	
13	1	1495	Male	137	267	Medium	1	
15	1	1431	Female	42	68	Small	6	
45	1	1438	Female	142	222	Medium	0	
48	1	1430	Female	200	350	Large	0	
52	1	1443	Female	149	267	Horizontal	4	
60	1	1493	Male	250	315	Large	4	
Total				**1254**	**1969**		**26**	**75%**
Mean				**156.75**	**246.125**		**3.25**	
4	2	1511	Male	145	185	Medium	3	
6	2	1451	Female	196	300	Horizontal	6	
14	2	1458	Female	52	95	Small	2	
27	2	1465	Female	228	325	Large	0	
43	2	455	Male	102	122	Small	6	
46	2	1506	Male	288	335	Large	4	
59	2	1507	Male	178	340	Horizontal	6	
61	2	1459	Female	130	225	Medium	7	
Total				**1319**	**1927**		**34**	**87.5%**
Mean				**164.875**	**240.875**		**4.25**	
3	3	1517	Male	205	240	Medium	7	
17	3	1472	Female	240	243	Large	7	
30	3	1518	Male	232	282	Large	6	
33	3	1516	Male	120	150	Small	5	
38	3	1475	Female	142	137	Small	7	
47	3	1474	Female	146	230	Medium	0	
51	3	1471	Female	237	465	Horizontal	5	
56	3	1513	Male	215	322	Horizontal	1	
Total				**1537**	**2069**		**38**	**87.5%**
Mean				**192.125**	**258.625**		**4.75**	
11	4	1525	Male	335	335	Large	5	
18	4	1485	Female	194	220	Horizontal	8	
28	4	1481	Female	95	168	Small	5	
31	4	1480	Female	135	210	Medium	6	
54	4	1524	Male	165	165	Medium	6	
62	4	1515	Male	56	80	Small	0	
63	4	1484	Female	235	395	Large	0	
64	4	1520	Male	212	405	Horizontal	0	

Total				**1427**	**1978**		**30**	**62.5%**
Mean				**178.375**	**247.25**		**3.75**	
22	5	1406	Male	150	145	Medium	6	
29	5	1420	Male	150	242	Horizontal	6	
36	5	645	Female	204	280	Large	0	
39	5	710	Female	165	243	Medium	7	
42	5	1395	Male	145	157	Small	2	
44	5	1377	Male	254	317	Large	4	
50	5	709	Female	101	80	Small	3	
53	5	758	Female	160	137	Horizontal	4	
Total				**1329**	**1601**		**32**	**87.5%**
Mean				**166.125**	**200.125**		**4**	
5	6	644	Female	235	197	Large	0	
8	6	539	Female	107	102	Small	5	
20	6	1285	Male	180	213	Horizontal	1	
21	6	1199	Male	183	213	Medium	2	
26	6	504	Female	162	232	Horizontal	0	
49	6	1367	Male	253	215	Large	5	
57	6	1289	Male	93	145	Small	0	
58	6	577	Female	117	155	Medium	0	
Total				**1330**	**1472**		**13**	**50%**
Mean				**166.25**	**184**		**1.625**	
2	7	1566	Male	204	240	Large	6	
16	7	1154	Male	118	175	Small	0	
19	7	366	Female	102	173	Small	7	
32	7	466	Female	125	153	Horizontal	7	
35	7	1161	Male	188	240	Horizontal	0	
37	7	1150	Male	135	162	Medium	7	
40	7	371	Female	165	185	Medium	3	
41	7	365	Female	283	224	Large	6	
Total				**1320**	**1552**		**36**	**75%**
Mean				**165**	**194**		**4.5**	
1	8	41	Female	185	200	Horizontal	2	
9	8	811	Male	157	244	Horizontal	8	
10	8	883	Male	174	200	Medium	8	
23	8	56	Female	230	256	Large	4	
24	8	882	Male	120	140	Small	4	
25	8	141	Female	58	120	Small	4	
34	8	816	Male	223	224	Large	7	
55	8	119	Female	203	305	Medium	3	
Total				**1350**	**1689**		**40**	**100%**
Mean				**168.75**	**211.125**		**5**	

Key: Ht = Height, Crown diam = Crown diameter, Size character = Morphological crown characteristics of individual bushes

Ten SSR markers used in this study yielded 20 polymorphic bands. Eight of the SSR markers showed polymorphism while the remaining two; JMA02 and JMA05 did not show polymorphism while JMA04 showed the highest percent polymorphism (13.32) with JMA10 being the least (9.18) (Table 6). PIC and gene diversity showed a similar trend with the highest 0.3748 and 0.4995, respectively, while the least was 0.2583 and 0.3047, respectively. However, the reverse trend was showed by major allele frequency where JMA10 was the highest with 0.8125 while JMA04 was the least with 0.5156.

Table 6: Summary of gene statistics showing PIC and percent polymorphism for ten SSR markers (n = 64)

Marker	Major Allele Frequency	Allele No	Gene Diversity	PIC	% Polymorphism
JMA01	0.5313	2.0000	0.4980	0.3740	13.29
JMA02	-	-	-	-	-
JMA03	0.6094	2.0000	0.4761	0.3628	12.89
JMA04	0.5156	2.0000	0.4995	0.3748	13.32
JMA05	-	-	-	-	-
JMA06	0.5781	2.0000	0.4878	0.3688	13.11
JMA07	0.6250	2.0000	0.4688	0.3589	12.75
JMA08	0.6719	2.0000	0.4409	0.3437	12.21
JMA09	0.5469	2.0000	0.4956	0.3728	13.25
JMA10	0.8125	2.0000	0.3047	0.2583	9.18
Total	**4.8906**	**16.0000**	**3.6714**	**2.8140**	**100.0000**
Mean	**0.6113**	**2.0000**	**0.4589**	**0.3518**	**12.5000**

The mean allele number was 1.6 while the total was 16. The most informative primers were JMA01, JMA04 and JMA09 with PIC of 0.3740, 0.3748 and 0.3728, respectively. The least informative among the polymorphic primers was JMA10, with a PIC of 0.2583. The cluster dendrogram phylogenic tree (Fig. 9) clustered genotypes into 3 groups. A few genotypes did not cluster with the main groups including 20, 53 and 60 which remained distinct from the other genotypes but clustered together. The clusters were not formed by

male or female, neither by specific blocks hence not very definite. These genotypes which did not group together with others, hence can be considered distinct and most diverse, however, they constituted only 4.7% of the sampled genotypes which is negligible.

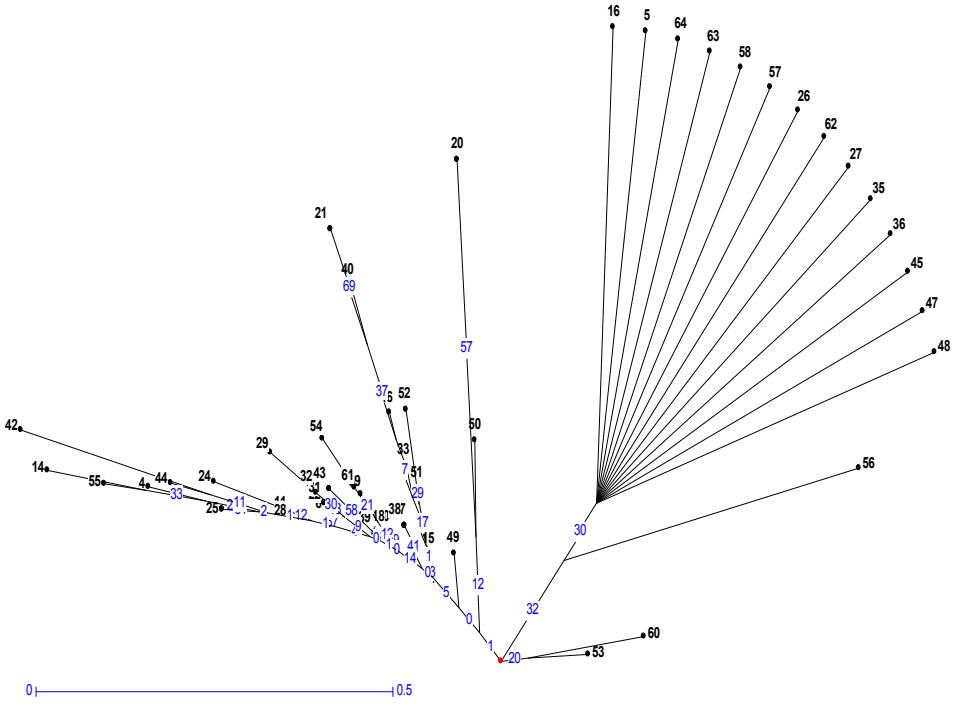

Figure 9: Cluster dendrogram showing the grouping of 64 jojoba genotypes

The results showed that the mature jojoba bushes had a low genetic diversity which was shown by PIC range of 0.2583-0.3748.

4.2 Identification of Sex Using Morphological Traits in Jojoba Seedlings and Cuttings

4.2.1 Use of morphological traits in identifying sex in jojoba seedlings

Shoot morphological characteristics did not show any significant difference for male and female sex for jojoba nursery seedlings (Table 7 and Appendix 7). However, male

seedlings showed higher growth in internode length, number of shoots and total fresh plant biomass relative to female seedlings by 16%, 10% and 1.6%, respectively. Similarly, female seedlings showed greater performance in number of internodes, node density, shoot length and height compared with male seedlings by 6.3%, 4.3%, 3.1% and 2.1%, respectively but they were not significantly different. High node density and number of internodes in females indicates stunted growth while long internodes translate to faster growth in males.

Table 7: Shoot morphological characteristics of male and female jojoba seedlings

Sex	Height (cm)	Number of shoots	Shoot length (cm)	Number of internodes	Internode length (mm)	Node density	Total fresh plant biomass (g)
Male	29.2	5.5	19.3	12.7	19.6	0.47	25.7
Female	29.8	5.0	19.9	13.5	16.9	0.49	25.3
CV	7.2	17.6	10.0	3.2	41.5	4.75	15.0
LSD	7.4	3.2	6.9	1.5	26.6	0.08	13.4
P value	0.7743	0.5979	0.7577	0.1472	0.7015	0.4639	0.9096

No significant difference among the means in each column at $p < 0.05$

Foliage morphological traits showed single leaf area for male seedlings (4.4 cm^2) to be significantly higher ($p < 0.05$) compared to the female seedlings (3.2 cm^2) (Table 8 and Appendix 7). However, all the other foliage variables were not significantly different although male seedlings were greater in leaf length, leaf width, number of leaves, total leaf area and leaf area/0.3 m relative to female seedlings by 13%, 14%, 19%, 63% and 69%, respectively. The females were superior in only leaf shape index by 1.4% compared with the males. The results show that male seedlings were superior in foliage traits relative to female seedlings except leaf shape index with single leaf area showing significant differences.

Table 8: Foliage morphological characteristics of male and female jojoba seedlings

Sex	Number of leaves	Leaf length (cm)	Leaf width (cm)	Leaf shape index	Single leaf area (cm^2)	Total leaf area (cm^2)	Leaf area/0.3 m
Male	71.3	3.4	1.6	2.17	4.4a	310.0	1627.3
Female	59.7	3.0	1.4	2.20	3.2b	190.7	965.7
CV	21.8	9.0	12.8	1.89	7.5	19.7	30.3
LSD	50.2	1.0	0.7	0.14	1.0	173.3	1377.9
P value	0.4226	0.2567	0.3206	0.4226	0.0351	0.0975	0.1748

Significant difference among the means in each column are shown by different letters at $p < 0.05$

Results indicated in Table 9 and Appendix 7 show that root morphological traits differences were not significant for male and female seedlings. However, females showed higher values compared with males in root length, root collar diameter and number of roots by 1.9%, 2% and 6.6%, respectively but the differences were not significant.

Table 9: Root morphological characteristics of male and female jojoba seedlings

Sex	Root collar diameter (mm)	Root length (cm)	Number of roots
Male	5.07	36.6	24.1
Female	5.17	37.9	25.7
CV	7.0	11.9	19.4
LSD	1.3	15.5	17.0
P value	0.8399	0.7593	0.7244

No significant difference among the means in each column

4.2.2 Use of morphological traits in identifying sex in jojoba cuttings

Male cuttings did not show any significant difference compared with the female cuttings in all the variables measured (Table 10 and Appendix 7). However, males were superior in root collar diameter, root length, height, internode length and height of new growth by 3%,

5%, 25%, 30% and 92%, respectively relative to the females, whereas, females were higher in number of roots by 14% compared with the males.

Table 10: Shoot and root morphological characteristics of jojoba cuttings

Sex	Height (cm)	Height of new growth (cm)	Internode length (mm)	Root collar diameter (mm)	Root length (cm)	Number of roots
Male	13.8	10.2	25.3	3.1	30.2	36.0
Female	11.0	5.3	19.5	3.0	28.8	41.0
CV	41.3	7.3	24.2	9.7	17.9	53.4
LSD	60.9	6.7	64.4	3.5	61.5	235.5
P value	0.7121	0.0792	0.4290	0.5000	0.5884	0.9906

No significant difference among the means in each column

Foliage traits for cuttings were not statistically significant in the entire variables measured (Table 11 and Appendix 7). However, males were higher relative to females in leaf length, leaf width, single leaf area, number of leaves and total leaf area by 37%, 43%, 95%, 155% and 458%, respectively.

Table 11: Foliage morphological characteristics of jojoba cuttings

Sex	Number of leaves	Leaf length (mm)	Leaf width (mm)	Leaf shape index	Single leaf area (cm^2)	Total leaf area (cm^2)
Male	14.0	35.6	15.0	2.4	3.7	56.4
Female	5.5	26.0	10.5	2.5	1.9	10.1
CV	32.6	2.8	11.0	12.3	8.4	11.8
LSD	40.0	10.4	16.8	3.5	2.9	51.6
P value	0.3593	0.0608	0.1780	0.6257	0.0855	0.0713

No significant difference among the means in each column

4.3 Effect of Management Regimes on Macro-Propagation of Jojoba Cuttings

4.3.1 Effect of management regimes on the survival and rooting percentages and root growth of jojoba cuttings

ANOVA results on effect of management regimes on jojoba cuttings (Table 12 and Appendix 8) showed significant differences ($p < 0.05$) among the management regimes in all the variables measured. However, there were no significant differences between the highest values relative to the control. The latter showed highest values in rooting percentage, height and root length among the variables measured while the others were shared among the management regime combination. However, micro catchment alone showed significantly the lowest values in all the variables.

