

**THE ASSESSMENT OF THE GENETIC DIVERSITY PRESENT IN
Jatropha curcas L GERMPLOSM CULTIVATED IN KENYA AS A POTENTIAL
BIOFUEL FEEDSTOCK**

By

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(A56/76042/2009)



**A thesis submitted in partial fulfilment of the requirements for the award of the
degree of Master of Science in Plant breeding and biotechnology**

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2012

DECLARATION

I do declare that, except references to other people's work which have been duly cited, this work is a result of my own investigation, and that it has, neither in whole nor in part, been submitted elsewhere for another degree.

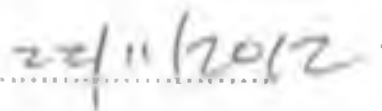


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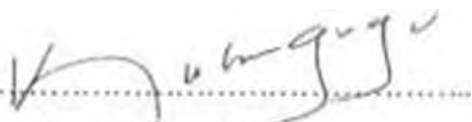
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I dedicate this thesis to my parents- Mrs. Consolata Naswa Kundu and the late Mr. Francis Kundu Nabiswa.

ACKNOWLEDGEMENTS

Praises and adoration be unto the almighty God for his protection, guidance and divine inspiration granted me during the pursuit of this degree. I therefore take this opportunity to record my indebt gratitude and appreciation to my supervisors, Dr. Kahi Ngugi and Dr. J. Kinama all of the Department of Plant science and Crop Protection, University of Nairobi for opening-up and sharing their experiences with me during the supervision of this thesis. To my parents, Mrs. Consolata Naswa Kundu and the late Mr. Francis Kundu Nabiswa, I say thank you for your encouragement and support, may the almighty God richly bless you. My heart-felt gratitude to my wife Agnes Nthenya, my sons Warren and Benjamin for moral support and patience during the course of my study and research. My profound gratitude goes to Dr. Jacob Kithinji of Department of Chemistry, University of Nairobi for his kind assistance in the analysis of the chemical aspect of the study. Much appreciation goes to the Kenya National Council of Science and Technology for financially supporting the study. To all the friends I could not mention may the almighty God bless you all and reward the support you gave me.

ABSTRACT

Jatropha curcas L has the potential of becoming an important feedstock for biodiesel and bioenergy in Kenya. There has been a concerted effort in collaboration from the sector industry to promote large scale farming of jatropha. However, to date there has been no program to improve *J. curcas* traits and thus its full potential has not been realized. Hence knowledge on the presence of genetic diversity and genotype x environment (GxE) interactions of *J. curcas* germplasm are vital source for the genetic improvement of seed yield, seed oil quality and quantity as well as other agronomic traits for viable commercial exploitation of the plant. The objective of this study was to assess genetic diversity and evaluate the effects of genotype-environment (GxE) interaction of the currently grown genotypes. A field trial consisting of 49 genotypes was laid out in a lattice design of two replications in two contrasting agro-ecological environments, namely Thika and Kihwezi for two years. Based on the multivariate Mahalanobis D² statistics, the genotypes were grouped into four clusters: III, IV, I and II comprised 20, 14, 9 and 6 genotypes, respectively. The analysis further indicated that the genotypes of common geographical origin or same location were grouped into different clusters, suggesting a lack of relationship between genetic and geographical diversity. The highest inter-cluster distance was observed between II and IV followed by I and III which may serve as potential parents for new gene combination. It has been shown that leaf type (22.12%), branching (19.24%) and days to flowering (12.10%) contributed most to the genetic divergence among the genotypes. The Eberhart and Russell stability method was used to measure the performance of yield components of the 49 genotypes. Environmental variance influenced the performance of genotypes for all the traits

measured and genotype x environment interactions was important in determining their performance. The environmental parameters that were important in influencing performance were minimum temperature and soil pH while the stability parameters that were important for adaptation were height and leaf type for $\beta_i=1$; leaf type, days to flowering and oil content for $\beta_i>1$; while height, leaf type, days to flowering, seed yield and oil content for $\beta_i<1$. Genotypes KJ1, KJ2, KJ5, KJ7, KJ10, KJ11, KJ15, KJ18, KJ20, K22, KJ23, KJ24, KJ27, KJ28, KJ31, KJ34, K36, KJ39, KJ40, KJ44, K47 and KJ49 showed high mean values, regression coefficients close to unity and least mean square deviation from regression for oil content in high responsive environments, whereas genotypes KJ4, KJ12, KJ16, KJ17, KJ25, KJ29, KJ32, KJ 33 and KJ 41 would be stable and have high mean oil yields under low-responsive environments such as the Kibwezi site.

Keywords: Genetic diversity, *Jatropha curcas*, multivariate analysis, Stability method.

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ABBREVIATIONS AND ACRONYMS

ANOVA- Analysis of variance

AOAC- Association of Official Analytical Chemist

FAO- The Food and Agriculture Organization of the United Nations.

GCV- Genotypic coefficient of variation

GHG- Greenhouse gas

G x E- Genetic x environment

IEA- International Energy Agency

KARI- Kenya Agricultural Research Institute

PCV- Phenotypic coefficient of variation

rRNA- Ribosomal ribonucleic acid

TAGs- Triacylglycerides

USDA- United States Department of Agriculture

CHAPTER 1: INTRODUCTION

1.1. Background

In Kenya, as the rest of the world, *Jatropha curcas* L is being considered as the most promising biofuel feedstock that can help us gain a measure of liquid fuel independence and decrease our carbon dioxide footprint; other feed-stocks are sucrose, starch, lignocelluloses and triacylglycerides (TAGs or plant oils). For several of these, for example, conversion of lignocelluloses to glucose or alkanes and production of oil from algae, considerable technology development is still needed (Gressel, 2008). Conversion of TAGs to fatty acid methyl esters (biodiesel) is a mature technology and the challenge is to produce large amounts of TAGs in a way that does not compete with food production. *Jatropha* is as yet undomesticated oil “crop” that is an excellent candidate for the production of biodiesel both on commercial plantations and for small community projects (Achten et al., 2009, Jongschaap et al., 2007).

Biodiesel runs any diesel engine and does not require modification. Since biodiesel is chemically similar to fossil diesel fuel, it can replace it into the fuel tank of any diesel vehicle (Sheehan et al., 1998). Biodiesel has advantages as a transport fuel; it has lower emissions and does not affect engine performance (Hill et al., 2006). Biodiesel reduces carbon dioxide emissions, the primary cause of the greenhouse gas effect, considerably. It can be used alone or mixed with petroleum diesel fuel, is safe for transport and is biodegradable.

Among the many species in *Jatropha* genus, only one species *Jatropha curcas* L. has been exploited in agriculture (Heller et al., 1996)). The physic nut (*Jatropha curcas* L.) has been cultivated for biodiesel production, whereas its seed cake can be used as organic fertilizer (Achten et al., 2010). As *Jatropha curcas* L. seed is rich in hydrocarbon molecules, it is used as straight vegetable and or as raw material for the transesterification process to produce a variety of products including biodiesel, glycerine and animal feed (Henning, 2007; Jongschaap et al., 2007). The plant is well adapted to marginal soils with low nutrient content but in order to support a high biomass production the crop shows a high demand for nitrogen and phosphorus fertilization (Henning, 2007; Dacy Ouwens et al., 2007). Harnessing the potential of jatropha would therefore help in meeting energy needs for rural communities to a large extent while at the same time expanding household income base in a bid to alleviate protracted rural poverty.

1.2. Problem Statement

Non-renewable energy has been a subject of debate globally because the supply is currently mainly based on fossil fuels. The use of fossil fuels has significantly added to the amount of carbon dioxide in the earth's atmosphere (Carbon Dioxide Information Analysis Center, 1997). Most scientists agree that this has contributed significantly to the greenhouse gas effect creating the condition for climate change that threaten life on this planet and causes serious environmental effects and economic problems. Fossil fuels produce substantial environmental pollution contributing too many health hazards (Epstein et al., 1998).

High fossil fuel prices, the risks of fossil fuel dependence and the increasing greenhouse gas (GHG) emissions derived from this kind of fuels are the main reasons to find new and renewable energy sources for the coming years (FAO, 2008). It is now evident that oil consumption is drastically increasing, while the reserves are rapidly diminishing (Dowlatabadi, 2006). Energy sources have become commodities that are not accessible to the poor. In Kenya 83% of the population relying on traditional biomass for cooking use fire-wood (IEA, 2011). With the current fluctuations in fuel supply, kerosene prices continue to rise and fire wood is getting scarcer.