Table 12: Effect of management regimes on the survival and rooting percentages and root growth of jojoba cuttings

Management regime	Survival%	Rooting%	Root collar diameter (mm)	Number of roots	Root length (cm)
Manure	66.7a	0.0d	-	-	-
Micro-catchment	60.0a	3.3cd	0.7bc	4.3c	10.7bc
Irrigation	26.7b	0.0d	-	-	-
Manure and Micro-catchment	90.0a	23.3ab	2.7a	27.5a	26.9a
Manure and Irrigation	70.0a	23.3ab	2.7a	16.8ab	29.9a
Micro-catchment and Irrigation	86.7a	13.3bcd	3.0a	23.0a	24.9ab
Manure, Micro-catchment and Irrigation	70.0a	16.7bc	1.7ab	10.7bc	19.4ab
Control	83.3a	33.3a	2.3ab	24.2a	33.1a
CV	26.3	65.1	60.2	46.9	50.1
LSD	31.9	16.2	1.7	10.9	15.9
P value	0.0183	0.0038	0.0062	0.0002	0.0018

Means with the same letter(s) in each column are not significantly different to each other at $p < 0.05$

Manure and micro catchment combination showed the highest survival (90%) which was significantly higher ($p < 0.05$) to irrigation alone (26.7%), whereas all the other regimes

were similar. Management regime combinations showed higher survival compared to single regimes although they were not significant. However irrigation, micro catchment and manure applied singly were lower by 68%, 28% and 20%, respectively relative to the control. On the other hand, the control showed the highest rooting (33.3%) which was significantly higher ($p < 0.001$) than all the other management regimes except manure and micro catchment combination (23.3%) and manure and irrigation combination (23.3%). The latter regime combinations were also significantly higher for rooting than all the single regimes namely, manure, micro catchment and irrigation.

Separate application of manure and irrigation treatments did not show any rooting hence no data is available for growth variables as indicated in Table 12. Micro catchment and irrigation combination showed the highest root collar diameter. The highest root collar diameter (3.0 mm) was significantly higher ($p < 0.01$) compared to micro catchment alone (0.7 mm). On the other hand, micro catchment alone showed significantly lower ($p < 0.01$) root collar diameter compared to combination of manure and micro catchment (2.7 mm) as well as manure and irrigation (2.7 mm).

Manure and micro catchment combination showed the highest number of roots (27.5) which were significantly higher than micro catchment alone (4.3) as well as the combination of manure, micro catchment and irrigation (10.7). Micro catchment alone showed the lowest number of roots which were significantly lower than all the irrigation combinations as well as the control. The latter was higher than micro catchment alone by 82%.

The control showed the highest root length (33.1 cm) which was significantly higher ($p < 0.01$) than micro catchment alone (10.7 cm). However, the latter was significantly lower

than manure and irrigation combination (29.9 cm) as well as manure and micro catchment combination (26.9 cm).

4.3.2 Effect of management regimes on the shoot and foliage growth of jojoba cuttings

ANOVA results in Table 13 and Appendix 8 indicate that the highest height was shown by the control (17.6 cm) which was significantly higher ($p \leq 0.001$) compared to micro catchment and irrigation combination (9.2 cm) as well as micro catchment alone (3.7 cm).

Table 13: Effect of management regimes on the shoot and foliage growth of jojoba cuttings

Management regime	Height (cm)	Height of new growth (cm)	Internode length (mm)	Total fresh plant biomass (g)	Leaf length (mm)	Leaf width (mm)	Number of leaves	Single leaf area (cm^2)	Total leaf area (cm^2)
Manure	-	-	-	-	-	-	-	-	-
Micro-catchment	3.7cd	2.7bc	10bc	0.7cd	10.3bc	5.7cd	2.3c	0.9cd	6.3cd
Irrigation	-	-	-	-	-	-	-	-	-
Manure and Micro-catchment	14.5ab	10.1a	28.3a	3.8a	34.8a	17.3a	14.7ab	3.9a	64.1a
Manure and Irrigation	13.9ab	10.7a	20.7ab	2.7ab	31.3a	13.2ab	22.0a	3.0ab	64.7a
Micro-catchment and Irrigation	9.2bc	7.7ab	21.3ab	3.3ab	27.3ab	11.3abc	11.3b	2.1bc	23.4cd
Manure, Micro-catchment and Irrigation	12.4ab	8.8a	18.0ab	2.0bc	25.5ab	8.7bc	12.2b	1.9bc	34.1abc
Control	17.6a	9.0a	26.3a	3.2ab	30.4a	12.2abc	15.2ab	2.7ab	41.2ab
CV	50	56	51.1	48	48.9	48	51.7	51.5	64
LSD	7.8	6	14	1.7	17.1	7.2	8.8	1.6	32.7
P value	0.0010	0.0045	0.0026	0.0005	0.0016	0.0011	0.0006	0.0012	0.0022

Means with the same letter(s) in each column are not significantly different to each other at $p < 0.01$

On the other hand micro catchment alone showed significantly the lowest height ($p \leq 0.001$) compared to manure and micro catchment combination, manure and irrigation combination and manure, micro catchment and irrigation combination.

Manure and irrigation combination showed significantly highest ($p < 0.01$) height of new growth (10.7 cm) compared to micro catchment alone (2.7 cm). Micro catchment alone showed significantly lower height of new growth compared to all the other management regime combinations including the control except micro catchment and irrigation combination.

Manure and micro catchment combination showed the highest internode length (28.3 mm) and total fresh plant biomass (3.8 g). However, the highest internode length was only significantly superior ($p < 0.01$) to micro catchment alone (10 mm) while the latter was also significantly lower than the control by 62%. On the other hand, the highest total fresh plant biomass was significantly higher compared to micro catchment alone (0.7 g) and the combination of manure, micro catchment and irrigation (2 g). Similarly, the lowest total fresh plant biomass was showed by micro catchment alone which was significantly lower than all the other management regime combinations including the control except manure, micro catchment and irrigation combination.

Manure and micro catchment combination showed the highest leaf length, leaf width and single leaf area compared to the other management regimes. However, the highest leaf length (34.8 mm) was only significantly greater ($p < 0.01$) than micro catchment alone (10.3 mm). On the other hand, the highest leaf width (17.3 mm) was significantly higher than micro catchment alone (5.7 mm) as well as the combination of manure, micro catchment and irrigation (8.7 mm). The highest single leaf area (3.9 cm^2) was significantly

higher than micro catchment alone (0.9 cm^2) and the combinations of micro catchment and irrigation (2.1 cm^2) as well as manure, micro catchment and irrigation (1.9 cm^2).

Manure and irrigation combination showed the highest number of leaves (22) which were significantly superior ($p < 0.001$) compared to all the other management regimes except manure and micro catchment combination (14.7) and the control (15.2). Micro catchment alone showed significantly the lowest number of leaves (2.3) compared to all the other management regimes.

Manure and irrigation combination showed the highest total leaf area (64.7 cm^2) which was significantly higher than micro catchment and irrigation combination (23.4 cm^2) as well as micro catchment alone (6.3 cm^2). The latter was also significantly lower than manure and micro catchment combination and the control by 90% and 83%, respectively. Although ANOVA results (Appendix 8) of root, shoot and foliage growth variables showed significant differences ($p < 0.01$) among the treatments, there were no significant differences between the highest values relative to the control.

4.4 Macro-Propagation of Jojoba Cuttings Using Different PGRs, Genotypes and their Interactions

4.4.1 Effect of different PGRs on the survival, rooting and growth of jojoba cuttings

Anatone showed significantly higher ($p < 0.05$) rooting percent (24.2) relative to the control (11.5) (Table 14 and Appendix 9a). Otherwise, there were no significant differences among the PGRs (IBA, Anatone and Roothom) including the control in all variables measured with the exception of rooting percent. On the other hand, Roothom had the highest number of roots (28.8) as well as the highest root collar diameter (2.3 mm)

whereas IBA showed the biggest root length value (19.9 cm). However, for root length, number of roots and root collar diameter, all the PGRs showed better performance relative to the control though not significant. Among the PGRs tested, IBA showed comparable performance with Anatone and Roothom for survival, rooting percent and root growth variables.

Table 14: Effect of different PGRs on the survival and rooting percentages and root growth of jojoba cuttings under polythene sheet tunnel

PGR type	Survival%	Rooting%	Root length (cm)	Number of roots	Root collar diameter (mm)
IBA	62.7	21.0ab	19.9	24.8	2.1
Anatone	70.0	24.2a	16.3	18.2	2.0
Roothom	62.0	14.8ab	16.2	28.8	2.3
Control	64.8	11.5b	9.8	11.8	1.5
CV	26.9	70.1	75.8	79.4	76.1
Std Dev	24.3	18.9	14.2	18.7	1.7
P value	0.6738	0.0071	0.2209	0.1890	0.6650

Means with the same letter(s) in each column are not significantly different to each other according to DMRT at p < 0.01

Results for the foliage and shoot (Table 15 and Appendix 9a) showed that out of the nine variables tested, there were no significant differences among the treatments. On the other hand, the control showed the lowest values for all the variables tested. However, IBA was highest in 5 variables namely: leaf length, leaf width, number of leaves, total leaf area and height of new growth which were higher than the control by 27%, 33%, 39%, 43% and 47%, respectively.

Roothom had the highest height, total fresh plant biomass and internode length which were higher than the control by 38.8%, 38.9% and 39.2%, respectively. Similarly, Anatone showed the highest single leaf area which was higher than the control by 37.5%.

Table 15: Effect of different PGRs on the foliage and shoot growth of jojoba cuttings under under polythene sheet tunnel

PGR type	Leaf length (mm)	Leaf width (mm)	Number of leaves	Single leaf area (cm^2)	Total leaf area (cm^2)	Height (cm)	Height of new growth (cm)	Internode length (mm)	Total fresh plant biomass (g)
IBA	23.0	9.3	8.4	2.1	28.8	8.7	6.0	15.9	1.7
Anatone	21.4	8.8	5.9	2.4	22.8	9.1	4.8	14.7	1.6
Roothom	21.6	9.2	6.7	2.3	23.8	9.8	4.6	16.8	1.8
Control	15.5	6.8	5.1	1.5	16.5	6.0	3.2	10.2	1.1
CV	68.3	65.9	90.5	66.0	93	80.5	74.3	74.2	89.3
Std Dev	18.1	7.7	6.9	2.0	26.9	7.5	4.4	12.8	1.5
P value	0.5640	0.6582	0.5034	0.4060	0.5754	0.5382	0.2964	0.4474	0.5745

No significant differences among the means in each column according to DMRT at $p < 0.05$

4.4.2 Effect of genotypes on the survival, rooting and growth of jojoba cuttings

Results on propagation of genotypes (Table 16 and Appendix 9a) showed significant differences ($p < 0.05$) among all the genotypes in survival percent, rooting percent and root growth variables. F2 genotype showed significantly the highest ($p < .0001$) survival (91.2%) compared with the other genotypes; M1, F1 and M2 which showed 59.6%, 57.6% and 51.2%, respectively.

For rooting percent, M2 genotype showed significantly higher ($p < 0.01$) percentage (37.6%) compared with M1, F2 and F1 genotypes which showed 24.2%, 7.6% and 2.2%, respectively. M1 genotype was also significantly higher than F2 and F1 genotypes. M2 genotype showed the highest root growth in all the variables observed. For root collar diameter and number of roots, M2 genotype showed significantly higher values ($p < 0.05$) compared with F1 and F2 genotypes. Similarly, for root length, M2 genotype showed significantly greater performance compared with M1, F2 and F1 genotypes. On the other hand, M1 genotype was significantly higher compared to F1 in all the measured root

growth variables. But F2 genotype was significantly higher ($p < 0.01$) only in the number of roots compared with F1. Despite M2 recording the lowest survival percent, it emerged as the best genotype for rooting percent and root growth in jojoba cuttings followed by M1, F2 and F1, respectively.

Table 16: Effect of different genotypes on the survival and rooting percentages and root growth of jojoba cuttings under polythene sheet tunnel

Genotype	Survival%	Rooting%	Root collar diameter (mm)	Number of roots	Root length (cm)
F1	57.6b	2.2c	0.7c	3.2c	5.2c
F2	91.2a	7.6c	1.7bc	16.7b	12.2bc
M1	59.6b	24.2b	2.4ab	26.3ab	17.1b
M2	51.2b	37.6a	3.1a	31.4a	27.7a
CV	26.7	70.1	76.1	79.4	75.8
Std Dev	24.3	18.9	1.7	19.7	14.2
P value	<.0001	0.0026	0.0152	0.0084	0.0040

Means with the same letter(s) in each column are not significantly different to each other according to DMRT at $p < 0.05$

Results on the genotype effect on shoot and foliage (Table 17 and Appendix 9a) showed significant differences ($p \leq 0.05$) among the genotypes in all the shoot and foliage variables. M2 genotype was significantly higher ($p < 0.05$) than M1, F2 and F1 genotypes for leaf width and single leaf area. M2 genotype also showed significantly higher performance in leaf length, number of leaves, internode length, total leaf area, total plant biomass and height of new growth compared with F2 and F1 genotypes. Also M2 genotype showed significantly higher ($p < 0.05$) height (12.1 cm) relative to F1 genotype (2.5 cm).

On the other hand, M1 genotype was also significantly higher ($p \leq 0.05$) than F2 and F1 genotypes in leaf length, leaf width, number of leaves and height of new growth while

single leaf area and total leaf area were significant higher at $p < 0.01$. M1 genotype was significantly higher than F1 genotype in height, internode length and total fresh plant biomass. M2 genotype gave the best growth followed by M1, F2 and F1 genotypes in decreasing order for root, shoot and foliage variables tested.

Table 17: Effect of different genotypes on the shoot and foliage growth of jojoba cuttings under polythene sheet tunnel

Genotype	Height (cm)	Height of new growth (cm)	Internode length (mm)	Total fresh plant biomass (g)	Leaf length (mm)	Leaf width (mm)	Number of leaves	Single leaf area (cm^2)	Total leaf area (cm^2)
F1	2.5b	1.2b	4.2c	0.5c	5.0b	2.0c	1.6b	0.5c	4.7b
F2	7.4ab	2.5b	10.3bc	1.2bc	12.3b	4.8c	4.0b	0.9c	6.6b
M1	11.5a	7.6a	18.7ab	2.0ab	27.4a	10.5b	11.2a	2.6b	39.7a
M2	12.1a	7.2a	24.3a	2.6a	36.7a	16.7a	9.5a	4.3a	40.8a
CV	80.5	74.3	74.2	89.3	68.3	65.9	90.5	66	93
Std Dev	7.5	4.4	12.8	1.5	18.1	7.7	6.9	20	26.9
P value	0.0212	0.0521	0.0171	0.0293	0.0127	0.0174	0.0492	0.0016	0.0097

Means with the same letter(s) in each column are not significantly different to each other according to DMRT at $p \leq 0.05$

4.4.3 Effect of PGR × genotype interaction on macro-propagation of jojoba cuttings

Results showed significant interaction ($p < 0.05$) between PGRs and genotypes in all the variables measured which included survival percent, rooting percent and root growth (Table 18 and Appendix 9b). The highest survival percentage was shown by Roothom × F2 interaction (93.3%) while the lowest was Roothom × M2 interaction (29.3%). The former was significantly greater ($p < 0.01$) than IBA × F1, IBA × M2, Roothom × F1, Roothom × M2, Anatone × M1, Control × F1, Control × M1 and Control × M2. On the other hand, F2 interactions gave outstanding superior performance in survival percent (87.7-93.3%) compared with the overall mean of 64.9% whereas F1 genotype × PGR

interactions showed the lowest rooting percent (0-4.3%) compared with the overall mean of 17.9%.

Table 18: Effect of interaction between PGRs and genotypes on the survival and rooting percentages and root growth of jojoba cuttings

PGR × G Interaction	Survival%	Rooting%	Root length (cm)	Number of roots	Root collar diameter (mm)
IBA × F1	46.0cd	0.0f	-	-	-
IBA × F2	91.7a	8.7def	19.2abc	27.3abcd	2.0ab
IBA × M1	63.0abc	37.7b	30.0a	43.0a	3.0a
IBA × M2	50.0cd	37.7b	30.3a	28.9abcd	3.2a
Roothom × F1	58.7bcd	0.0f	-	-	-
Roothom × F2	93.3a	13.0cdef	22.3ab	29.7abc	3.7a
Roothom × M1	66.7abc	25.3bcd	12.7abc	21.8abcd	2.2ab
Roothom × M2	29.3d	21.0bcde	29.8a	39.5ab	3.3a
Anatone × F1	71.0abc	4.3ef	12.9abc	5.0cd	1.3ab
Anatone × F2	92.0a	8.7def	7.2bc	9.7cd	1.0ab
Anatone × M1	50.0cd	21.0bcde	16.8abc	28.5abcd	2.7ab
Anatone × M2	67.0abc	62.7a	28.3ab	29.7abc	2.8a
Control × F1	54.7cd	4.3ef	7.7bc	7.7cd	1.3ab
Control × F2	87.7ab	0.0f	-	-	-
Control × M1	58.7bcd	0.7cdef	9.0abc	12.0bcd	1.8ab
Control × M2	58.3bcd	29.0bc	22.4ab	27.7abcd	3.0a
Mean	64.9	17.9	15.5	19.4	2
CV	26	56.6	72.8	77	2.2
Std Dev	24.3	18.9	14.2	18.7	1.7
P value	0.0014	<.0001	0.0073	0.0083	0.0251

Means with the same letter(s) in each column are not significantly different to each other according to DMRT at p < 0.05. G= genotype

Rooting percentage was highest for Anatone × M2 interaction (62.7%) which was significantly higher (p <.0001) to all the PGR × genotype interactions considered. Rooting did not occur in IBA × FI, Roothom × F1 and also Control × F2, hence excluded from the other growth variables. IBA × M2 interaction showed the highest root length (30.3 cm)

which was significantly higher ($p < 0.01$) than IBA × M1, Anatone × F2 and Control × F1 interactions. Similarly, IBA × M1 interaction showed the highest number of roots (43) which were significantly superior ($p < 0.01$) to Anatone × F1, Anatone × F2, Control × F1 and Control × M1 interactions. On the other hand, Roothom × F2 interaction showed the highest root collar diameter (3.7 mm) though not significant compared with the other interactions while Anatone × F2 interaction showed the lowest (1.0 mm).

All the PGR × genotype interactions were comparable in performance for survival percent, rooting percent and root growth. However, F2 interactions gave outstanding superior performance in survival percent compared with the other interactions. For root length and root to shoot ratio, IBA × M2 interaction gave the best results, while Anatone × M2 interaction also showed the best rooting percent. For number of roots and root collar diameter, IBA × M1 and Roothom x F2 interactions gave the highest values respectively.

Significant interaction ($p \leq 0.01$) was showed by PGRs × genotypes for shoot and foliage growth (Table 19 and Appendix 9b) with the exception of IBA × FI, Roothom × F1 and Control × F2 since they did not root. For all the shoot and foliage variables measured, IBA × M1 interaction showed the highest growth in leaf length, number of leaves, total leaf area, height of new growth and total plant biomass.

Leaf length for IBA × M1 interaction was significantly higher ($p < 0.01$) than Anatone × F1, Anatone × F2, Control × F1 and Control × M1 interactions. IBA × M1 interaction showed significantly higher number of leaves (21.3) relative to all the other PGR × genotype interactions. IBA × M1 interaction showed significantly greater ($p < 0.01$) total leaf area (78.5 cm^2) compared with all the other PGR × genotype interactions except the Control × M2 interaction (49.9 cm^2) which was not significant. IBA × M1 interaction

showed significantly higher height of new growth in cuttings (13.2cm) compared with all the other PGR × genotype interactions except the Anatone × M2 interaction (8.3 cm) which was not significant. IBA × M1 interaction showed significantly higher ($p < 0.01$) total plant biomass (3.7g) compared with IBA × F2, Anatone × F1, Anatone × F2, Control × F1 and Control × M1 interactions.

Table 19: Effect of PGRs and genotype interactions on the shoot and foliage growth of jojoba cuttings

PGR×G Interaction	Height (cm)	Height of new growth (cm)	Internode length (mm)	Total fresh plant biomass (g)	Leaf length (mm)	Leaf width (mm)	Number of leaves	Single leaf area (cm^2)	Total leaf area (cm^2)
IBA × F1	-	-	-	-	-	-	-	-	-
IBA × F2	7.4abc	3.5bcd	13.0abc	0.7bc	20.7abcd	7.0bcd	3.7bc	1.2cdef	3.5def
IBA × M1	16.3ab	13.2a	27.7a	3.7a	42.5a	15.5abc	21.3a	3.8ab	78.5a
IBA × M2	11.3abc	7.1bc	22.9ab	2.6ab	28.7abc	14.6abc	9.6bc	3.6abc	33.2bcdef
Roothom × F1	-	-	-	-	-	-	-	-	-
Roothom × F2	17.6a	5.4bcd	22.3ab	3.3a	23.7abcd	10.3abcd	9.7bc	2.0cdef	20.1bcdef
Roothom× M1	8.1abc	6.1bcd	17.3abc	1.5abc	25.3abc	10.2abcd	7.3bc	3.1abcd	33.8bcdef
Roothom× M2	13.6ab	6.9bc	27.3a	2.3ab	37.3ab	16.3ab	9.8bc	4.2ab	41.4bc
Anatone × F1	4.7bc	3.0bcd	8.0abc	1.0bc	11.3cd	5.7cd	3.3bc	1.3cdef	12.7cdef
Anatone × F2	4.7bc	1.1cd	6.0bc	0.7bc	5.0cd	2.0d	2.7bc	0.3ef	2.7ef
Anatone × M1	14.6ab	6.6bc	16.8abc	2.0abc	28.3abc	9.5abcd	10.0bc	2.6bcde	37.0bcde
Anatone × M2	12.3ab	8.3ab	27.8a	2.7ab	40.7a	17.9a	7.4bc	5.2a	38.7bcd
Control × F1	5.3bc	1.7cd	8.7abc	1.0bc	8.7cd	2.3d	3.0bc	0.7def	6.3cdef
Control × F2	-	-	-	-	-	-	-	-	-
Control × M1	7.3abc	4.5bcd	12.8abc	0.7bc	13.3bcd	6.7bcd	6.0bc	1.1cdef	9.6cdef
Control × M2	11.4abc	6.7bc	9.2abc	2.7ab	39.8a	18.0a	11.3b	4.2ab	49.9ab
Mean	8.4	4.6	14.4	1.5	20.3	8.5	6.6	2.1	23
CV	72.6	67.8	71.8	75.3	62.4	63.1	79.6	63.7	80
Std Dev	77.5	4.4	12.8	1.5	18.1	7.7	6.9	2	26.9
P value	0.0108	0.0006	0.0028	0.0028	0.0003	0.0002	0.0023	<.0001	0.0002

Means with the same letter(s) in each column are not significantly different to each other according to DMRT at $p \leq 0.01$. G= genotype

For leaf width, the Control × M2 interaction gave the highest width (18 mm) which was significantly larger than IBA × F2, Anatone × F1, Anatone × F2, Control × F1 and Control × M1 interactions. Single leaf area was highest for Anatone × M2 interaction (5.2 cm^2) and was significantly higher ($p < .0001$) than all the other PGR × genotype interactions except IBA × M1, IBA × M2, Roothom × M1, Roothom × M2 and Control × M2 interactions.

Height was highest for Roothom × F2 interaction (17.6 cm) which was significantly superior ($p \leq 0.01$) compared with Anatone × F1, Anatone × F2 and Control × F1 interactions. Similarly, internode length was highest for Anatone × M2 interaction (27.8 mm) which was significantly higher than Anatone × F2 interaction (6 mm).

IBA × M1 interaction showed the highest performance in most of the variables measured for shoot and foliage growth. Male genotypes especially M1 showed the best performance in shoot and foliage growth compared with the female genotypes. This was clearly demonstrated in total fresh plant biomass, leaf width, single leaf area and internode length. IBA also showed the highest performance compared with the other PGRs in the shoot and foliage growth.

4.5 Effect of Propagule Type and Management Regimes on the Early Growth of Field Planted Jojoba Cuttings and Seedlings

4.5.1 Effect of propagule type on the early growth of field planted jojoba cuttings and seedlings

The results of ANOVA for wholeplot representing the propagule type were highly significant ($p < .0001$) for most of the variables for the 10 months period (Appendix 10). However, subplot and wholeplot x subplot interaction did not show any significant

differences in most variables except in root collar diameter for subplot and survival percent for the wholeplot x subplot interactions.

All the variables measured which included survival percent, height, root collar diameter, number of leaves and shoots showed significant differences ($p < 0.05$) during the first month of growth where seedlings were higher compared with the cuttings (Figures 10a; 10b; 11; 12; 13; 14; Appendices 10; 11 and Plate 4). Seedlings showed a survival range of 91.3-97.3% (81.9-87.1% after arcsine transformation) which was significantly higher ($p < 0.01$) relative to the cuttings which ranged from 59.8-83.4% (53.3-73.7% after arcsine transformation) over 10 months period (Figures 10a and 10b).

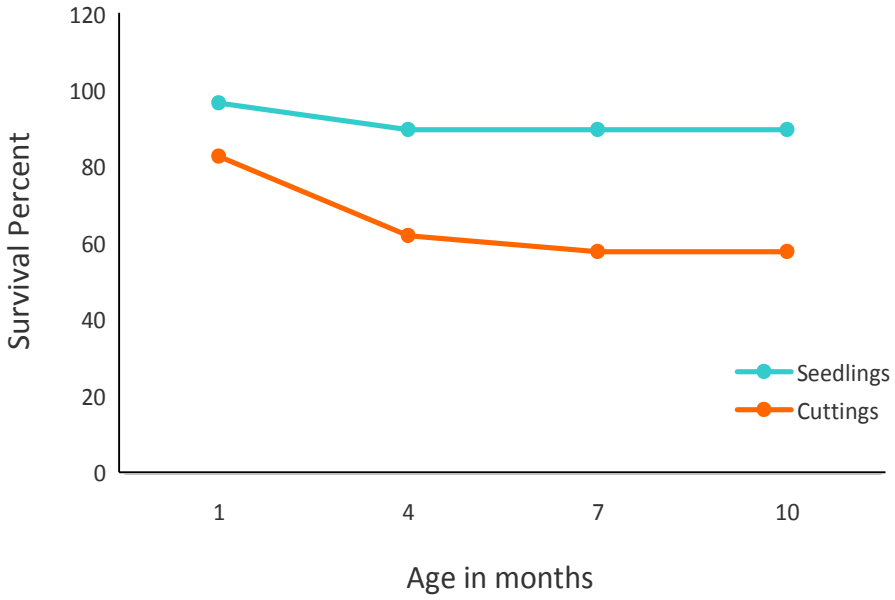

Figure 10a: Trend in survival percent (before arcsine transformation) for jojoba cuttings and seedlings over 1-10 months period

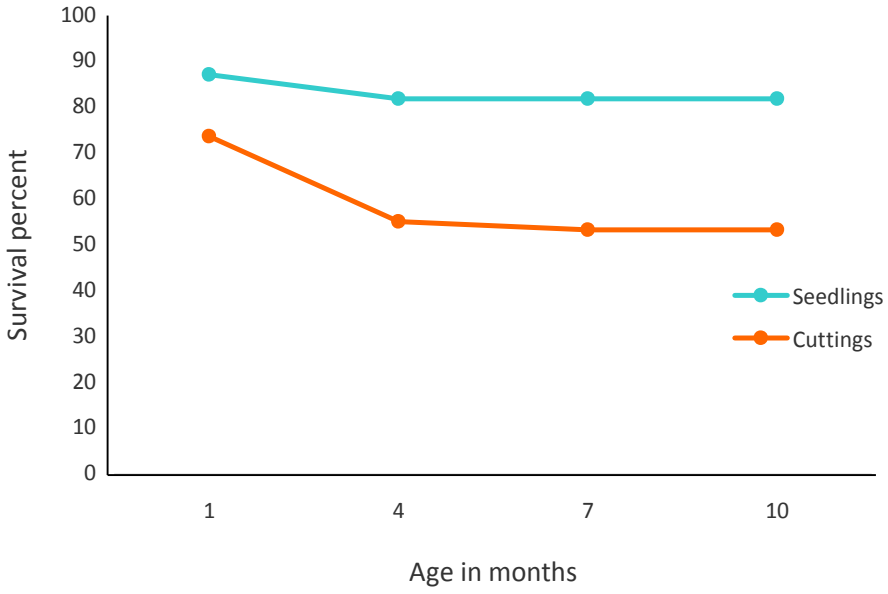

Figure 10b: Trend in survival percent (after arcsine transformation) for jojoba cuttings and seedlings over 1-10 months period

Height and root collar diameter were significantly higher ($p < .0001$) in seedlings compared with the cuttings over the 10 months period (Figures 11; 12 and Appendix 10; 11). However, height growth rate varied between cuttings and seedlings over 1, 4, 7 and 10 months by 63, 49 and 42%, respectively for cuttings and 24, 32 and 28%, for seedlings. On the other hand, root collar diameter showed a growth rate of 50, 90 and 62% for cuttings and 32, 93 and 47% for seedlings.