There is now a more urgent search for sustainable sources of energy that are renewable and affordable. *Jatropha* has promising potential as a biofuel feedstock but both seed and oil yields cannot yet be predicted at any degree of accuracy. The major factor hindering the improvement of *jatropha* is the lack of characterization of the genetic diversity of the various key traits of economic importance and therefore its potential for biodiesel production remains largely untapped until this is done (Achten et al., 2008). The exact effects of genotype by environmental interactions on flowering, fruit setting, seed yield, oil yield and quality are not yet known.

1.3. Justification

Comprehensive knowledge of germplasm diversity and genetic relationship among wild and cultivated *jatropha* in Kenya is invaluable aid in the crop improvement programme. The progress in developing a superior variety depends largely on the genetic basis of selection of diverse parents and the breeding approach followed. In any crop most of the economic characters, including yield, are metric in nature and being polygenically

controlled. These are highly influenced by environmental factors. The progress in breeding for such characters is determined by the magnitude and nature of interactions between their genotypic and phenotypic variability under varying conditions of soil and climate. Hence partitioning the overall variability into its heritable and non-heritable components with the help of genetic parameters such as genetic co-efficient of variation, heritability and genetic advance constitutes an important step in analyzing genetic diversity in a plant breeding programme (Falconer, 1981).

Information about nature and extent of genetic variability present in the jatropha germplasm and association of various morphological characters is a pre-requisite in planning successful breeding programme. Further seed yield in a crop plant is the sum of effects of several yield component characters, which are governed by a large number of genes and also by environment (Falconer, 1990). For a rational approach towards the improvement of seed yield, selection has to be made for the components of yield. However, the heritable variation is often masked by non-heritable variation, which creates difficulty in selection programme. This suggests the need for partitioning the overall variability into heritable and non-heritable components, which enables the breeder to evolve suitable breeding procedures (Falconer et al., 1996). Knowledge of association between yield and its attributes obtainable through estimation of genotypic and phenotypic correlation helps a great deal to formulate selection strategies to develop suitable genotypes. Realizing the importance and need for such a comprehensive study in jatropha the present investigation was undertaken with the following objectives:

1.4. General Objective

The general objective of the study was to generate basic information on genetic diversity and to evaluate the effect of genotype-environment interaction of *Jatropha curcas* genotypes growing in Kenya.

1.4.1. Specific objectives

1. To assess genetic diversity among the jatropha germplasm and grouping the genotypes in order to understand the nature and magnitude of genetic divergence
2. To evaluate genotype-environment interaction and determine the stability of performance of the currently grown genotypes.

1.5. Hypotheses

1. There are statistically significant differences in the diversity among and within jatropha genotypes used as biofuel feedstock in Kenya.
2. There is significant environmental, genotype and genotype-environment (GxE) interactions influence for all the traits measured.

CHAPTER 2: LITERATURE REVIEW

2.1. Importance and distribution

Jatropha was first described by Swedish botanist Carl Linnaeus in 1753. *Species Plantarum* (USDA, 2010). It is one of many species of the genus *Jatropha*, a member of the large and diverse Euphorbiaceae family. Many of the Euphorbiaceae are known for their production of milky white sap. The genus *Jatropha* has some 175 species particularly common in tropical America and Africa, with a few species native to Southwest Asia and one species in Madagascar. None are native to Southeast Asia, Australasia or Oceania. Several species are cultivated, mainly in the tropics as ornamental and as hedge plants. The species mainly planted for bioenergy the world over include: Nicaraguan (with larger but fewer fruits), Mexican (distinguished by its less-toxic or non-toxic seeds) and Cape Verde. The Cape Verde variety is the one commonly found throughout Africa and Asia. From Caribbean where the species was already used by Mayas (Schmook and Serralta-Peraz, 1997), *Jatropha* was probably distributed by Portuguese Ships via Cape Verde Islands and Guinea Bissau to other countries in Africa and Asia (Heller, 1996). *Jatropha* has become naturalised throughout arid and semiarid tropical regions of the world with an average annual rainfall of between 300 and 1000 mm (Heller, 1996; Palgrave, 1983). In Kenya, *Jatropha* is found distributed in the 6 floral regions (Coast, Eastern, Rift Valley, Western, Central and Nyanza provinces). Its favored niche in these regions is in bushlands and along river-banks (Maundu and Tengnas, 2005).

2.2. Origin and Cytogenetics

Jatropha is a diverse and widespread genus of 175 species (Airy Shaw, 1972) and family Euphorbiaceae. The members of the genus are particularly common in tropical America and Africa, with a few species native to Southwest Asia and one species in Madagascar. None are native to Southeast Asia, Australasia or Oceania. Several species are cultivated, mainly in the tropics (Henning, 2007). Among the various species of the genus, *Jatropha curcas* is becoming an increasingly popular oleaginous crop in several developing countries for its proposed value in the lipids, biopharmaceuticals, cosmetics and biopesticide industry. It is thought to have originated in Mexico and continental Central America (Heller, 1996). The genus name *jatropha* derives from the Greek *jatros* (doctor), *trophe* (food), which implies medicinal uses. *Curcas* is the common name for physic nut (*jatropha*) in Malabar, India (Ramanathan, 2004). Puangpaka and Thaya (2003) conducted an experiment on the karyology of five *Jatropha* species including *J. curcas* in Thailand. Their finding indicated that *Jatropha curcas* has chromosome numbers of $2n = 2x = 22$ and a basic chromosome number of $x = 11$. The result of this study revealed that meiotic configuration of *Jatropha curcas* chromosome was found to be similar with *Jatropha multifida*. Carvalho et al., (2008) found that the same number of metacentric and sub-metacentric chromosomes in *Jatropha curcas* and base composition of 38.7% GC. Carvalho and his colleagues also indicated that the chromosomes are relatively small size (ranges from 1.24 to 1.7 μm) which is in the same size range as that of rice. They also further indicated an average genome size (1C) value to be 416 Mbp which is smaller than that of other species of euphorbiaceae that were reported to vary between 1.3 and 28.6 pg. (Arumuganathan, 1991). This is relatively small to plant genome (Zonneveld

et al., 2005) and could make *Jatropha curcas* an attractive candidate for genome sequencing. The level of genetic diversity and genetic differentiation in *Jatropha* populations deserves special attention due to its introduction history as an exotic species in many countries.

2.3. Description of *Jatropha curcas* L.

2.3.1. Botanical description

Jatropha curcas L. is a small tree or a large shrub which can achieve a height of 5 meter and rarely can attain a height of 10 meter under favourable conditions. The plant show articulated growth, with a morphological discontinuity at each increment. It is drought resistant species and known as an arid and a semi-arid plant species (Heller, 1992).

Jatropha curcas is a deciduous plant and the stem has smooth grey bark which exudes white watery latex during injury. *Jatropha* leaves have narrow lobes and are arranged on stem and branches in an alternate manner. The length and width of the leaves highly vary and morphologically it is similar to *Ricinus communis* leaves. It grows mainly primary and secondary branches which are arranged alternately.



Plate 1 Above ground part of the *Jatropha curcas* (a) Inflorescence (b) fruits (c) Leaf shape and sizes (d) Stem and branch arrangement

Jatropha curcas is monoecious with unisexual flowers formed at the terminal of branches and leading stem (Deng, et al , 2008) Dehgan and Webster (1979) reported occasional existence of hermaphrodite plant with incomplete growth of pistil. The inflorescences are complex and botanically described as a cyme type. Deng and his associates designated that the male flowers are numerous (80- 90%) and occupies the subordinate position of the inflorescences. Ten stamens are arranged in two distinct whorls of five each in a single column in the androecium's, and in close proximity to each other.

Pollination of the *Jatropha curcas* is by insects. However, the hermaphrodite flowers can be self pollinating. Usually flowers open for a period of 8-10 days in the inflorescence. Male flowers open for the whole day whereas female flowers open for only 2-4 days usually 2 to 3 hours in the morning (Raju et al., 2002). However, Munch (1986) did not observe this chronological order in Cape Verde. It seems that such mechanism is influenced by environment. Therefore, both genotype and environment can affect pollinating mechanism. The flower inflorescence yields a bunch of ovoid fruits which mature into yellowish color fruits ready for harvesting. The blackish thin-shelled seeds are 2cm long and 1cm thick, oblong in shape and resemble small castor seeds (Rao et al., 2008).