Plate 4: Jojoba propagules in polythene pots before transplanting. From left; male cutting, female cutting and seedling

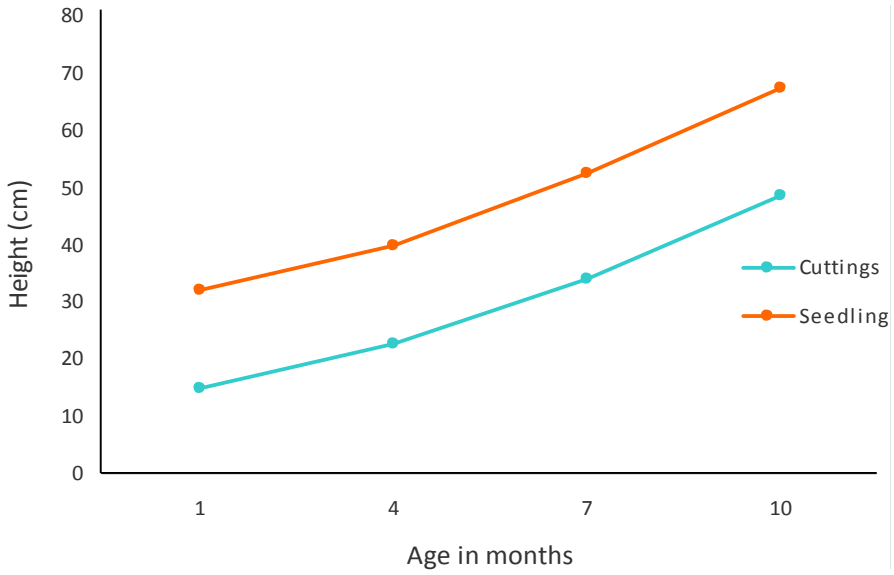

Figure 11: Trend in height growth for jojoba cuttings and seedlings over 1-10 months period

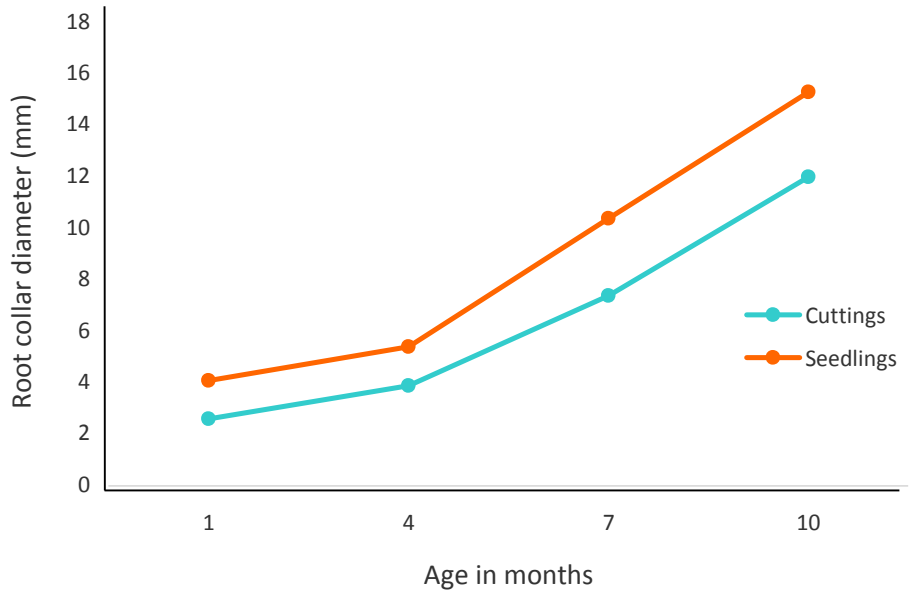

Figure 12: Trend in root collar diameter growth for jojoba cuttings and seedlings over 1-10 months period

Number of leaves in seedlings was significantly higher ($p < 0.05$) compared with the cuttings for the period of 1, 4 and 7 months but did not show any significant difference at 10 months. On the other hand, number of shoots for seedlings was significantly higher ($p < 0.05$) compared with cuttings at 1 and 4 months while 7 and 10 months were not significant (Figures 13; 14 and Appendix 10).

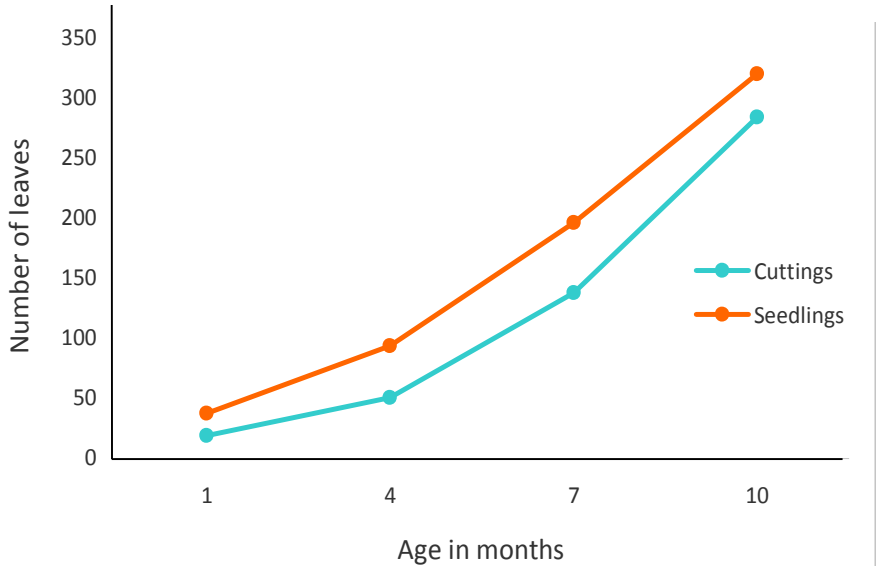

Figure 13: Trend in number of leaves for jojoba cuttings and seedlings over 1-10 months period

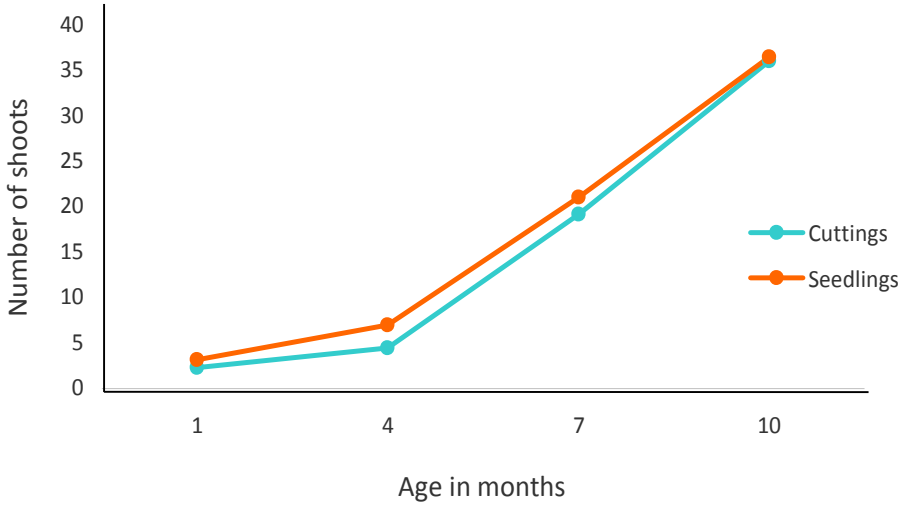

Figure 14: Trend in number of shoots for jojoba cuttings and seedlings over 1-10 months period

Cuttings showed higher growth rate in all the variables compared with the seedlings over the period of 10 months. Both root collar diameter and height growth in cuttings was 9% higher compared with the seedlings, while number of shoots and leaves were higher by 37 and 59%, respectively.

4.5.2 Effect of management regimes on the early growth of field planted jojoba cuttings and seedlings

Management regimes of 10 month old cuttings (Table 20 and Appendix 12) showed significant differences ($p < 0.05$) in height and root collar diameter. However, survival percent, number of leaves and shoots were not significant. The tallest height (55.3 cm) was shown by the control, which was significantly different ($p < 0.05$) relative to micro catchment and irrigation combination (29.8 cm).

Table 20: Effect of management regimes on the survival and growth of 10 months old jojoba cuttings

Management regime	Survival%	Height (cm)	Root collar diameter (mm)	Number of leaves	Number of shoots
Manure	44.3	41.6ab	11.0abc	314.8	27.3
Microcatchment	55.3	39.7ab	8.7bc	236.9	30.5
Irrigation	44.3	49.8ab	14.7a	310.0	51.8
Manure and Microcatchment	67.0	49.8ab	13.2abc	283.2	29.5
Manure and Irrigation	67.0	48.6ab	14.8a	339.7	43.5
Microcatchment and Irrigation	55.7	29.8b	7.5c	197.7	24.0
Manure, Microcatchment and Irrigation	78.0	49.8ab	10.6abc	247.0	30.5
Control	55.7	55.3a	12.7abc	247.0	38.2
CV	51.4	30	31.1	51.8	52
LSD	52.6	24	6.1	247	31.3
P value	0.0645	0.0445	0.0178	0.0919	0.0586

Means with the same letters in each column are not significantly different to each other using LSD at $p < 0.05$

On the other hand, manure and irrigation combination showed the thickest root collar diameter (14.8 mm) which was significantly different ($p < 0.05$) relative to micro catchment alone (8.7 mm) as well as micro catchment and irrigation combination (7.5 mm). The thickest root collar diameter was higher by 16.5% relative to the control. Micro catchment and irrigation combination showed the smallest root collar diameter which was

also significantly different in relation to irrigation alone. However, the highest survival (78%), number of leaves (339.7) and shoots (51.8) were shown by manure, micro catchment and irrigation combination, manure and irrigation combination and irrigation alone, respectively. The best management regime was shown by the manure and irrigation combination whereas the least was the micro catchment and irrigation combination. The 10 months old seedlings showed significant differences ($p < 0.05$) in height, root collar diameter and number of leaves (Table 21 and Appendix 12).

Table 21: Effect of management regimes on the survival and growth of 10 months old jojoba seedlings

Management regime	Survival%	Height (cm)	Root collar diameter (mm)	Number of leaves	Number of shoots
Manure	89.0	64.8b	15.0ab	268.9ab	30.2
Microcatchment	89.0	61.4b	12.2b	246.5b	24.0
Irrigation	100.0	62.2b	15.0ab	199.4b	29.1
Manure and Microcatchment	100.0	60.7b	14.7ab	282.8ab	33.7
Manure and Irrigation	100.0	69.9ab	16.5a	462.7a	43.3
Microcatchment and Irrigation	77.7	86.4a	15.4ab	315.7ab	47.7
Manure, Microcatchment and Irrigation	66.7	71.6ab	17.1a	328.7ab	43.7
Control	100.0	61.5b	16.2ab	385.0ab	39.0
CV	23.5	17.7	15.3	38.1	52.5
LSD	37.2	20.9	4.1	199.2	33.4
P value	0.0854	0.0245	0.0375	0.0239	0.0681

Means with the same letter(s) in each column are not significantly different to each other using LSD at $p < 0.05$

The highest height (86.4 cm) was shown by micro catchment and irrigation combination which was significantly higher than all the other management regimes with the exception of manure and irrigation combination (69.9 cm) as well as manure, micro catchment and irrigation combination (71.6 cm). On the other hand, the biggest root collar diameter (17.1 mm) was shown by manure, micro catchment and irrigation combination which were significantly higher ($p < 0.05$) than micro catchment alone (12.2 mm). The latter was also significantly lower than manure and irrigation combination (16.5 mm).

Similarly, the highest number of leaves (462.7) was shown by manure and irrigation combination which was significantly higher ($p < 0.05$) than micro catchment alone (246.5) as well as irrigation alone (199.4). The latter two treatments were also significantly lower than manure and micro catchment combination (282.8). The highest height was significantly higher by 37.6% relative to the control, whereas the highest root collar diameter, number of leaves and shoots were higher in relation to the control by 5.6%, 20.2% and 12.1%, respectively. The best growth performance was shown by manure, micro catchment and irrigation combinations whereas the lowest was micro catchment alone.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Genetic Diversity of Jojoba Using SSR Molecular Marker Technique

Genetic diversity that was mapped by the SSR markers in this study was narrow. SSR markers which were polymorphic produced a PIC range of 0.2583 and 0.3748 with an average of 0.3518 demonstrating low genetic diversity of the studied genotypes. Gene diversity was termed as the probability of two randomly chosen alleles being different from a population (Weir, 1996) while PIC was defined as the measure to calculate the discrimination power of markers (Botstein *et al*., 1980).

Similar studies on jojoba by Hosseini *et al*. (2011) and Heikrujam *et al*. (2015) reported a PIC range of 0.32-0.89 and 0.38-0.43, respectively. Other studies on papaya cultivars (Ratchadaporn *et al*., 2007) reported a PIC range of 0.35 to 0.40 and concluded that all the cultivars were genetically closely related to each other. This is indicative of the posibility of similar pedigree of the genotypes in the studied plantation. The cluster dendrogram also demonstrated closeness of the genotypes to each other and to the parentage. The observations in this study are in line with the findings by Nei (1987), who stated that to determine the nucleotide sequence of a DNA fragment is a reliable application at population and taxonomic levels and provides the ultimate solution for detecting genetic variation.

Dodds (1991) reported that transition from primitive to advanced cultivars has had the effect of narrowing the genetic base as observed in this study. Through selection over time, the average genetic constitution of the population changes (Wright, 1976). Broadening the genetic base is possible through increasing the number of superior shrubs

for selection leading to an increase in the selection intensity (Balocchi, 1990). Introduction of new genotypes should be tested over a wide range of environments since there is a high genotype by environment interaction.

Jojoba in Voi was established through seedlings and cuttings from the primary block with a few introductions, all from a similar source (Thagana *et al*., 2003). Studies on oil palm (Ting *et al*., (2010) and jojoba (Hosseini *et al*., 2011) showed high PIC range. This is expected because these crops have not been fully exploited in most areas and thus have a broad gene pool. They are also mainly established through seeds while jojoba in the present study was established through seeds and cuttings.

Futher studies using seed established stands may give divergent results. Ince and Karaca (2011) reported a PIC of 0.08-0.29 while Naek *et al*. (2011) revealed a PIC of 0.379 for *Jatropa curcas* in Brazil and concluded that few differences were found among the plant cultivars. These findings corroborate with the results of this study. However, Bhardwaj *et al*. (2010) reported average PIC of 0.16 and 0.18 using RAPD and ISSR, respectively in jojoba germplasm which were lower than the findings of the present study indicating use of clonal cuttings in jojoba propagation.

A high degree of relatedness was demonstrated by the low PIC range found in jojoba, which is the only species in Simmondsiaceae family. However, PIC more than 0.5 are efficient in discriminating genotypes (Sharma *et al*., 2009b; Ganapathy *et al*., 2012; Sharifova *et al*., 2013), a factor that was missing in this study. This corresponds with the reports that the plantation may have been mainly made of clonal cuttings which were propagated from the earlier introductions (Thagana *et al*., 2003) and that the different blocks were made for management purposes and not to separate different clones. The

present study is consistent with earlier studies by Stewart (2004) and Halkett *et al*. (2005) who reported that clonal plants generally have low genetic diversity similar to that found in self-pollinated plants.

More recent studies by Heikrujam *et al*. (2014; 2015) using CBDP markers have classified male and female jojoba separately by use of dendrograms and males showed higher PIC compared with the females. This was consistent with earlier studies by Sharma *et al*. (2009a) although contradictory to the present study, where dendrograms were not able to distinguish the sexes.

PIC in this study was low and so was gene diversity. This further confirms that the study population was relatively similar. The similarities did not classify the 64 genotypes into distinct groups, just like what was observed by Alves *et al*. (2013). The markers used may not have sampled the genomic control of observed phenotypes, as also reported by Sunil *et al*. (2011).