Jatropha curcas can be grown both from seeds and cuttings. It grows five roots when it is propagated by seeds, one central and four peripheral. A tap root is not usually formed by vegetatively propagated and such plantations have lower production period. Recently tissue culture propagation is used especially in multiplication of high oil yielding genotypes *Jatropha curcas* starts producing seeds within 12-18 months but reaches its maximum productivity level after 4 to 5 years (Henning, 1996). At maturity Becker and Makkar (2000) reported 2- 3 tons of seeds/ha in semi-arid areas, although yields of 4 to 5 tons/ha are routinely achievable under more favorable (wetter) conditions (Foidl et al.,1996; Matsune et al.,1984). With oil content of approximately 35% (Henning, 1996), this equates to an average yield of approximately 1.75 tons of oil per hectare. Recently, the high yield of seed from the tree (5 tons /ha year) and the high oil content of its seeds through genetic modification attracted global attention for the development of *Jatropha curcas* as a source for biofuel (Chen et al., 2006; Li et al., 2008; Openshaw, 2000).

2.3.2. Toxicity

All parts of the *Jatropha* plant contain toxins such as phorbol esters, curcins and trypsin inhibitors (Gubitza et al., 1999; Makkar et al., 1997; Makkar et al., 1998; Martinez-Herrera et al., 2006). Germplasm commonly found in Africa and Asia has seeds that are toxic to humans and animals, whereas germplasm found in Mexico and Central America are known to be non-toxic (Makkar et al., 1998). The poisonous and anti-nutrient properties of the seeds are exploited in traditional medicine for de-worming and as a purgative (Gubitza et al., 1999). Just one to three seeds can produce toxic symptoms in humans, mainly those associated with gastro-intestinal irritation. There is acute abdominal pain and a burning sensation in the throat shortly after ingestion of the seeds, followed by nausea, vomiting and diarrhoea. Children are more susceptible.

Toxicity is chiefly due to the presence of phorbol esters, as inferred from the fact that non-toxic Mexican cultivars are deficient in these compounds. A toxic protein was isolated from *Jatropha curcas* seed designated as curcin and it is ribosome inactivating protein (Juan et al., 2003). Juan and his colleagues reported that curcin has antitumor activity and this is related to N-glycosidase action, which cleaves the N-glycosidic bond of adenine A 4234 of 28S rRNA. According to Makkar and Becker (1998) phorbol esters decompose rapidly - within six days- as they are sensitive to light, elevated temperatures and atmospheric oxygen and *Jatropha curcas* seed meal treatment by heat reduce significantly the amount of lectins and trypsin inhibitors. The decomposition of these and other toxic compounds in the field needs further evaluation before insecticide or molluscicide oil extracts can be widely used, or before the widespread application of seed

cake as fertilizer, particularly on edible crops, given that there is no information as to whether such compounds are taken up by plants. Mexico has varieties of jatropha that are not poisonous. Using these varieties in future breeding programmes is the most likely route to non-toxic jatropha products.

2.4. Jatropha production constraints

The growing and management practices of jatropha are poorly documented. Some of the current strategies used to promote jatropha may be sub-optimal, since there is no proper experimental evidence. To promote jatropha productivity in the rural communities, the major goals would be to use jatropha plants and their products for economic and environmentally sustainable development and to make marginal areas self-sufficient in energy, especially liquid fuels. Where possible, this is to be achieved without displacing other agricultural crops or competing for land with other high value or food security crops.

However, arguments that jatropha may displace food crops, may be as result of lack of availability of scientific evidence (Jongschaap et al., 2007). The main knowledge gaps are because of very little genotypic characterization, sub-optimal agronomic practices and limited information on the genome of jatropha. There is need for major research initiatives in agronomy, breeding and provenance in order to fully realize potential of jatropha.

In jatropha provenances available in India only modest levels of genetic variation were observed, while wide variation was found between the Indian and Mexican genotypes (Basha and Sujatha, 2007). Collections of jatropha plants from the same geographical area showed very little genetic variability (Pamidamam et al., 2009). The plants developed from wild seed collections also tend to have diverse morphological features and chemical composition (Sunil et al., 2008).

2.5. Genetic diversity and genotype-environment interaction

2.5.1. Variability, Heritability and Genetic Advance.

For plant breeders to achieve improvement in any crop they depend heavily on the magnitude of genetic variability. Heritability is a concept that summarizes how much of the variation in a trait is due to variation in genetic factors. Often, this term is used in reference to the resemblance between parents and their offspring. In this context, high heritability implies a strong resemblance between parents and offspring with regard to a specific trait, while low heritability implies a low level of resemblance. Phenotypes that vary between the individuals in a population do so because of both environmental factors and the genes that influence traits, as well as various interactions between genes and environmental factors. Phenotypic variability expressed by a genotype or a group of genotypes in any species can be partitioned into genotypic and phenotypic components. The genotypic component being the heritable part of the total variability, its magnitude in yield and its component characters, influences the selection strategies to be adopted by the breeder (Falconer et al., 1996).

Broad-sense heritability, defined as $H^2 = V_G/V_P$, captures the proportion of phenotypic variation due to genetic values that may include effects due to dominance and epistasis. On the other hand, narrow-sense heritability, $h^2 = V_A/V_P$, captures only that proportion of genetic variation that is due to additive genetic values (V_A). Given its definition as a ratio of variance components, the value of heritability always lies between 0 and 1.

Studies of the quantitative genetic variation within populations are sparse in jatropha. Phenotypic studies are several, but they are often made for seed properties of genotypes standing at different sites, making it impossible to separate the effect of the genotype from the effect of the site. Kaushik et al., (2007), reported divergence in seed oil traits of jatropha from a limited number of locally collected accessions. Ginwal et al., (2005), compared plants from ten Indian landraces after 6–24 months, and found large significant variations, attributing more than 80% of the total phenotypic variance to seed sources. This is a relatively high level of genetic variability compared with what is usually found in tree species of tropical dry zones. Basha and Sujatha (2007) observed that initial variations in fruit and seed yields of candidate plus trees of jatropha were found to be insignificant when the plants were grown on common site in India, indicating low variability. Rao et al., (2008) conducted a thorough and extensive wild germplasm exploration survey on 32 high yielding candidate plus trees of jatropha from different locations considering latitudes and longitudes. They found significant trait differences in all the seed characters such as seed morphology, oil content, growth, female to male flower ratio and seed yield in the progeny trial

High broad-sense heritability and a small phenotypic variation were found for plant height and root collar diameter in ten accessions by Kumar et al., (2003). In an experiment carried out by Rao et al., (2008) with cuttings from 29 candidate trees evaluated after 34 months, the broad-sense heritability was high (ranging between 0.63 and 0.88) for height, number of branches, number of flowers, ratio between female and male flowers, days from initiation of flowering to fruiting, days from fruiting to maturity and seed yield per plant.

2.5.2. Correlation coefficient analysis

Grain yield is a function of two or more component traits. The study of genetic correlation between yield and its components help in making indirect selection for yield. The correlations between observed characters may exist due to various reasons; because of pleiotropy, genetic linkage, the association of loci or blocks of loci governing variability for different characters located on different chromosomes. Segregation that will result normally reflects the nature of correlations between different traits. The association of characters can be measured as the co-efficient of correlation (Galton, 1889).

The correlation co-efficient analysis helps to determine the nature and degree of relationship between any two measurable characters. It resolves the complex relationships between events into simple forms of association. But the measure of correlation does not consider dependence of one variable over the other. The main purpose of correlation and regression analysis in crop plants is to have detailed understanding of complex characters, such as yield per plant

Yield being dependent on morphological features of the plant, it is desirable to know the individual contribution of these characters for developing effective selection strategies. The direct contribution of each component to the yield and the indirect effects it has through its association with other components cannot be differentiated by simple correlations.

Genetic correlations between different traits have been investigated in jatropha. Rao et al., (2008) found that the number of branches was moderately correlated with seed yield per plant (genetic correlation of 0.61). The genetic correlation between number of flowers and seed yield was 0.29 and the yield per plant was moderately correlated (0.32) with days from fruiting to maturity.

2.5.3. Genetic divergence studies through multivariate analysis

Several multivariate techniques can be used to predict genetic divergence, such as clustering methods and analysis of principal components and canonical variables. These techniques have been applied successfully for diverse crops such as cassava (Nick et al., 2008). Cluster analysis is used to group plants based on their traits, so the within and between differences is prominently shown. Mahalanobi's generalized distance (D^2), especially the hierarchical method, has been widely used for the study of dissimilarity in plants. This method computes a distance measure between comparable plants by coefficient of determination in regression (Hair et al., 2006). The canonical variables method is used to evaluate the similarity between genotypes based on geographical dispersion, generally based on two Cartesian axes (Cruz and Regazzi, 1997).