This study was divergent from other previous studies which showed high to moderate genetic diversity. A study on jojoba involving five primers showed highly polymorphic nature of the studied plants in the natural habitat of Egypt, an indication that they belong to different genotypes (Gaber *et al*., 2007). Al-Soqeer (2010) investigated 8 jojoba genotypes and clones in the central region of Saudi Arabia and reported high genetic variability among the Jojoba clones and this could permit improvement by selection and breeding for commercial plantation establishment. However, further investigations by Osman and AboHassan (2013) in western and northern Saudi Arabia showed moderate genetic variability among jojoba clones. The cluster analysis did not group the diffent genotypes by different blocks. This could be due to the fact that they came from

populations that may be related in other ways hence could not be picked by the variables studied. The character that led the populations to be classified into three groups using the pedigree tree need further investigation. Collard *et al*. (2005) stated that SSR markers have limited use in predicting phenotypic diversity of individuals especially in complex traits and this phenomenon was observed as a limitation in this study.

5.2 Identification of Sex Using Morphological Traits in Jojoba Seedlings and Cuttings

Literature on use of morphological traits to identify sex before flowering in plants is quite limited. There are no earlier reported studies on the use of morphological traits for sexing in young seedlings of jojoba. In the present study, both male and female seedlings and cuttings performed fairly equally in the overall growth since the male seedlings were compensated by the higher foliage growth while the female seedlings by root growth. This shows that there are no clear morphological characters that can be used to identify sex of young jojoba seedlings except the single leaf area, which was significantly higher for males relative to the females. However, a few studies have been reported by some authors to characterize other crops such as vanilla (Mantengu *et al*., 2007) and papaya (Reddy *et al*., 2012) before the flowering stage. Leaf morphology has been used to identify papaya sex at seedling stage where male leaves are 3-lobed while females are 5-lobed (Reddy *et al*., 2012). The males were slower growing compared to females in papaya.

However, several authors have reported studies in mature plants (Culley *et al*., 2005; Wheelwright *et al*., 2012; Barrett and Hough, 2013) and also genotype/clonal differentiation within similar sex (Benzioni, 1997; Tobares *et al*., 2004; El-Baz *et al*., 2009; Nderitu *et al*., 2014). Hoffman and Alliende (1984) reported different male trees/shrubs species having larger size and faster growth rate (*Fragaria chiloensis)* and

greater biomass (*Laretia acauli*, *Lithrea caustic* and *Peumus boldus)* than females. These studies are in agreement with the present study but they are based on mature plants. Studies on *Baccharis halimifolia*, a dioecious shrub as reported by Krischik and Denno (1990) showed that males possessed longer shoots and more tender leaves, grew faster, flowered and senesced earlier than female plants. The tenderness of leaves made males to be herbivore targets by beetles than females, hence resulting to a more female bias in the field.

The results of this study corroborate with other studies by Kohorn (1994) on jojoba in California who stated that females were smaller than the males. Kohorn (1995) also reported that females had larger leaves than males which are contradictory to this study. In xeric sites, males have smaller leaves and more compact canopies than females. In more mesic sites, populations of jojoba do not differ in vegetative morphology (Kohorn, 1994). Spatial segregation of sexes associated with microhabitat differences is common in dioecious tree species (Zhang *et al*., 2010).

Studies by Dawson and Bliss (1989) on *Salix arctica* in different sites, reported that males showed faster growth rate in dry site while females were faster in wet site. This may change the sex ratio in the field depending on the adaptive ability (Hultline *et al*., 2008), since males are more adapted for warmer climates (Zhao *et al*., 2012). Hence a climate change phenomenon can lead to more male bias. Dioecious plants are vulnerable to change in population size and structure, thus sensitive to habitat fragmentation through human or livestock encroachment (Yu and Lu, 2011). Male plants of perennial species are either larger, grow faster, have more ramets or have higher biomass than female plants though the growth rate could be habitat dependent (Jing and Coley, 1990). Gao *et al*. (2010) reported that climatic sensitivity in male and female trees of dioecious species is

different, yet this difference is not stable through time. Elevated $C0_2$ concentration and increased air temperature caused males to increase leaf expansion rate leading to higher biomass accumulation than females (Zhao *et al*., 2012).

However, the current study was conducted in a semi arid environment, hence this could account for some differences in the findings. Studies show that jojoba can display some level of phenotypic plasticity which is the ability to develop different phenotypes in response to environmental conditions (Winn, 1996), is heritable and plays an important role in species evolutionary strategy (Agrawal, *et al*., 2008).

Allocation differences in dioecious species have been found in species that are markedly dimorphic, such as *Simmmondsia chinensis* (Wallace and Rundell, 1979; Kohorn *et al*., 1994) as well as in *Siparuna grandiflora* that is less obviously dimorphic (Oyama and Dirzo, 1988; Nicotra, *et al*., 2003). Several studies have demonstrated that if there are no mechanisms to compensate for resource allocation to reproduction, males achieve greater growth than females (Lloyd and Webb, 1977; Popp and Reinartz, 1988; Garcia and Antor, 1995; Forero-Montana and Zimmerman, 2010; Zhao *et al*., 2012; Barrett and Hough, 2013). These findings corroborate with the results of the present study. Morphological studies on female jojoba genotypes by El-Baz *et al*. (2009) found considerable variability among all parameters studied ranging from seed, foliage to shoot. These variations observed in the morphological parameters were principally due to genotype differences.

The existence of apparent differences among multiple genotypes resulted in a wide range of phenotypes or a combination in almost all of the studied parameters. Morphological differences were not prominent in the seedlings as observed in the current study, except single leaf area. Previous studies on growth characteristics between males and females of

dioecious plants have shown that females are smaller than the males (Hoffman and Alliende, 1984; Vasiliauskas and Aarsseen, 1992) and the females grow more slowly (Jing and Coley, 1990; Cipollini and Whigham, 1994; Reddy *et al*., 2012). This study is consistent with these studies. Other studies on *Schiedea salicaria* showed evidence of sexual dimorphism where males had higher mass based photosynthetic rate and specific leaf area than females (Culley *et al*., 2005), although this is in contrast to that predicted if females have higher reproductive costs.

Work by Lambers *et al*. (1998) noted that females had thicker and denser leaves leading to lower specific leaf area, which could potentially slow their growth rate. Studies on *Silene latifolia* by Gehring and Monson (1994) and Laporte and Delph (1996) agree with this study since higher leaf size and leaf area lead to higher photosynthesis and more carbon accumulation in males than females of dioecious plants. These differences are thought to exist because females allocate more resources to reproduction than males and therefore should have fewer resources for vegetative growth (Wilson, 1983). Work by Li *et al*. (2007) on *Hippophae rhamnoides*, a deciduous shrub in south west China showed that females had a higher specific leaf area than males along an altitudinal gradient which contradicts with the current findings. Many authors found significant variability in different morphological traits of productive characteristics, such as node density, branching, leaf shape and area, seed size and weight and wax content per seed (Yermanos and Duncan, 1976; Hogan *et al*., 1980; Nagvi and Ting, 1990; Gaber *et al*., 2007; El-Baz *et al*., 2009; Inoti *et al*., 2015).

Further work by Cepedo-Cornejo and Dirzo (2010) on Neotropical palms reported that asymmetrical allocation to reproduction by females may lead to reproduction-growth tradeoff, where female plants grow less than male plants, but invest more in defense and

thus experience lower herbivory than male plants. Studies in *Corema album*, a dioecious plant in Iberian Peninsula, showed that reproductive effort was 3 times higher in females than in males or hermaphrodite plants (Zunzunegui *et al*., 2006).

Most studies of dioecious species have focused on mature individuals (Gehring, 1990) and very little work is reported on cuttings at the nursery stage. Dudley (2006) stated that comparisons of short term studies can be misleading because correlations of fitness and growth to physiological traits varies widely among plant species, environmental conditions and life stages.

Hence long term growth studies rather than short term physiological studies are recommended. The present study demonstrates sex-based differences in growth at the nursery stage where foliage morphological traits were superior for male compared to the females. However, similar work by Nicotra (1999) reported studies on *Siparuna grandiflora*, a dioecious shrub on the vegetative traits for sex-based differences in both cuttings and mature plants.

Results on pre-reproductive males showed larger leaves and leaf area than females while mature females had smaller leaves and allocated less biomass per unit stem length than males (Nicotra, 1999). These findings are in agreement with the present study where single leaf area was higher for males compared to females in seedlings. Females allocate more biomass to reproduction and the latter has negative effect on the growth of females. Females of *Ocotea tenera* compensated for higher costs of reproduction and diminished photosynthetic capacity by producing larger leaves (Wheelwright *et al*., 2012). Jing *et al*. (2008) reported that net photosynthetic rate of female ginkgo was significantly higher than males. Similarly, studies on *Rumex hastatulus*, a wind pollinated plant, provides support

for high male reproductive costs (Teitel *et al*., 2015) which is contradictory to the present study. The variation could probably be explained by the fact that the current study used a long-lived shrub which can display different growth patterns due to climatic conditions.

5.3 Effect of Management Regimes on Macro-Propagation of Jojoba Cuttings

This study did not show significant differences between the highest values relative to the control. The latter showed highest values in rooting percentage, height and root length among the variables measured while the others were shared among the other management regime combinations. However, micro catchment alone showed significantly the lowest values in most of the variables. On the other hand, irrigation alone showed significantly the lowest survival which was 68% below the control. This implies that propagation of jojoba cuttings does not need any special prior management regime on the mother plants.

The relevance of mother plant status in propagation has often received little attention (Da Costa *et al*., 2013), yet it is a key determinant in rooting propensity of cutting derived from it. Da Costa *et al*. (2013) reported that adventitious rooting in cuttings is a multifactorial response leading to new roots at the base and establishment of an autonomous plant. It occurs in two phases: a) induction, with a requirement of high auxin and occurs within 96 hours after harvesting the cutting, b) formation, inhibited by high auxin and in which anatomical changes take place. The combined increased accumulation of basipetally transported auxins from the shoot apex to the cutting base is sufficient for adventitious rooting in easy - to - root species. Hence, control of environmental variables around the stock plant is quite relevant for clonal propagation process. Despite the importance of the mother plant management to rooting of cuttings, the findings of the present study were contradictory showing that there is no need for mother plant management.

The most plausible explanation for this study on the low performance of management regimes is that application of manure alone without enough water through irrigation or rainfall could have resulted to toxicity to the mature jojoba bushes hence poor quality of the cuttings. There were isolated cases of partial and total death of the bushes with manure application, despite having been stored for over 6 months before application. This experiment was conducted in 2012 during a period of severe drought, since the rainfall received was 34% below average compared to the long term annual rainfall (458 mm) for the region (Table 2). The optimum rainfall for jojoba is between 500-600 mm (rainfall plus irrigation). However, the range of rainfall in jojoba natural habitats is between 220-400 mm, according to Undersander *et al*. (1990). Yermanos (1979) also recommended supplemental irrigation for areas where rainfall does not exceed 400 mm yr^{-1} in order to maximize production. The supplemental irrigation in the current study was 6 litres $bush^{-1}$ per fortnight which was not adequate for a drought year and this could explain the low performance of irrigation in this study.

Management techniques applied to the mother plants can exert positive effects on the rooting potential of cuttings (Loreti and Morini, 1983). The ability to root also depends on the endogenous balance of carbohydrates, auxins and rooting co-factors according to Sadhu (1989) who reported that endogenous auxin accumulation helps to initiate rooting and this varies with species and season. Season of harvesting the cuttings has been ranked higher than variation within jojoba plants in initiating rooting (Low and Hackett, 1981). However, this was not investigated by this study. Carbohydrate allocation distribution within the cutting could be more important than the content itself (Druege, 2009; Ruedell *et al*., 2013). Light and current photosynthesis of cuttings is vital in this scenario. Rooting hormone such as IBA promotes rooting during periods of high rooting potential but can have no effect or become slightly inhibitory during periods of low rooting potential (Low

and Hackett, 1981). These findings corroborate with this study since IBA was not significantly different compared with the control in rooting.

Ability to root is also affected by the physiological condition of the stock plant (Low and Hackett, 1981), cultural factors (Foster *et al*., 1984) and maturation (Ozel *et al*., 2006). The cuttings in the present study were onbtained from old bushes which were over 25 years, which were not able to root effectively, and this can be considered as a limitation in this study. Cultural practices such as pruning, fertilizing and watering during the dry season can encourage sprouting of shoots for cuttings (Longman, 1993). The presence of auxins, enzymes and phenolic compounds in the mother plant affected rooting in cuttings, whereas the physiological and biochemical quality could limit rooting in cuttings. Physiological condition is affected by environmental conditions such as light, temperature, water and nutrients (Moe and Andersen, 1988). Endogenous auxin, carbohydrate content, mineral nutrients and other biochemical components such as phenolics which act as auxin transport modulators may be affected by environmental factors (Da Costa *et al*., 2013).

The superior performance of the control, in this study, corroborates with a study by Undersander *et al*. (1990) who stated that manuring is not necessary for mature bushes since addition of N and P does not show any improvement in vegetative growth. This is also consistent with work by Benzioni and Ventura (1998) who reported that low P resulted in a decrease in Mg and Ca content in the leaves but this had no effect on shoot growth or chlorophyll concentration. On the other hand, since Jojoba can thrive under marginal nutrient soils, it is likely that the inherent soil fertility in the study site was adequate for normal growth hence no further addition of nutrients was necessary. The events leading to adventitious rooting strongly depend on the mother plant nutritional status, both in terms of minerals and carbohydrates, as well as sink establishment at the

cutting base (Da Costa *et al*., 2013). High levels of amino acid build up were observed under canopies of jojoba bushes (Burman *et al*., 2002), due to nutrient recycling making it more suitable for companion crops under parkland agroforestry system. Jojoba has a deep rooting habit which is usually about 5 times the plant height as reported by Forster and Wright (2002). This helps the plant to tap water from deep aquifers for its survival in arid environments and also to return the leached nutrients to the soil surface.

The negative effect of the micro catchments in this study can best be explained by low rainfall received during the year (300 mm) of the experiment making runoff harvesting less effective. In addition, creation of micro catchments opened the soil surface to more water loss from the soil as compared to minimal disturbance for the control. This phenomenon agrees with previous studies by Al-Kaisi *et al*. (2000). The authors showed that soil surface disturbance through tillage causes moisture loss. Every soil disturbance on the top 5 cm depth causes soil moisture loss of 6 mm although this varies with texture, percent OM and amount of surface residue (Al-Kaisi *et al*., 2000). When moisture is very limited, soils tend to have massive structure and any disturbance could damage newly formed root systems.

Under dry conditions, it is recommended to scrap small weeds on the surface without disturbing the soil too deeply (Al-Kaisi *et al*., 2000). According to Ramos and Martinez-Casasnovas (2007), highly disturbed soils had lower soil moisture than less disturbed soils and accompanied by less infiltration and more sealing. After a long dry spell, highly disturbed soils dry faster.