Mahalanobis' - D^2 statistic (Mahalanobis, 1936) has been put to use for discriminating divergent populations (Mischner and Sokal, 1957; Morashima and Oka, 1960; Murthy and Quadri, 1966). Estimation of degree of divergence between biological population and computation of relevant contribution of different components to the total divergence is done completely by Mahalanobis generalized distance estimated by D^2 statistics (Nair and Mukherjee, 1960; Maurya and Singh, 1977). Selection of parents in hybridization programme based on Mahalanobis's D^2 statistic provides knowledge about the diversity of many traits prior to crossing. Nair and Mukherjee (1960) were the pioneers to use D^2 statistic as a measure of genetic divergence in the field of plant breeding in the classification of teak tree. Mahto and Verma (1998) evaluated fifty-nine genotypes of linseed for genetic diversity and clustered in ten groups. Cluster I was biggest and contained nineteen genotypes. There was no parallelism between clustering pattern and geographic origin. Maximum genetic diversity was obtained between cluster VI & VIII.

2.5.4. Stability method for genotype - environment interaction

The stability methods can be divided into two major groups: univariate and multivariate stability statistics. Knowledge on the components of the genotype environment (GxE) interaction is of great importance for genetic breeding but provides no detailed information on the performance of each cultivar under varying environmental conditions (Cruz et al., 2004).

The analyses of adaptability and stability are therefore extremely important and necessary for the identification and recommendation of superior genotypes in different environments. Different biometrical methods have been used for Genotype x

Environment interaction in crop plants by several workers. Among the most important are the G X E analyses of Finlay and Wilkinson (1963), Eberhart and Russell (1966), Perkins and Jinks (1968), and Freeman and Perkins (1971). Other G X E analyses include the AMMI by Gauch-Jnr H.G (2006) and centroid analysis of (Rocha et al., 2005). Most of these methods give information mostly about the genotype (varieties) constitution and the role of the mega environment.

Jatropha is cultivated under variable conditions. In general, a wide genetic base is essential to prevent inbreeding depression and allow for adaptation to changing environmental conditions (Dawson et al., 2009).

CHAPTER 3: MATERIALS AND METHODS

3.1. Collection of germplasm

A total of 49 *Jatropha curcas* accessions were collected from different agroecological areas of East Africa , with some procured from Madagascar (three) , India(1) and Mexico (four). Tables 1 list the accessions, place of collection, region and country. The criteria for selecting the mother plants were that: the tree had to be old enough, as indicated by height and girth diameter; the trees were free from pest and diseases; and the tree stands had to be more than 100km apart. However, in certain cases, there were special considerations for unique ecoregions to collect at 50km between provenance stands. The seeds were cleaned, air dried to 7% moisture content and germinated in the nursery. Thereafter, robust seedlings that germinated in the first 10 days were selected for transplanting.

3.2. Experimental location

The experiment was conducted on the University of Nairobi Experimental Farm in Kibwezi and Kenya Agricultural Research Institute (KARI) in Thika. Thika is about 2000 m above sea-level, with a maximum and minimum day temperatures of 24°C and 10°C respectively whereas Kibwezi is about 850m above sea-level with maximum and minimum temperatures of 35°C and 24°C. Thika represented the high rainfall, high altitude area (agro-ecological zone 3) whereas Kibwezi represented, low rainfall, semi-arid and low altitude agro-ecological zone (agro-ecological zone 5). The growing seasons were late rainy and dry periods.

Table 1: *Jatropha curcas* germplasm collected from Kenya and other parts of the world

Accession code	Place of collection	Region	Country
KJ1	Kirinyaga	Central	Kenya
KJ2	Meru	Central	Kenya
KJ3	Nguruman	Rift valley	Kenya
KJ4	Kibwezi	Eastern	Kenya
KJ5	Namanga	Rift valley	Kenya
KJ6	Kangundo	Eastern	Kenya
KJ7	Tana river	Coast	Kenya
KJ8	Shimba Hills	Coast	Kenya
KJ9	Bissil	Rift valley	Kenya
KJ10	Homabay	Nyanza	Kenya
KJ11	Murang'a	Central	Kenya
KJ12	Bungoma	Western	Kenya
KJ13	Busia	Western	Kenya
KJ14	Maseno	Nyanza	Kenya
KJ15	Kakamega	Western	Kenya
KJ16	Kitui	Eastern	Kenya
KJ17	Rachuonyo	Nyanza	Kenya
KJ18	Ndhiwa	Nyanza	Kenya
KJ19	Mtito Andei	Eastern	Kenya
KJ20	Nakuru	Rift valley	Kenya
KJ21	Ardhi river	Eastern	Kenya
KJ22	Arusha	Arusha	Tanzania
KJ23	Mombo	Moshi	Tanzania
KJ24	Korogwe	Tanga	Tanzania
KJ25	Zanzibar	Zanzibar	Tanzania
KJ26	Pemba	Pemba	Tanzania
KJ27	Masaka	Masaka	Uganda
KJ28	Iganga	Iganga	Uganda
KJ29	Kahale	Kabale	Uganda

Table 1 continued

Accession code	Country of origin
KJ30	Madagascar
KJ31	Madagascar
KJ32	Madagascar
KJ33	India
KJ34	Mexico
KJ35	Mexico
KJ36	Mexico
KJ37	Mexico
KJ38	Mexico
KJ39	Mexico
KJ40	Mexico
KJ41	Mexico
KJ42	Mexico
KJ43	Mexico
KJ44	Mexico
KJ45	Mexico
KJ46	Mexico
KJ47	Mexico
KJ48	Mexico
KJ49	Mexico

3.3. Field trials

Seedlings raised in the nursery were used as planting materials. The trials were laid in lattice 7 x 7 design with two replications in each of the environment (Fig1). In each replication, the accessions were planted in plots with 7 rows each of which had a spacing of 1m x 1m between and within rows. There was a two row guard around the unit and the central rows were used for data collection. The treatment consisted of 49 jatropha genotypes whose seeds were collected from different locations within the country. The fields were primarily disc-ploughed one time and secondary ploughing was done once.

Di-ammonium phosphate fertilizer at the rate of 3g and organic manure at the rate of 3kg per plant was incorporated into the soil at the planting time. Manual weed control was carried out 14 days after transplanting. Pruning was not done in this trial and seeds were harvested by hand. During the trial period, observations and data collection was done fortnightly to monitor the occurrence of insect pests and diseases; no major insect pest or disease was observed that caused any economic damage and, therefore, there was no need for spraying. However, the following were recorded: Powdery mildew and flea beetles were observed during the cool months of May-August when humidity and temperature changes were highest; leaf miners occurred during mid-season drought prone periods between the short and long rains; while *Scutelleridae* bugs were observed during fruiting period. Irrigation at the rate of 5 litres / plant per week was done when the soil moisture fell below field capacity or the plants showed apparent wilting. Harvesting was done at 7 months after planting and was repeated in the second year.

x	x	x	x	x	x	x	x	x
x	40	34	24	35	19	22	18	x
x	47	13	33	12	1	26	48	x
x	11	30	10	45	44	42	28	x
x	15	49	41	2	36	9	3	x
x	43	17	6	29	27	7	37	x
x	46	21	38	31	32	16	14	x
x	4	25	8	20	23	5	39	x
x	x	x	x	x	x	x	x	x

Figure 1: Experimental layout

NB: X – guard rows indicated by 'x'; Numbers 1-49 represent genotypes

3.4. Measurement of agronomic traits

The following experimental data was recorded on five randomly selected plants per plot/accessions: (i) Plant height at maturity (ii) Girth diameter (iii) Leaf type (iv) Number of branches per plant (v) Days to 50% flowering (vi) Number of male flowers per plant (vii) Number of female flowers per plant (viii) Number of fruits per plant (ix) 100 seed weight (x) Seed yield plant (xi) Seed moisture (xii) Oil content (Xiii) Seed oil yield.

3.4.1. Procedure for oil extraction and analysis

The oil extraction was done following the method described by AOAC (1994). For each sample the seeds were weighed and cracked to obtain the shell and kernel which were weighed separately. They were then ground and a sample of 1 g of each taken in duplicate to determine the dry matter in the seeds. For each sample the seeds were cracked to obtain the kernel which was then ground and 5g of each of the sample put on a 22X80mm paper. A small ball of cotton wool was then placed on the thimble to prevent loss of the sample. Anti-bumblng granules were put in a clean and dried 250ml round bottom flask. 150ml of hexane at a boiling point between 60-80 °C was then added and assembled. Quickfit condenser was then connected to the soxhelt extractor and refluxed for 6hrs on a high heating mantle. The flask was removed and evaporated on a steam bath. The flask containing the oil was dried for 30min in an oven at 120°C and cooled unto room temperature in a desiccator. The weight of the flask and the oil collected were weighed after which the weight of the flask was subtracted to obtain the weight of the oil collected. The weight of the oil collected was then expressed as percentage in respect to total weight of seeds to estimate the oil content of each sample.

3.5. Data Analysis

The mean values of genotypes were used for the analysis of variance (ANOVA). The analysis of variance and covariance for individual characters and for the character pairs respectively, were carried out using the mean values of each plot following the method given by Panse and Sukhatme (1964). The significance of the differences among all the genotypes was determined by the F-test using the error variance.

Broad-sense heritability was calculated using both genotypic and phenotypic variances. The genetic (or genotypic) variance in a cultivar trial is the variance of the cultivar effects and is denoted by σ^2_G . The phenotypic variance in a cultivar trial is the variance of cultivar means across replications. It is denoted as σ^2_P and because means are based on plot measurements, which contain both G's and e's, σ^2_P contains both the genetic variance and a portion of the residual variance as:

$$\sigma^2_P = \sigma^2_G + \sigma^2_e/r$$

Where, σ^2_e is the plot residual or error variance from the ANOVA, and r is the number of replications. σ^2_G and σ^2_e were estimated from the ANOVA as follows:

Source of variation	Df	Mean square	Expected mean square
Replicates	$r-1$		
Genotypes	$g-1$	MS_G	$r\sigma^2_G + \sigma^2_e$
Residual	$(r-1)(g-1)$	MS_e	σ^2_e

Thus, to estimate σ^2_G :

$$\sigma^2_G = (MS_G - MS_e)/r$$

σ^2_e is estimated directly as the error variance of the experiment.

The repeatability of a variety trial is the proportion of the variation among line means that is due to the variation in genotype effects. This statistic, that also denoted broad-sense heritability (H), is calculated as:

$$H = \sigma^2_g / \sigma^2_p \\ = \sigma^2_g / (\sigma^2_g + \sigma^2_e/r)$$

3.5.1. Genetic diversity analysis

Multivariate analysis using D^2 statistics Mahalanobi's (1936) was used for assessing the genetic divergence between genotypes. The intra and inter cluster distances were calculated by the formula given by Singh and Choudhary (1977). Based on the genetic distance, all the genotypes were grouped into different clusters using Tocher's method (Rao, 1952). In addition to clustering the mean intergroup distances corresponding to the groups formed was estimated and the relative contribution of each character to genetic divergence was established using the analysis of canonical variables.

3.5.2. Stability analysis

The data was analyzed using the stability analysis model of Eberhart and Russell (1966). Using this analysis, the sum of square due to G x E were portioned into individual genotypes (X_i), regression of environmental means (β_i) and deviation from regression (S^2_d). The regression coefficients (β_i) and mean square deviation from regression (S^2_d) were used to define genotype stability.

The environmental mean was the mean of all genotypes in each environment. The pooled error was used to test the hypothesis that the mean square deviation did not differ significantly from 0 at 0.05 and 0.01% probability levels. The t-test employing the standard error of regression coefficient was used. It was assumed that genotype effects were fixed and year effects were random.

All tests were performed using software Genes (Cruz, 2001) and Microsoft Windows 2010.

CHAPTER 4: RESULTS

4.1. Genetic divergence in *Jatropha curcas*, a biofuel feedstock in Kenya

4.1.1. Introduction

In Kenya, as the rest of the world, biofuels have attracted scientists, investors and policy makers as a substitute to the depleting fossil fuels by offering a measure of liquid renewable fuel that has the potential to reduce greenhouse gas effect and offer energy security. The important biofuel generated from plant biomass are biodiesel and bioethanol. There are a number of plant species yielding oil, which can provide biodiesel. In this plant category, the property of the tropical physic nut (*Jatropha curcas*) is being promoted in Kenya by several sectors of the economy.

It is believed it was first introduced into Kenya from Central America by Portuguese sailors in the 16th century, but there are no records of the exact source or where it was first planted. In recent years, subsequent introductions have been made but no reliable information is available. Both seed and oil yields of this crop have steadily remained unpredictable and low and do not represent the full genetic potential of the species. A number of factors are responsible for these low yields, include, the use of genotypes with low genetic potential, lack of proper agronomic practices and the effect of environment on the genotypes.

The genetic improvement of *Jatropha* begins with the selection of parents and formation of the base population generating segregating populations in which superior lines are selected, making knowledge on dissimilarity between the parents particularly important. Studies on genetic diversity are therefore of great importance in breeding programs, since

they permit the identification of appropriate parents for hybrids with greater heterotic effect and with greater segregation in recombination, allowing the appearance of transgressive genotypes (Cruz and Carneiro, 2003).

In view of the importance of jatropha in Kenya, the purpose of this study was undertaken to identify suitable breeding parents having diverse characters through grouping according to Mahalanobis dissimilarity measure in order to indicate the relative contribution of the response variables to genetic divergence analysis.

4.1.1.1. Analysis of variance

The analysis of variance indicated highly significant differences for all the 14 characters.

4.1.1.2. Means and coefficient of variations

The genotypes were subjected to summary statistics for the quantitative traits and a table of means was obtained (Table 2).

Table 2: Mean values for various plant characteristics of different *J. curcas* accessions

Genotypes	PH(cm)	G.D(cm)	NB	LF	D.F	F:M	F/P	SY(Kg)	SW(100)	SM (%)	Oil content (%)
KJ1	106.9	5.9	5.4	9.7	118	18.2	32	0.2	51.8	6.2	35.5
KJ2	116.5	7.9	6.2	12.3	104	24.3	26	0.18	47.5	5.8	28
KJ3	112.5	4.9	3.1	8.1	110.5	47.4	47	0.46	64.2	6.1	42.8
KJ4	70.5	6.2	3.2	7.9	116	20.9	33	0.32	45.2	5.6	36.5
KJ5	64.5	5.8	4.9	8.2	104	17	31	0.21	66.2	5.8	33.6
KJ6	69.8	7.9	5.4	12.4	116	24.6	72	0.25	66	6	32.4
KJ7	125.2	7.8	5	11.7	110.5	22.2	21	0.21	59.7	6	31.7
KJ8	116.1	5.9	4.6	7.8	107	16.2	16	0.17	58.6	5.7	38.8
KJ9	112.4	6.6	6.7	13.4	118	23.6	15	0.26	60.4	6.1	29
KJ10	117.4	4.7	3.2	6.1	104	19.5	15	0.26	55.4	6	29.2
KJ11	113.3	6.9	6	12.4	110.5	24.2	19	0.23	56.9	5.2	30.4
KJ12	76.5	5.4	3.2	8	116	15.6	13	0.22	56.9	6.6	38.3
KJ13	116.9	6.4	4.3	8.1	104	16.9	35	0.22	57.6	6.2	36.2
KJ14	121.1	6	6	7.7	110	17.7	13	0.25	60	6.2	36.7
KJ15	123.4	5.5	2.7	3.7	113	38.9	12	0.19	64.2	6.2	23.7
KJ16	125.4	7.7	5	14.9	105	29.5	16	0.25	58.3	6.3	34.9
KJ17	116.5	7.9	6.2	9.5	111	24.3	16	0.26	57	6.4	36.1
KJ18	120.5	4.9	3.1	4	110	27.4	16	0.28	54.9	6.6	27.3
KJ19	80.5	6.2	3.2	12.3	108	20.9	13	0.32	62	8.2	38.6
KJ20	114.5	5.8	4.9	8.1	100	47	31	0.21	63	7.1	40.9
KJ21	149.8	7.9	5.4	7.9	111	24.6	22	0.25	48.6	6.4	36.4
KJ22	95.2	7.8	5	8.2	107	22.2	21	0.23	33.7	6	19.3
KJ23	78	5.9	4.6	12.4	110	16.2	16	0.17	31.4	5.9	36.6
KJ24	112.4	6.6	6.7	11.7	104	23.6	15	0.26	37.1	6.1	36.9
KJ25	117.4	4.7	3.2	7.8	116.5	19.5	25	0.16	35.5	6.7	35.4
KJ26	123.3	6.9	6	13.4	110.5	24.2	19	0.23	55.4	6.5	36.2
KJ27	146.5	5.4	3.2	6.3	108	15.6	13	0.32	36.9	6.6	31.8