This study noted low performance of irrigation applied singly and this phenomenon can best be explained by the little water added through irrigation (6 litres per fortnight) which

was inadequate to sustain the water requirements of a mature jojoba bush for normal growth especially under harsh drought conditions. On average 1-2 litres of irrigation water plant^{-1} day^{-1} is recommended for jojoba (CJP, 2007). Little water application under high temperatures during drought can lead to scorching of the lateral roots at the top soil layer where 80% of the lateral roots are found.

Abscisic acid (ABA) accumulates under water stress conditions and is a known inhibitor of cell cycle progression (Wolters and Jurgens, 2009). Hence the level of water stress is a relevant factor for establishment of cuttings that should be minimized in order to avoid losses and slow establishment of plants. Husseini *et al.* (2013) reported that increasing the irrigation interval of jojoba increased the amount of proline soluble carbohydrates, chlorophyll a+b, as well as the value of succulence and osmotic potential but decreased all the growth characters. Leaves are also succulent and leathery in nature which reduces water loss. It takes about one quarter of the amount of water taken by olive plants according to Undersander *et al*. (1990).

However, manure and irrigation combination showed superior growth performance in most of the variables measured though not significant relative to the control. Manure supplies nutrients which are made available to plants through irrigation water. This phenomenon agrees with studies by (Sadhu, 1989) who observed that high carbohydrate to N ratio in stock plants favour rooting in cuttings. High accumulation of carbohydrate and starch at the rooting zone was associated with improved rooting in *Eucalyptus globulus* (Ruedell *et al*., 2013) and *Tectona grandis* cuttings (Husen and Pal, 2007).

Several authors have emphasized the importance of mineral nutrients such as P and trace elements namely, B, Zn and Mo in stimulating rooting (Weiser and Blaney, 1960;

Feldman *et al*., 1982). Feldman *et al*. (1982) further reported that jojoba leaf N and K content was positively correlated to root and shoot growth in spring and to shoot growth in summer. Studies on Peach by Blazich (1988) also reported significant effect on rooting of cuttings when mother plants were supplied with nutrients such as N, P, K and Ca. Mineral nutrition of the stock plant is important in determining rooting capacity. The auxin precursor requires Zn (Blazich, 1988; Marschner, 1995; Tromas *et al*., 2010). Peroxidases is vital in rooting and it is composed of Mn and Fe according to Fang and Kao (2000). High N supply to stock plant has been seen to promote adventitious rooting in herbaceous cuttings (Zerche and Druege, 2009). Further investigation is necessary on the effect of mineral nutrition of mother plant for jojoba propagation since the present study shows improved performance which however was not significantly different relative to the control.

5.4 Macro-Propagation of Jojoba Cuttings Using Different PGRs

5.4.1 Effect of PGRs on the survival, rooting and growth of jojoba cuttings

The tested PGRs exhibited a pattern of higher growth with Anatone showing significantly higher rooting percent compared with the control. However, IBA and Roothom were higher in all the variables relative to the control but the difference was not significant. The findings of this study are consistent with studies by Hasanuzzaman *et al*. (2007) using *Capsicum annum* in Bangladesh who reported that the Milstin and Litosen synthetic hormone treated plants exhibited a trend of higher yield compared to control plants with non-significant differences and recommended use of higher doses of hormones in future research.

Kebede *et al*. (2013) reported propagation of leafy stem cuttings of *Prunus africana* and *Syzygium guineense* in Ethiopia using both IBA and NAA at 0.0, 0.2 and 0.4%

concentrations in a non-mist poly-propagator. Humidity was 85% inside the poly-propagator while temperature ranged from 7 to 22.5°C. The results showed that the auxins had no significant effect on rooting percent of both species. However, the effect of auxin treatment on root number, root and shoot length was significant at $p < 0.01$. These findings showed the reverse trend of the present study. This could be probably be explained by differences in the concentrations of the hormones used compared to the present study.

Successful rooting of jojoba cuttings can be achieved by the use of different auxins but their performance varies greatly among them. Work by Kebede *et al*. (2013) indicated that *Prunus africana* and *Syzygium guineense* rooted successfully without hormone application in the control treatment. Successful rooting without auxin application has been reported in a number of tropical tree species such as *Nauclea* and *Vochysia* (Leakey and Coutts, 1989). These findings partially agree with the present study since IBA and Roothom were not significantly different relative to the control in rooting percent. This could be explained by the presence of inherent IAA in the plant tissues.

Rooting hormones cause a greater percentage of cuttings to root, hasten the formation of roots, induce more roots of cutting and increase root uniformity (Tchoundjeu *et al*., 2002). The results of the present study showed that the effect of IBA was similar to Anatone and Roothom in propagation of jojoba cuttings. Earlier findings have reported IBA use for rooting of cuttings to be superior to NAA (Tchoundjeu *et al*., 2002; Berl and Trigiano, 2011; Ngeno *et al*., 2013). The use of Anatone was also reported by Gitonga *et al*. (2010) to be comparable to NAA in initiating the rooting of *in vitro* bananas. The present study found that Anatone was similar to IBA in rooting of jojoba cuttings. However, Gitonga *et al*. (2010) recommended that further research should be conducted to analyze the active ingredients of Anatone and other non conventional PGRs.

Several studies have reported varying rooting percent depending on auxin type and concentration, humidity and temperature. Creating an atmosphere of 100% humidity contributed to high degree of rooting (Brown and Campbell, 1985). Thomson (1982) reported 30-70% rooting with 4000 mgL^{-1} of IBA under intermittent misting after every 4 minutes. About 56% and 61% rooting in jojoba was reported by Bashir *et al*. (2001) by using 1500 and 4000 mgL^{-1} of IBA, respectively in polyethylene sheet having 90-95% humidity and temperature of 15 to 30°C. Very high rooting rates of cuttings treated with IBA, NAA and IAA (100 mgL^{-1} each) of 82%, 80% and 76%, respectively were reported by Zhou (2002) who used young semi-lignified shoots of jojoba under appropriate temperature and humidity control. These results are higher than the ones in the present study and the most plausible explanation could be the use of young shoots which regenerate easily compared to the old bushes in the present study.

According to Singh *et al*. (2003), a concentration of 5 000 mgL^{-1} of IBA with addition of 31 mgL^{-1} boric acid was found to be effective for rooting. Similarly, 5000 mgL^{-1} of IBA with 15.5 mgL^{-1} boric acid were used in the present study with temperatures of 23 to 28°C and 80-95% humidity. The optimum temperature for rooting is between 25 and 32°C (Berl and Trigiano, 2011). All these conditions applied in this study are within the range used previously by other authors but rooting was lower. The rooting chamber environment was manually controlled in the present study which could explain the low performance in rooting and this was observed as a limitation in this study.

This study showed rooting percent which ranged from 11.5 to 24.2% with IBA (21%) and was more comparable to a recent work by Eed and Burgoyne (2015) using jojoba cuttings treated with IBA, IAA and NAA at 4000 mgL^{-1} and culturing under plastic tunnel conditions showed that IBA had the highest rooting (37.3%) in the media containing sand

and peat compared with the other PGRs. These results were considered good when no modern tools such as intermittent mist propagation system are available in some countries.

The results of this study are consistent with those of other studies using nonconventional rooting hormones such as 150 mgL^{-1} of Seradix 2 which was reported by Ngeno *et al.* (2013) to show significantly higher mean rooting percent in *Strychnos heeingsii* cuttings compared with NAA and IAA but was similar to IBA. Similarly, Araya (2005) also reported Seradix 2 to increase root numbers in *Athrixia phylicoides* (Bush tea) in South Africa. Seradix 2 was reported to have 0.3% IBA, NAD carrier and Thiram fungicide which could have contributed to its good rooting performance (Ngeno *et al*., 2013). Semi-hardwood cuttings require 0.1-0.5% of rooting hormone (Amri, 2009). However, the concentration of the Anatone used in the present study was 1 000 mgL^{-1} IBA whereas that of Roothom was 0.6% IBA.

Bashir *et al*. (2009) reported that the highest level of IBA (10 000 mgL^{-1}) was the most effective and therefore, could be applied to jojoba cuttings for mass multiplication from a selected strain/clone/plant. El-Deen *et al*. (2014) reported studies on carob propagation and noted that IBA at 8000 mgL^{-1} + NAA at 200 mgL^{-1} gave the highest values of the parameters measured followed by IBA at 6000 mgL^{-1} + NAA at 200 mgL^{-1}. Gehlot *et al.* (2014) reported 80% rooting in *Azadirachta indica* using 250 mgL^{-1} IBA with sand media. High auxin concentration has been reported to be inhibitory to root growth in several tropical tree species such as *Dalbergia sissoo* (Leakey and Coutts, 1989) and *Pingamia pinnata* (Kesari *et al*., 2010).

However, for jojoba, high concentrations have been reported to increase rooting (Bashir *et al*., 2009). Seasonal effects on ortet and rooting chamber environment affects rooting

(Marino, 1982; Bashir *et al*., 2013). Rooting environment includes: carefully controlled mist, supplemental CO_2, bottom heat and photoperiod extension (Marino, 1982; Berl and Trigiano, 2011). These environmental factors are controlled in a glass or green house under which the propagation experiments are conducted. The use of polyethylene sheet tunnel for Jojoba cuttings propagation is a successful cheaper technique compared with greenhouse or mist propagation chambers (Garrity, 2004; Bashir *et al*., 2008; Amri, 2010).

5.4.2 Effect of genotypes on the survival, rooting and growth of jojoba cuttings

Although jojoba is a difficult to root plant, propagation through cuttings is the most commonly used asexual method, but with limited success (Palzkill and Feldman, 1993). Jojoba genotypes differed significantly from each other in their effect on survival, rooting and growth variables in this study. M2 genotype gave the highest performance followed by M1, F2 and F1 genotypes, respectively in that decreasing order. Although results show high survival for F2, it will eventually die without root formation, hence the importance of rooting in propagation of cuttings (Berl and Trigiano, 2011).

Other similar results (Mohamed *et al*., 2013; Mousa and Bakhashwain, 2014) reported that jojoba genotypes/clones differed in their response to rooting and growth when cultured *in vitro*. Rogalski *et al*. (2003) reported that genotype differed significantly for survival in *Prunus* sp. which was being acclimatized. Several authors have reported that ability to root is affected by the genotype (Kesari *et al*., 2010; Hassanpour and Ali Shiri, 2014). Bashir *et al*. (2008) reported that the jojoba cuttings of PKJ-3 strain were the most responsive to the various levels of auxins, followed by those of PKJ-6. Hence, both strains are the most suitable for multiplication through cuttings. Similar findings are reported by the present study where genotype M2 was the best followed by M1 in their response to various PGRs.

Rooting of cuttings ranged from 2.2 to 37.6% in this study which was lower than the findings of other authors who had reported results of between 15 to 95% depending on clone type and the season (Greenwood *et al*., 1980 and Sadhu, 1989), but the season has been seen to have the greatest effect (Bashir *et al*., 2009). This difference could be explained by the fact that the season was not considered in this study and also genotypes were used instead of clones.

Low rooting can also be explained by the fungal and bacterial infection at the nursery stage which showed symptoms of shedding of leaves and darkening of the lower portions of the cuttings. This observation is in keeping with earlier findings by Benzioni (1997) and Cother *et al*. (2004) who reported that fungal and bacterial diseases, are common during the propagation of rooted cuttings and can lead to low survival and rooting of the cuttings if not well managed.

The ability of cuttings to form roots tends to decrease with the age of the mother plant (Paton *et al*., 1970; Zhou, 2002; Mourao *et al*., 2009; Awang *et al*., 2011) due to increase in rooting inhibitors such as essential oils and phenolic compounds (Kibbler *et al*., 2002). This could explain the low ability to root in the present study. This can be reversed by de-topping of the mature plant to encourage new sprouts near the base assuming juvenile characteristics (Sadhu, 1989). However, this management technique was not applied in the present study hence the poor rooting performance.

The time period for rooting in this study was 5 months which was probably not adequate for rooting and growth of cuttings in a polythene sheet tunnel. This could have reduced the performance of the slow growing genotypes such as F1 and F2 as observed from the study, hence a limitation in the present study. However, Zhou (2002) reported that the rooting

time for jojoba cuttings ranged from 4 to 9 months although even shorter periods between 2 weeks to 2 months under ideal conditions have been documented by Benzioni (1997). Consequently, increasing the period of rooted cuttings in the greenhouse was reported by Osburn *et al*. (2014) to increase the chances of diseases infection due to misting which weakens the cuttings.

Some of the differences noticed in this study could be attributed to differences among the genotypes, variation of the concentration of the PGRs used and the infrastructure (Polythene sheet tunnel) which was manually controlled to create some favourable environment for rooting. These observations are consistent with earlier findings by Bashir *et al*. (2008). Environmental factors such as shade and high humidity inside the polysheet can even be more important than the auxin concentration. Partial shading was provided by *Delonix elata* tree which gave 50-70% shade in the present study.

5.4.3 Effect of PGR × Genotypes interaction on the survival, rooting and growth of jojoba cuttings

Anatone × M2 interaction was the best for rooting (62.7%) and was significantly higher relative to all the interactions. All the PGR × genotype interactions showed significant differences relative to the control in all the variables measured with the exception of root collar diameter and internode length. However, there are limited studies on auxin × genotype interactions in jojoba and other semi-hardwoods in the tropics.

This study is consistent with the work by Bashir *et al*. (2008) who reported effect of jojoba strain × auxin interaction to be significant for all the root parameters as well as for number of leaves, length and diameter of primary shoot. Strain × auxin concentration was also significantly different for diameter of primary root, number of leaves and shoot length.

Similarly, Bashir *et al*. (2007) reported significant effect by interactions of jojoba strains and growth regulator combinations on number of shoots and primary root length *in vitro*.

Other studies by Owais (2010) reported that rootability of pomegranate is influenced by the interactive effect of cutting age, IBA concentration and variety. Significant interaction effect was observed in rooting percent, number of roots and weight of roots. Further work by Ansari (2013) and Sarrou *et al*. (2014) reported significant interaction effect between time of cutting collection, media, auxin and cutting thickness on rooting characteristics in pomegranate. Sarrou *et al*. (2014) observed that melatonin can be substituted for IBA to produce rooting. Studies by Rogalski *et al*. (2003) reported significant interaction effects between genotype and IBA concentration in *Prunus* rootstocks for survival which corroborates with the present study. Khattab *et al*. (2014) showed significant effect on rooting due to interaction between auxin, cutting date and wounding in jojoba cuttings which was consistent with a study reported by Hegazi *et al*. (2010) on olive cultivars. Further research by Bashir *et al*. (2013) reported significant differences between jojoba genotypes when combined with IBA which is in agreement with this study.