Table 2 continued

Genotypes	PH(cm)	GD(cm)	NB	IF	D.F	F:M	F/P	SY(Kg)	SW(100)	SM (%)	Oil content (%)
KJ28	136.9	6.4	4.3	12.4	109	21.9	15	0.22	36.9	6.5	34.4
KJ29	76	6	6	8	111	17.7	13	0.25	37.6	6.5	29.4
KJ30	243.4	5.5	2.7	8.1	107.5	18.9	12	0.29	30	7.2	27.5
KJ31	285.4	7.7	5	7.7	110	19.5	18	0.25	34.2	7.2	27.4
KJ32	220.5	4.9	3.1	7.9	104	27.4	16	0.28	38.3	6.8	31.1
KJ33	170.5	6.2	3.2	8.2	107.5	30.9	13	0.12	57	6.8	19.6
KJ34	154.5	5.8	4.9	12.4	115.5	27	11	0.21	34.9	7	32.3
KJ35	269.8	7.9	5.4	11.7	116	34.6	12	0.25	32	6	31.4
KJ36	285.2	7.8	5	7.8	120.5	32.2	12	0.23	33	6.9	27.7
KJ37	246.3	5.9	4.6	13.4	113	26.2	12	0.27	38.6	6.8	37
KJ38	292.4	6.6	6.7	6.3	116	23.6	12	0.26	33.7	6.3	37.8
KJ39	217.4	4.7	3.2	12.4	120	19.5	12	0.16	31.4	6.8	29.3
KJ40	251.1	6.9	6	8	119	34.2	13	0.13	37.1	6.9	36.7
KJ41	236.5	5.4	3.2	8.1	116.5	25.6	13	0.2	45.5	6.4	32.7
KJ42	236.9	6.4	4.1	7.9	114	21.9	13	0.22	63	6	31.2
KJ43	251.1	6	6	8.2	113	17.7	13	0.25	48.6	6.3	27.3
KJ44	243.4	5.5	2.7	11.4	114	18.9	12	0.19	43.7	6.4	31.3
KJ45	225.4	7.7	5	11.7	121.5	29.5	12	0.25	41.4	6	32.3
KJ46	236.5	5.4	3.2	7.8	117.5	25.6	13	0.12	47.1	6.3	24.4
KJ47	236.9	6.4	4.1	13.4	120	21.9	15	0.22	45.5	6.9	27.7
KJ48	143.4	5.5	2.7	7.8	121	18.9	12	0.19	63	6.8	37
KJ49	285.4	7.7	5	12.4	119	24.3	18	0.25	58.6	6.7	37.8
Grand mean	161.03	6.3	4.5	9.5	112.2	24.5	19	0.23	48.5	6.4	32.7
CD (5%)	21.2	1.4	n.s	3.5	14.8	3.7**	2.5**	n.s	13.9	1.4	1.7

The details about the range, mean, genetic variability, heritability and genetic advance are presented in Table 3. The characters are discussed in the following sections.

4.1.1.3. Plant height (cm)

As shown in Table 3 the genotypes exhibited a wide range of variability for this trait with overall mean of 161.01 cm. The range in the collection varied from 64.5 cm to 285.5 cm. The genotypic and phenotypic coefficients of variations for this trait were 17.7% and 18.7% respectively. This trait exhibited a high heritability of 89 with expected genetic advance of 37.6 and a mean genetic advance of 23.3%.

4.1.1.4. Girth diameter (cm)

As shown in Table 3 the girth diameter recorded overall mean of 6.29cm. The range collection varied from 4.65cm to 7.85cm. The genotypic and phenotypic coefficients of variations for this trait were 15 % and 18% respectively. This trait showed heritability of 68.9 with expected genetic advance of 1.1 and a mean genetic advance of 17.4 %.

4.1.1.5. Leaf type

As shown in Table 3 the germplasm accessions displayed a wide range of variation for this character with overall mean of 9.514. The range in the collection varied from 3.7cm to 14.9cm. The GCV and PCV were 28.3% and 34.4% respectively. This character exhibited a moderate heritability of 67.7 with moderate expected genetic advance of 3.11 and a genetic advance expressed over mean of 32.7 %.

4.1.1.6. Number of branches per plant

As shown in Table 3 a moderate range of variation was observed for this character. The Number of branches varied from 2.7 to 6.65 in the collection with overall mean of 4.52 branches per plant. The GCV and PCV for this trait were 22.1% and 31.7% respectively. This trait showed a very a low heritability of 48.5 and low expected genetic advance of 1 with mean genetic advance of 21.5%.

4.1.1.7. Days to (50%) flowering

As shown in Table 3 the genotypes displayed a wide range of variation for this character with overall mean of 7.66 days. The range in the collection varied from as early as 104 days to as late as 185.5 days. The GCV and PCV were 31.3% and 37.8% respectively. This character exhibited a high heritability of 68.6% with moderate expected genetic advance of 2.78 and a genetic advance expressed over mean of 36.3%

Table 3: Genetic parameters of growth and yield components in *Jatropha curcas*

Character	Mean	Range	PCV (%)	GCV (%)	Heritability (Broad sense)	GA (20%)	GA (%Mean)	F-Value
Plant height (cm)	161.02	64.5-285.5	18.7	17.7	89	37.6	23.3	17.24**
Girth diameter (cm)	6.32	4.65-7.85	18	15	68.9	1.1	17.4	5.45*
Number of branches	4.52	2.70-6.65	31.7	22.1	48.5	1	21.5	2.88
Leaf type	9.51	3.70-14.9	34.4	28.3	67.7	3.11	32.7	5.19*
Days to flowering (M ² -)	112.2	104.0-121.5	37.8	31.3	68.6	2.78	36.3	5.36*
Female: Male flower ratio	24.47	15.5-47.3	47.8	41.5	75.2	5.25	50.5	7.08*
Number of fruits per plant	18.56	11.2-72.0	24.6	21.8	78.6	2.52	27	8.38*
Seed yield (Kg)	0.231	0.052-0.281	0.18	0.11	0.38	0.066	0.99	0.225
Seed weight(100) (g)	48.4	33.0-66.7	45.3	34.3	91.1	28	57.9	21.48**
Seed moisture (%)	6.4	1.6-2.6	18.8	10.6	31.9	0.18	8.53	1.94
Oil content (%)	32.74	48.9-98.3	18.1	17.8	95.8	18.1	24.2	46.68**

4.1.1.8. Female: Male flower ratio

As shown in Table 3 the germplasm accessions displayed a wide range of variation for this character with overall mean of 24.7. The range in the collection varied from 15.5 to 47.3. The GCV and PCV were 41.5% and 47.8% respectively. This character exhibited a high heritability of 75.2% with moderate expected genetic advance of 2.52 and a genetic advance expressed over mean of 50.5%.

4.1.1.9. Number of fruits per plant

As shown in Table 3 the genotypes displayed a wide range of variation for this character with overall mean of 18.56 fruits. The range in the collection varied from 10 to 25. The GCV and PCV were 21.8% and 24.6% respectively. This character exhibited a high heritability of 78.6 with moderate expected genetic advance of 2.52 and a 27% advance expressed over mean of 27%.

4.1.1.10. Oil content

As shown in Table 3 the genotypes displayed a wide range of variation for this character with overall mean of 32.74 fruits. The range in the collection varied from 10 to 50. The GCV and PCV were 17.8% and 18.1% respectively. This character exhibited a high heritability of 95.8 with moderate expected genetic advance of 18.1 and a 55% advance expressed over mean of 24.2%.

4.1.2. Character association

The genotypic and phenotypic correlation coefficients were computed between characters studied and the data is presented in Table 4. Graphical representation of genotypic and phenotypic correlations between yield and yield components and explained in the following sections where genotypic correlation is indicated by 'g' and phenotypic correlation with 'p':

4.1.2.1 Association between seed yield and yield components

Significant positive genotypic and phenotypic correlations were recorded between seed yield with number of branches per plant ($g = 0.413, p = 0.616$), seed yield with flowering ($g = 0.686, p = 0.690$), seed yield with total dry matter yield

$p = 0.434$), seed yield with number of fruits per plant ($g = 0.5580$, $p = 0.186$), seed yield with seed moisture ($g = 0.459$, $p = 0.469$) and seed yield with oil yield per plant ($g = 0.692$, $p = 0.639$) as shown in Table 4.

The magnitude of correlation with seed yield was highest in case of oil yield per plant with correlation values of ($r = 0.692$ and $r = 0.639$) at genotypic and phenotypic levels respectively.

4.1.2.2. Associations among yield components

Table 4 further shows that days to 50% of flowering had a positive and significant genotypic and phenotypic correlation with female: male flower ratio ($g = 0.302$, $p = 0.325$), days to 50% of flowering had a positive and significant genotypic and phenotypic correlation with number of fruits per plant ($g = 0.554$, $p = 0.561$) and days to 50% of flowering had a positive and significant genotypic and phenotypic correlation with seed yield ($g = 0.686$, $p = 0.690$).