Hasanuzzaman *et al*. (2007) noted significant effect between genotypes and synthetic hormones (Milstim and litosen) interaction in *Capsicum annum* for number of leaves which is consistent with the findings of this study. However, they found that height and number of branches were not significant which was contradictory to the current findings. However, work by Kesari *et al*. (2010) contradicted this study by stating that interaction among auxins, genotypes and month of collection had no significant effect on root induction and differentiation in *Pongamia pinnata*. Some bacteria such as those belonging to the genus *Agrobacterium* and rhizobia release auxin and can have positive effect on rooting of cuttings (Sezai *et al*., 2003). Dodd *et al*. (2010) reported interaction between

bacteria isolates and apple rootstock genotype which resulted in elongation of roots. Similar results were reported by Gosal *et al*. (2010).

5.5 Effect of Propagule Type and Management Regimes on the Early Growth of Field Planted Jojoba Cuttings and Seedlings

5.5.1 Effect of propagule type on the early growth of field planted jojoba cuttings and seedlings

The range of survival in cuttings for the present study was 59.8-83.4% which was lower but comparable to that reported by Zhou *et al*. (2002) of 75-95%. These variations can be explained by the varying environmental conditions. According to Zhou *et al*. (2002), cuttings tend to produce more fibrous rooting system which is not able to sustain the plants under stressful conditions but can be successfully established under irrigated conditions (Palzkill, 1988).

Propagation of cuttings is unsuccessful in ASALs due to limited moisture and hostile climatic conditions (Harsh *et al*., 1987). Early differences in the propagules are attributed to variation in rooting (Foster *et al*., 1987) and genetic material (Pangou *et al*., 2011). However, other studies by Jahromi and Fard (2013) reported survival in cuttings was higher (90%) compared with seedlings (82%) which was the reverse in relation to the present study since seedlings were higher (91.7%) compared to the cuttings (59.8%) at 10 months period.

Seedlings showed higher survival in this study compared with the cuttings. This can be explained by lack of proper acclimatization in the latter which was done for a period of 2 weeks. Lee (1988) reported that insufficient acclimatization can lead to high mortality in field planted cuttings. During the first month of growth, seedlings showed significantly

higher performance compared with the cuttings. This can be explained by the short period (5 months) that the cuttings were raised in the polythene sheet tunnel compared to the 7 months taken by seedlings in the nursery coupled with enough time for acclimatization.

Field planted cuttings in the present study grew faster compared with the seedlings by 9% for root collar diameter and height and 37 and 59% in number of shoots and leaves, respectively. These findings are consistent with the work by Paul *et al*. (1993) in *Terminalia superba* and Lambeth *et al*. (1994) in *Eucalyptus grandis* who found a clear superiority of improved cuttings over unimproved seedlings in growth. Clones are expected to out-produce shrubs raised from seed by 300 to 500% (CJP, 2007) and also produce bushes which bear seed 1-2 years earlier than the seedlings which start seeding at 3-4 years (Chaturvedi and Sharma, 1989).

Similar work comparing jojoba cuttings and seedlings was reported by Jahromi and Fard (2013) in Iran where height, crown diameter and seed yield were significantly higher in cuttings compared with seedlings over a 5 year period. The findings of the present study are consistent with this study since all the variables measured which included; height, root collar diameter, number of leaves and shoots were increasing faster in cuttings compared to seedlings over the 10 months period. However, seed yield could not be obtained in the present study due to the short peiod of growth, hence a limitation in this study. Stelzer *et al*. (1998) working on loblolly pine cuttings and seedlings grown for 10 years revealed that despite initial differences in size, the relative growth rates of both propagules stabilized and were equal at the age of 7 years. At 10 years, there was virtually no difference in height, diameter, stem taper and volume between the propagules. These findings are consistent with the present study since number of leaves and shoots were not significantly different at 10 months period.

Similar results were reported by Pangou *et al*. (2011) who showed that there were no significant differences in growth between field planted *Carapa procera* cuttings and seedlings. Some studies reveal that the early differences tend to decrease over time as the trees mature (Goudet, 2002). These results are consistent with the present study where the earlier findings were higher for seedlings compared with cuttings but compensated later to be similar for number of leaves and shoots.

5.2.2 Effect of management regimes on the early growth of field planted jojoba cuttings and seedlings

Combination of management regimes which included manure and irrigation showed superior performance in jojoba plants. The results of this study are consistent with the findings of other authors who reported that manure or compost application can improve N and P levels as well as soil structure and moisture retention leading to increased crop production for one year (Murwira *et al*., 1995; Nelson, 2001) and influenced soil properties for several years (Mugwira, 1979; Mortimore *et al*., 1995; Eghball *et al*., 2002; Eghball *et al*., 2004; Kihanda *et al*., 2006). Increase of OM by 1% has been reported by Sullivan (2002) to raise water storage by 120 m^3 ha^{-1}. This reduces the severity of drought and also the need for irrigation. Manure is necessary to provide nutrients and improve soil structure for fast growing jojoba seedlings, since the pots used to raise seedlings were not large enough to provide further nutrients for growth in the field considering the fast growth of roots at 2.5 cm day^{-1} according to CJP (2007).

Feldman (1982) reported that early growth of transplanted jojoba seedlings in the field was significantly greater for inorganic fertilized plants in the nursery. Benzioni and Nerd (1985) observed the increase in jojoba growth and yield due to irrigation as well as large additional effect through NPK fertilizer application, although FYM was used in the

present study to provide the nutrients. CJP (2007) recommended application of NPK to Jojoba at a rate of 75, 37.5 and 75 kg for NPK ha^{-1} yr^{-1}, respectively, whereas Pinoyfarmer (2007) reported response of jojoba to N and Zn and but recommends little fertilization. According to Undersander *et al.* (2009) and Hussein *et al.* (2013), fertilization has limited impact on jojoba productivity although foliar application of K and Zn help the plants to tolerate drought through accumulation of ABA (Ferriere *et al*., 1989) leading to stomatal closure. The low requirement for nutrients especially for mature bushes can partially be due to the deep rooted nature of jojoba (Osman and AboHassan, 2013). Heterogeneous nature of jojoba population might have contributed to the lack of positive continuous response to NPK fertilizer application as earlier observed by Nerd and Benzioni (1988) and Al-Soqeer (2010) and as observed in the present study.

The low performance in growth of jojoba seedlings through use of micro catchment alone in the present study can be due to inadequate infiltration rate due to surface sealing and failure to dig out the soil around the tree after each runoff event (Critchley and Siegert, 1991). McGarry *et al.* (2007) observed that tillage leads to pulverization of soil particles while soil surface disturbance through tillage causes moisture loss (Al-Kaisi *et al*., 2000). However, Schiffner (2012) stated that soil moisture storage efficiencies of 40-60% in ASALs are achieved when tillage is minimized or eliminated. Benites and Castellanos (2003) reported that minimal soil disturbance leads to increase in soil moisture resulting to increased yields if nutrients are available.

Albedo or soil spectral reflectance decreases with increase in SOM, surface roughness (Matthias *et al*., 2000) and soil moisture (Vinogradov, 1983). Roots absorb more water when soil temperature increases up to a certain maximum which depends on the crop. High temperatures can also restrict water absorption leading to adverse effect on seedling

establishment, crop growth and also microbial population development (McGarry *et al*., 2007). Vegetative growth in jojoba varied with drought stress (Osman and AboHassam, 1998). Increase in OM improves the water holding capacity by increasing the number of micropores and macropores resulting to less water needed for irrigation of the same crop.

In ASALs, most crops will require watering in areas with rainfall below 250 mm yr^{-1}. Jojoba plants failed to flower below 109 mm of rain yr^{-1}. According to El-Bassam (2010), jojoba requires 750 mm yr^{-1} for adequate growth which is critical for early stages, flowering and seeding. Irrigation ensures good crop establishment, shortens time to maturity, doubles the number of roots, increases number of buds, allows more dense plantings and increases time of photosynthesis (Pinoyfarmer, 2007). Over 80% of the lateral roots of jojoba are found within the top 1 m soil depth (Pinoyfarmer, 2007). Average annual irrigation in USA is 2 000- 4 000 litres $plant^{-1}$ in mature plantations whereas 320 litres $seedling^{-1}$ yr^{-1} was applied in the present study. Irrigation is applied daily in the first month and once a week in sandy soils and then once in 2-3 weeks during summer. The soils in the current study were sandy loam which could retain more soil moisture compared with sandy soils, hence less water applied for irrigation. Water for effective irrigation is about one third of that lost through evaporation in ASALs and NPK fertilizers are added in the water (Gentry, 1958). However, the actual water requirement for young jojoba plants has not been studied.

This study shows that combinations of management regimes are superior relative to single applications. These results are consistent with findings of on-farm trials in Arusha and Arumeru districts in Tanzania by Rockstrom (1997) and semi-arid areas of Kenya by Kihanda *et al*. (2006) who reported that combined water and fertility management can greatly improve crop yields as opposed to either of the two. Rapid seedling establishment

is an important requirement for successful crop farming in ASALs, since it helps the plants to escape from drought (Balkan, 2012).

Incorporation of animal manure is necessary to combat decline in soil fertility and structure (Rowland, 1993; Kihanda and Gichuru, 1999) and the recommended rate is 5-10 t ha^{-1} (Kihanda, 1996). According to Andrews and Foster (2007), manures release 40-70% nutrients within 4 - 8 weeks after application. However, in drylands, the quantity of manure applied is restricted due to burning of the crop when insufficient moisture is available at the time of application (FAO, 2004). These observations are consistent with the present study since single manure applications are discouraged. This burning effect was observed in mature bushes outside the experimental plot in this study. Farmers wait until onset of rains to apply manure in ASALs.

Securing a water source during dry spells through irrigation can be an incentive needed for investment on improved soil moisture, which in turn can result in progressively increasing yields and profits (Cooper *et al*., 1987; Rowland and Whiteman, 1993; Figueres *et al*., 2003; Itabari *et al*., 2011). There was also accelerated decomposition of OC resulting from higher soil moisture maintained throughout the growing season. Depletion of SOM was reported by Du Preez *et al*. (2010) to be relatively higher in irrigated compared to non-irrigated in drylands.

The findings of Sullivan (2002) and FAO (2005) reported yield increase of 300% compared with yields without runoff harvesting in drylands. NAS (1974) observed the beneficial effects of 32 m^2 micro-catchments for growing pasture shrubs in Negev, Israel and reported maximum response which increased the yields of shrubs 15 times those of untreated land. However, the results of the present study were contradictory to these

finding probably due to the small size of microcatchments (3 m^2) used which were able to harvest minimal runoff. Water harvesting has also been equated to irrigation since it gives similar yields.

Weber and Stoney (1986) mentioned the need to have wide spacing in marginal areas in order to have sufficient area for micro-catchment. Water harvesting can improve production and carrying capacity of drylands. Other contrasting studies have reported that micro catchments have been used to establish trees such as prosopis successfully in northern Kenya (Koohafkan and Stewart, 2008). Micro-catchments have been used successfully in Near east, Africa and America and especially in the Negev desert in Israel. This technique is more widely applicable than irrigation and may have useful environmental benefits as regards erosion control. It offers more hope for increasing production in ASALs for which irrigation is not a viable option due to capital limitations (Critchley and Siegert, 1991; Mati, 2005).

Only 15-30% of rainfall is actually used in productive food making, while 70-85% of rainfall in water-scarce farming is lost through runoff, evaporation and drainage which mean that there is a high volume of water that can be tapped for use for productive purposes (Rockstrom, 1997). The present study was not able to tap this runoff potential due to low and poor distribution of rainfall hence rendering micro catchments to be ineffective.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The jojoba plantation in Voi, Kenya has a low genetic diversity since the PIC ranged from 0.25 to 0.37 since it was established mainly from cuttings after selection.

Foliage traits for the males of jojoba cuttings and seedlings were higher relative to the females. Single leaf area was found to be significantly higher for the males as compared to the females hence used to determine sex in jojoba seedlings.

Different management regimes on jojoba mother plants before propagation did not show significant differences relative to the control. This therefore means that there is no special management regime required for jojoba mother plants before harvesting of cuttings.

Anatone was superior in rooting jojoba cuttings since it resulted into significantly higher rooting compared to the control under a polythene sheet tunnel. Propagation results of various genotypes tested showed significant differences within and between sexes in rooting. The males (M2 and M1) were superior compared to the females (F1 and F2).

Anatone × M2 interaction was the best for rooting (62.7%) and was significantly higher relative to all the interactions. Similarly, all the PGRs (IBA, Anatone and Roothom) × genotype interaction showed superior performance relative to the control in all the variables measured with the exception of root collar diameter and internode length.

Manure, micro catchment and irrigation combinations were superior compared to single application in early growth of field planted jojoba seedlings. This study reveals that jojoba can be grown successfully using the available resources in the ASALs.

6.2 Recommendations

i In spite of the low genetic diversity of the present plantations, in the short term the cuttings can be used for multiplication in order to supply farmers with rooted seedlings. Alternatively, since jojoba is dioecious, plants derived from seeds are different from each other and therefore seedlings can be used to supply planting material. Selected superior females should be pollinated using superior males in order to increase the level of genetic diversity. However, in the long term there is need to broaden the genetic base of the jojoba plantation by importing diverse superior germplasm for future multiplication.

ii Single leaf area morphological trait may be used for sexing of jojoba seedlings especially after stressing of the seedlings. Morphological studies can be improved in future by sampling at juvenile and mature stages. Since water stress initiated early flowering, research is needed to determine the ideal stress level. Further research should be carried out for a longer period of 9-12 months in the nursery on normal non stressed seedlings after which they can be stressed by reducing the watering regimes in order to identify their sexes. The current study was carried out for a period of 7 months. A further delay in the nursery through use of bigger pots up to 4 litres can help to identify sex by allowing flowering to occur before field planting, although this option is more expensive.

iii Anatone and the male genotypes (M1 and M2) be used in the propagation of jojoba cuttings in future. However, since female genotypes are more highly preferred due to their seed production compared with the males which produce pollen, further research is needed in selection of a wide range of superior high yielding female genotypes that can root easily for future propagation. Similarly, more research is

necessary to test a wide variety of cheaper PGRs in terms of their performance, active ingredients and their concentrations using various superior genotypes of jojoba cuttings.

iv Anatone × M2 interaction for future propagation of jojoba cuttings is recommended. However, there are more prospects for further interaction studies between various PGRs and female genotypes inorder to get the best combination for scaling up production. The study recommends the use of manure, micro catchment and irrigation combinations in early establishment of jojoba in the ASALs. Further investigation should be done on the effect of management regime on field planted seedlings in a normal year with adequate rainfall (450 - 600 mm) and properly distributed in order to ascertain the low performance of micro catchments noted in this study.

v Research on management regimes in the field planted seedlings should be conducted over a longer period in order to realize their effect on yields. There is also urgent need for research in the amount of water used for irrigation technology to supplement rainfall.