Table 4: Genotypic (G) and phenotypic (P) correlation coefficients between plant growth and yield components for selected *Jatropha curcas*.

Character	Genotype	GD	NH	LF	DF	F:M	NF	SY	SW	SM	Oil Content
	Phenotypic										
Plant Height	Genotypic	0.02	0.926*	0.133*	0.21	-0.203	0.114	0.327	0.132	0.05	0.286
	Phenotypic	0.69	0.909*	0.091	0.20	0.273	0.105	-0.304*	0.124	0.045	-0.253
Girth Diameter	Genotypic		0.649*	0.115	0.19	0.181	0.18	0.357*	0.064	0.011	0.318
	Phenotypic		0.614*	0.15	0.18	0.346*	0.166	0.382*	0.066	0.102	0.276
Number of Branches	Genotypic			0.175	0.21	0.068	-0.011	0.413*	0.107	0.018	0.348**
	Phenotypic			0.147	0.20	0.058	0.004	0.346*	0.096	0.017	0.322**
Leaf Type	Genotypic				0.04	0.187*	0.175	0.131	0.172*	0.065	0.098
	Phenotypic				0.07	0.250*	0.221*	0.028	0.064	0.151	-0.008
Days to Flowering	Genotypic					0.302*	0.554*	0.686*	0.306*	0.316*	0.055
	Phenotypic					0.325*	0.561*	0.690*	0.079	0.285	0.467**
Female: Male	Genotypic						0.961*	0.411*	0.254*	0.028	0.044
	Phenotypic						0.965*	0.434*	0.008	0.225*	0.198**
Number of Fruits	Genotypic							0.558*	0.311*	0.122	0.023
	Phenotypic							0.186	0.03	0.274*	0.302**
Seed Yield	Genotypic								0.144	0.459*	0.692**
	Phenotypic								0.095	0.469*	0.639**
Seed Weight (100)	Genotypic									-0.488	0.239**
	Phenotypic									0.219*	0.272
Seed moisture	Genotypic										-0.053
	Phenotypic										0.198
Oil content	Genotypic										1
	Phenotypic										1

(**, * at significant at the 0.5 and 1 % level, respectively)

NB. Plant height (cm), Girth diameter (cm), Days to flowering (50%), Seed yield (Kg), Seed Weight 100 (g), Seed moisture (%), oil content (%).

4.1.3. Assessment of genetic diversity through Mahalanobis D^2 .

The mean values for different quantitative characters were utilized for working out genetic distance between pairs of genotypes. The D^2 values between any two varieties were calculated as the sum of squares of the differences between the mean values of all the characters and used for final grouping of the genotypes. Estimation of D^2 values was carried out for 49 genotypes.

4.1.3.1 Contribution of different characters towards divergence.

The different quantitative characters with their respective per cent contribution towards divergence are presented in Figure 2 and Table 5. The relative ranking of different character components of D^2 has shown that among the studied variables, most genetic divergence was contributed by leaf type by 22.12%, number of branches by 19.24% and seed yield by 17.56%. The variables with lowest contribution to the genetic divergence were oil content by 2.42% and moisture content by 4.12%.

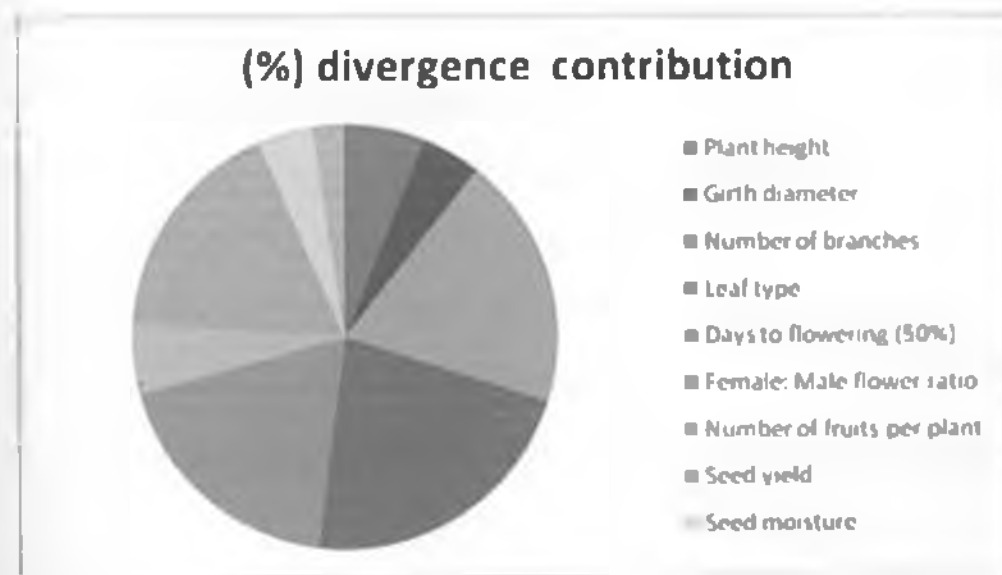


Figure 2: Percentage divergence contribution of variables under study

Table 5: Relative contribution of variables to genetic divergence in *Jatropha curcas* found in Kenya

Response variable	Relative contribution of the response variables to the divergence (%)
Plant height	6.12
Girth diameter	4.6
Number of branches	19.24
Leaf type	22.12
Days to flowering (50%)	12.10
Female: Male flower ratio	6.52
Number of fruits per plant	5.20
Seed yield	17.56
Seed moisture	4.12
Oil content	2.42

4.1.3.2. Grouping of the germplasm

Following the method of dissimilarity suggested by Tocher (Rao, 1952) 49 genotypes were grouped into four clusters by treating estimated D^2 values as the square of the generalized distance. The genotypes were collected from different regions across Kenya, East Africa, India, Madagascar and Mexico (Table 1 (a) and (b)). There is a wide distribution of genotypes in different groups, indicating wide diversity of genotypes.

Maximum number of 20 genotypes was included in cluster III. The Mexican genotypes seemed to congregate in cluster IV. Cluster II contained minimum number of 6 genotypes while cluster I with 9 genotypes contained collections from East Africa, Madagascar and one from Mexico. This pattern indicated that in certain cases there was no association between geographical distribution of genotypes and their genetic diversity. Genotypes from Africa, and Asia selected under diverse regions grouped together. However, in some cases, the genotypes especially from Mexico clustered together indicating that the genetic diversity in these genotypes is very similar. These genotypes have been isolated from their presumed progenies for many years and seem to have evolved diverse genetic diversity due to geographical isolation and genetic drift.

Table 6: Grouping of 49 jatropha genotypes

Group	Genotypes	Mean distances
I	KJ22,KJ23,KJ24,KJ28,KJ29 ,KJ30,KJ31,KJ32, KJ34	56.99
II	KJ2,KJ7,KJ8,KJ26, KJ27 , KJ36	126.24
III	KJ1,KJ3,KJ4,KJ5,KJ6,KJ9,KJ10,KJ11,KJ12,KJ13,KJ14,KJ15,KJ16,KJ17,KJ18KJ19,KJ20,KJ21,KJ25, KJ33	78.32
IV	KJ35,KJ37,KJ38,KJ39,KJ40,KJ41,KJ42,KJ43,KJ44,KJ45,KJ46, KJ47,KJ48,KJ49	52.12