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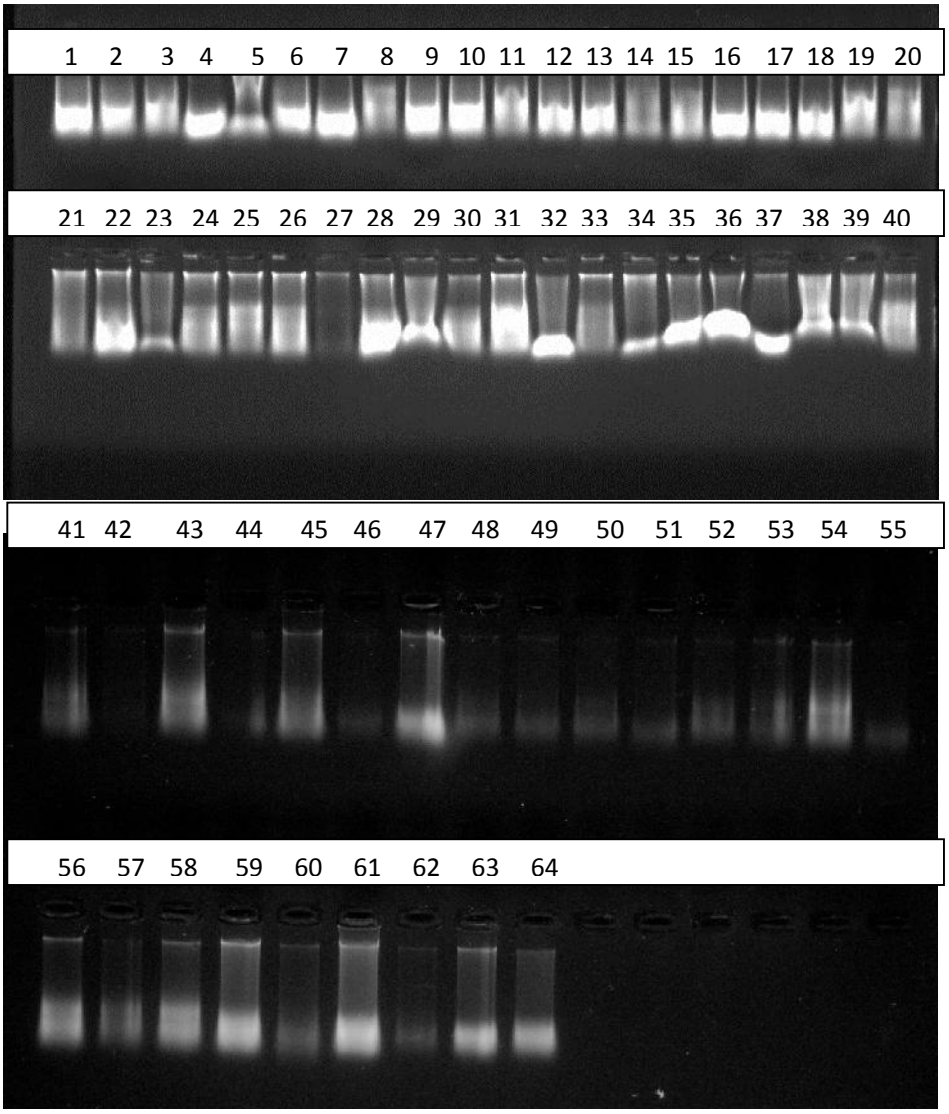
APPENDICES

Appendix 1: Mature jojoba bushes growing in Voi, Kenya

Appendix 2: DNA Extraction buffer preparation (2% CTAB)

Stock concentration	**Working concentration**	**Final volume (100 ml)**
1M Tris	100 mM	10 ml
0.5M EDTA	50 mM	10 ml
5M Nacl	700 mM	10 ml
CTAB	2%	
PVP	2%	
dH_20		70 ml

Appendix 3: DNA quantification for 64 jojoba leaf samples using gel electrophoresis



Appendix 4: PCR components and reagents

COMPONENTS	**X1 REACTION VOLUME**
PCR Premix	5 µl
SSR Primer-Forward	0.25 µl
SSR Primer-Reverse	0.25 µl
$Mgcl_2$	0.65 µl
DNA template	2 µl
	Final reaction volume 10 µl

Appendix 5: PCR Programme

STEPS	**TEMPERATURE**	**TIME**
Initialization step	94°C	5 Minutes
Denaturation step	94°C	30 Seconds
Annealing step	X	30 Seconds
Extension/elongation step	72^0C	2 Minutes
Final elongation	72^0C	10 Minutes
Final hold	4°C	Infinity

Appendix 6: Characteristics of ten markers used in jojoba diversity studies

Markers	Primer Forward	Primer Reverse	Repeat mortif	bps	Annealing temp.
JMA01	ACACCAGATTCCAGAGGCATA	ATTCGTCAAAGGGGATGATG	[CT]8	198	52°C
JMA02	AGAGTACGCGGGAAGCAGT	TGCTGGCAAGGGAGGTAATA	[AG]8	600	55°C
JMA03	AGTCGTTTCCCCTGCTTTTC	CTTCTGCTTATCCCCCTCATC	[CT]7	320	52°C
JMA04	GGACCTCTGCCCTTCTTCTT	TGGCGTCTTCACTGCTACTC	[GT]11	500	57°C
JMA05	CGGGGATTTATAGTCTTCACTCTC	GTCCAGGCTTCAGACCAGAG	[TC]13	214	56°C
JMA06	GCATCTGCCATTTTATGTTCAG	AACCCAGTTCCAGCTTCATC	[AAT]5	180	52°C
JMA07	GCCAAGTGGGGATGTAGAGA	GGGGACTGAACTCCACCAA	[GA]8	165	52°C
JMA08	GGAACCACAATGGCAACG	CGCAGGAAGGTCGTAAACTG	[TCT]9	185	52°C
JMA09	GCGGGGAAAGTGTTACGC	GATTAGCAGAGAAACCAAGGGACT	[AG]15	190	52°C
JMA10	AGTCAGAGTCACAGAGCAATGAA	AAGAGATTAGCAGAGAAACCAAGG	[TCT]5	700	55°C

Source: Ince *et al*. (2010)

Appendix 7: Summary of ANOVA (p > F) testing effects of morphological traits for identifying sex in jojoba cuttings and seedlings

Cuttings/Seedlings	Sources of variation	Degrees of freedom	Height	Height of new growth	No. of shoots	Shoot length	No. of nodes	Node density	Internode length	Leaf length	Leaf width	Leaf shape index
Cuttings	Sex	1	0.7121	0.0792	-	-	-	-	0.4290	0.0608	0.1780	0.6257
Seedlings	Sex	1	0.7743	-	0.5979	0.7577	0.1472	0.4639	0.7015	0.2567	0.3206	0.4226

Appendix 7 continued……..

Cuttings/ Seedlings	Sources of variation	Degrees of freedom	No. of leaves	Single leaf area	Total leaf area	Leaf area/0.3m	Root collar diameter	Root length	No. of roots	Total fresh plant biomass
Cuttings	Sex	1	0.3593	0.0855	0.0713	-	0.5000	0.5884	0.9906	-
Seedlings	Sex	1	0.4226	0.0351	0.0975	0.1748	0.8399	0.7593	0.7244	0.9096
	Rep	2	-	-	-	-	-	-	-	-
	Residual error	2	-	-	-	-	-	-	-	-
	Corrected total	6	-	-	-	-	-	-	-	-

There was no test statistic for replication as specified in the General Linear Model of SAS. Root and plant biomass variables were only measured for the young seedlings and rooted cuttings

Appendix 8: Summary of ANOVA (p > F) showing effects of management regime on survival and growth of jojoba cuttings in a polythene sheet tunnel

Sources of variation	Degrees of freedom	Survival %	Rooting %	Height	Height of new growth	Leaf length	Leaf width	Internode length	No. of leaves	Single leaf area	Total leaf area
Management regime	7	0.0183	0.0038	0.0010	0.0045	0.0016	0.0011	0.0026	0.0006	0.0012	0.0022

Appendix 8 continued…….

Sources of variation	Degrees of freedom	Root collar diameter	No. of roots	Root length	Total fresh plant biomass
Management regime	7	0.0062	0.0002	0.0018	0.0005
Replication	2	-	-	-	-
Residual error	14	-	-	-	-
Corrected total	23	-	-	-	-

There was no test statistic for replication as specified in the General Linear Model of SAS

Appendix 9a: Summary of ANOVA (p > F) showing effects of PGR and Genotype on survival and growth of jojoba cuttings in a polythene sheet tunnel

Sources of variation	Degrees of freedom	Survival %	Rooting %	Height	Height of new growth	Leaf length	Leaf width	Internode length	No. of leaves	Single leaf area	Total leaf area
PGR	3	0.6738	0.0071	0.5382	0.2964	0.5640	0.6582	0.4474	0.5034	0.4060	0.5754
Genotype	3	<.0001	0.0026	0.0212	0.0521	0.0127	0.0174	0.0171	0.0492	0.0016	0.0097

Appendix 9a continued……

Sources of variation	Degrees of freedom	Root collar diameter	No. of roots	Root length	Total fresh plant biomass
PGR	3	0.6650	0.1890	0.2209	0.5745
Genotype	3	0.0152	0.0084	0.0040	0.0293
Replication	2	-	-	-	-
Residual error	39	-	-	-	-
Corrected total	47	-	-	-	-

There was no test statistic for replication as specified in the General Linear Model of SAS

Appendix 9b: Summary of ANOVA (p >F) showing effects of PGR x Genotype interaction on survival and growth of jojoba cuttings in a polythene sheet tunnel

Sources of variation	Degrees of freedom	Survival %	Rooting %	Height	Height of new growth	Leaf length	Leaf width	Internode length	No. of leaves	Single leaf area	Total leaf area
PGR x Genotype	9	0.0014	<.0001	0.0108	0.0006	0.0003	0.0002	0.0088	0.0023	<.0001	0.0002

Appendix 9b continued…….

Sources of variation	Degrees of freedom	Root collar diameter	No. of roots	Root length	Total fresh plant biomass
PGR x Genotype	9	0.0251	0.0083	0.0073	0.0028
Replication	2	-	-	-	-
Residual error	18	-	-	-	-
Corrected total	29	-	-	-	-

There was no test statistic for replication as specified in the General Linear Model of SAS

Appendix 10: Summary of ANOVA (p > F) showing the effects of propagule type, management regimes and their interactions on survival and growth of field planted jojoba cuttings and seedlings over 1-10 months period

Period in months	Sources of variation	Degrees of freedom	Survival %	Height	Root collar diameter	Number of leaves	Number of shoots
1	Wholeplot	1	0.0110 (0.0088)	<.0001	<.0001	<.0001	0.0379
	Subplot	7	0.4682 (0.4243)	0.8326	0.1970	0.9389	0.5899
	Wholeplot x subplot	7	0.7307 (0.5264)	0.9224	0.2093	0.7312	0.9809
4	Wholeplot	1	<.0001 (<.0001)	<.0001	<.0001	<.0001	<.0001
	Subplot	7	0.3836 (0.3030)	0.2832	0.4621	0.8985	0.8943
	Wholeplot x subplot	7	0.0115 (0.0062)	0.5661	0.4116	0.7623	0.8014
7	Wholeplot	1	<.0001 (<.0001)	<.0001	<.0001	0.0107	0.4474
	Subplot	7	0.5975 (0.4500)	0.2133	0.6088	0.5607	0.8071
	Wholeplot x subplot	7	0.0518 (0.0225)	0.5210	0.3375	0.9547	0.9619
10	Wholeplot	1	<.0001 (<.0001)	<.0001	<.0001	0.2713	0.9337
	Subplot	7	0.5975 (0.4500)	0.3189	0.0387	0.3671	0.6825
	Wholeplot x subplot	7	0.0518 (0.0225)	0.2363	0.4337	0.8478	0.7332
	Rep	2	-	-	-	-	-
	Rep x wholeplot	2	-	-	-	-	-
	Residual error	28	-	-	-	-	-
	Corrected total	47	-	-	-	-	-

Key: Wholeplot comprised of propagule type (cuttings and seedlings) while subplot comprised of management regimes. Survival percent arcsine transformed is represented by brackets

Appendix 11: Survival and early growth performance of field planted jojoba cuttings and seedlings over 1-10 months period

Period in months	Propagule type	Survival%	Survival% (Arcsine transformed)	Height (cm)	Root collar diameter (mm)	Number of leaves	Number of shoots
1	Cuttings	83.4b	73.7b	14.8b	2.6b	18.9b	2.3b
	Seedlings	97.3a	87.1a	31.9a	4.1a	37.7a	3.2a
	CV	19.5	20.4	16.5	14.3	34.2	50.9
	SE	3.59	3.35	0.79	0.10	2.00	0.30
4	Cuttings	62.2b	55.0b	22.8b	3.9b	50.7b	4.5b
	Seedlings	91.7a	81.9a	39.7a	5.4a	94.1a	7.0a
	CV	22.7	21.4	17.4	21.9	33.7	31.1
	SE	3.58	2.99	4.98	0.21	4.98	0.37
7	Cuttings	59.8b	53.3b	34.0b	7.4b	138.4b	19.2a
	Seedlings	91.7a	81.9a	52.6a	10.4a	196.6a	21.1a
	CV	26.3	23.8	17.5	20.4	44.0	42.8
	SE	4.06	3.28	1.54	0.37	15.0	1.76
10	Cuttings	59.8b	53.3b	48.5b	12.0b	284.7a	36.1a
	Seedlings	91.7a	81.9a	67.3a	15.3a	320.4a	36.5a
	CV	26.3	23.8	18.2	17.5	36.4	48.8
	SE	4.06	3.28	2.16	0.49	36.50	3.61

Significant difference is shown by a different letter along each column using DMRT at $p < 0.05$ and SE is standard error

Appendix 12: Summary of ANOVA (p > F) showing effects of management regimes on survival and growth of field planted jojoba cuttings and seedlings over a 10 month period

Growth period	Propagule type	Sources of variation	Degrees of freedom	Survival %	Height	Root collar diameter	No. of leaves	No. of shoots
1 month	Cuttings	Management regime	7	0.0785	0.0998	0.0609	0.0861	0.0634
	Seedlings	Management regime	7	0.0600	0.0882	0.0164	0.0721	0.0937
4 months	Cuttings	Management regime	7	0.0566	0.0276	0.0070	0.0788	0.0605
	Seedlings	Management regime	7	0.0854	0.0616	0.0738	0.0850	0.0695
7 months	Cuttings	Management regime	7	0.0645	0.0603	0.0359	0.0996	0.0974
	Seedlings	Management regime	7	0.0854	0.0163	0.0374	0.0583	0.0898
10 months	Cuttings	Management regime	7	0.0645	0.0445	0.0178	0.0919	0.0586
	Seedlings	Management regime	7	0.0854	0.0245	0.0375	0.0239	0.0681
		Replication	2	-	-	-	-	-
		Propagule type	1	-	-	-	-	-
		Residual error	30	-	-	-	-	-
		Corrected total	47	-	-	-	-	-