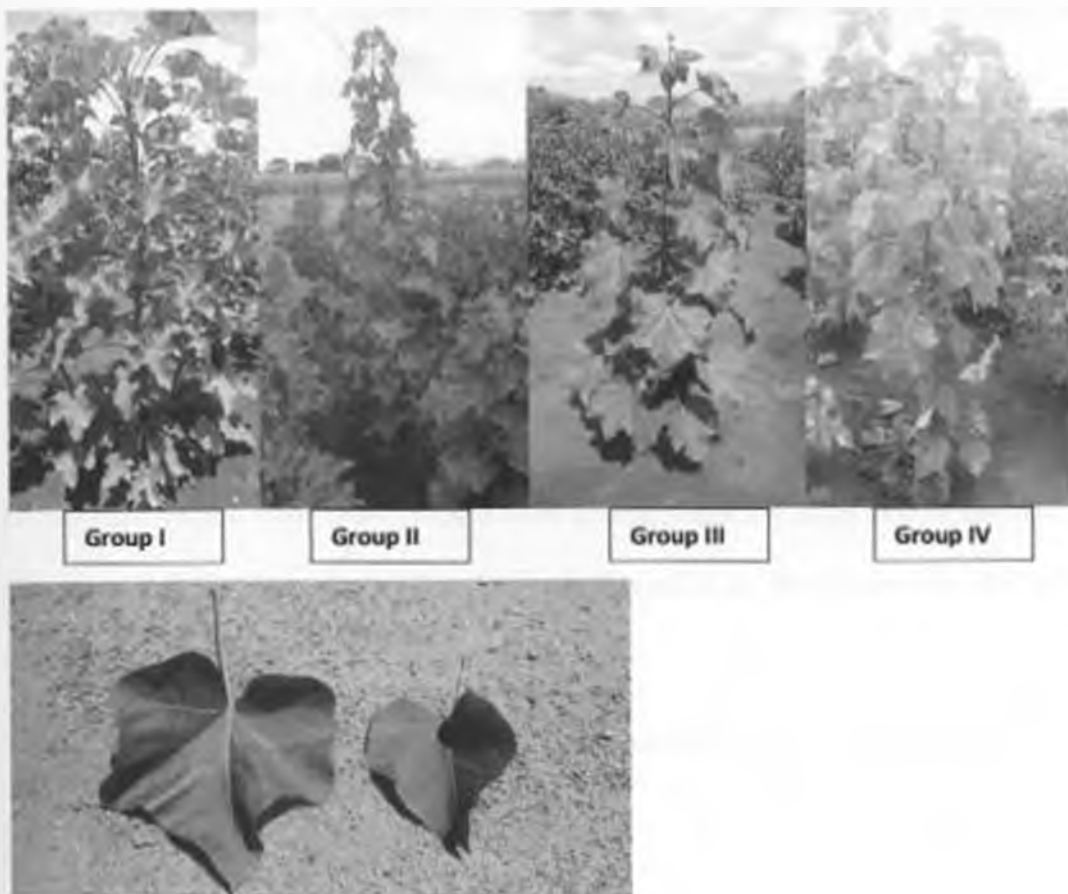


Plate 2: Variation in the germplasm found in Kenya

4.1.3.3 Interrelation of groups

The averages D^2 values of intra and inter clusters are given in Table 7. The intra and inter cluster D^2 values among the four clusters showed that, cluster II which comprised of 6 genotypes revealed the maximum genetic diversity with intra cluster distance of $D = 38.28$ among themselves, followed by cluster IV of $D = 33.28$ and cluster III with minimum intra cluster divergence of $D = 25.30$ while cluster I had the lowest intra cluster divergence of $D = 16.40$. As indicated by the inter cluster D^2 values of the four clusters, the highest inter cluster generalized distance of $D = 53.2$ was observed between cluster II

and cluster IV as well as between cluster I and II of 43.2 indicating that group II is the most diverse and would yield interesting parental materials for hybridization.

The identification of superior genotypes based on genetic divergence is the most appropriate strategy to start a breeding program according to Rahman et al., (2002). Crosses between highly divergent genotypes, in particular between groups II and IV and between groups I and II would yield segregants with a high genetic potential. The lowest D^2 distance values were observed between groups I and IV (6.5) and II and III (8.4) indicating that the intercross of the genotypes that make up the groups may not produce superior genotypes in segregating generations.

Table 7: Average intra and inter cluster Mahalanobis D^2 value and distance

	I	II	III	IV
I	16.40 (268.96)	43.2 (1,866.24)	28.20 (795.24)	6.5 (42.25)
II		38.28 (1465.34)	18.40 (338.56)	53.2 (2,830.24)
III			25.30 (640.09)	37.1 (1,376.41)
IV				33.28 (1,107.56)

*- figures in parenthesis are Mahalanobis' D^2 values

4.1.3.4. Cluster wise means

As shown by Table 8 cluster wise means revealed variation in the characters observed. Cluster IV had high mean value for plant height (188.36), girth (13.27), plant canopy (386.21), number of branches (4.64) and days to flowering (122.22). Cluster II had high means female: male ratio (38.04), number of fruits per plant (34.68) and seed yield (0.482). Cluster I had dwarf plants of height (78) and high oil content (37.5) while cluster III recorded genotypes with poor characters. Hybridization between genotypes in group I, II and IV may yield high genetic potential and would result into faster growing, high yielding plant of average height.

Table 8: Cluster wise mean values of 11 characters in *Jatropha curcas* L.

Groups	I	II	III	IV
Plant height (cm)	78	116.21	122.21	188.36
Girth diameter (cm)	12.27	12.14	10.02	13.27
Plant Canopy(cm)	254.2	268.2	248.12	386.21
Number of branches	2.72	3.24	2.11	4.64
Leaf type	132.7	149.42	112	126.02
Days to flowering (50%)	110.2	98.4	118.2	122.22
Female: Male flower ratio	15.21	38.04	18.5	12.0
Number of fruits per plant	10.46	34.68	8.6	12.18
Seed yield (Kg)	0.45	0.482	0.325	0.225
Seed moisture (%)	6.6	6.8	5.7	6.5
Oil content (%)	37.5	34.67	27.53	33.04

4.1.3.5. Principal canonical variable

Using canonical variable method, the correlation coefficient between the principal canonical variables and the response variables used in the study of genetic divergence in jatropha were determined as shown by Table 9. Significant correlations indicate contribution of each response variable to the genetic divergence in jatropha. It shows that the variables that were significant for more than one canonical variable were number of branches, leaf type and number of fruits. These variables contributed most to genetic divergence whereas seed yield, seed moisture and oil content contributed the lowest to the divergence.

Table 9 : Correlation coefficient between the principal canonical variables and the response variables used in the study of genetic divergence in jatropha.

Response variable	Canonical variables			
	1	2	3	4
Plant height (cm)	-0.16	0.36**	0.40	-0.43
Girth diameter (cm)	-0.47*	-0.27	-0.59	-0.05
Number of branches	-0.63*	0.07	0.49**	0.12
Leaf type	0.67**	0.12	-0.70**	0.14
Days to flowering (50%)	0.68**	-0.17	0.26	-0.26
Female : Male flower ratio	-0.49**	0.27	0.22	0.18
Number of fruits per plant	0.15	0.60**	-0.21	0.40*
Seed yield (Kg)	-0.44**	0.44	0.32	-0.18
Seed moisture (%)	0.37*	0.44	-0.10	0.56
Oil content (%)	-0.26	0.67	0.05	0.61*

* and ** Significant by the t test at 5% and 1 % probability, respectively.

4.1.4. Discussion of genetic divergence

All the genotypes displayed considerable amount of differences in their mean performance with respect to all the quantitative characters studied. This was exhibited by the analysis of variance, which indicated highly significant mean differences for these traits indicating that the genotypes under study were genetically diverse.

This variation provides scope for selection of superior and desired genotypes for the plant breeder for further crop improvement programs. It is apparent from Table 3 that the phenotypic coefficients of variation (PCV) were higher than genotypic coefficients of variation (GCV) for all the characters under study, which suggests that the traits considered under the present study are influenced more by the environment. High values of GCV and PCV were obtained for seed weight, female: male flower ratio, days to flowering (50%), and leaf type indicating that selection of these traits would yield the highest genetic advance. The wide range of variability observed in this investigation is in accordance with the work done by other researchers including Rao et al. (2008). The presence of abundant genetic variability as indicated by the above results for the said characters provides greater scope for improvement by direct selection. On the other hand oil content exhibited moderate variability in the present investigation with seed yield showing least variability.

The heritability estimates in 'broad sense' comprises both additive and non-additive gene effects. Knowledge of heritability of a trait is an essential tool, which can be employed by the breeder in improving the trait under specified situation. In the present study, all the

characters showed low to very high estimates of broad sense heritability ranging from 0.38% to 91.10% (Table 3). The characters namely, plant height, girth diameter, days to flowering (50%), and female: male flower ratio, number of fruits per plant, 100 seed weight and oil content showed very high estimates of broad sense heritability (Table 3). These results indicate that the selection based on these traits would be effective as they are likely to be controlled by additive genes. If a character has higher heritability estimate, in self-pollinated crop (*Jatropha curcas* is both self and cross pollinated), it is more likely to be controlled by additive genetic variation, thus selection could be more suitable through phenotypic means (Falconer, 1960). High heritability coupled with high percent of mean genetic advance was obtained for female: male flower ratio and seed weight (Table 3).

Selection of these characters would be effective as they are less likely to be influenced by the environment, have a large additive gene variance and are therefore the most heritable. High heritability coupled with moderate genetic advance over mean was observed for leaf type and days to 50% flowering (Table 3). However plant height, girth diameter, number of fruits per plant and oil content exhibited high heritability coupled with low genetic advance expressed over mean. In these two scenarios, are indicative of the presence of non-additive gene effects and high genotype - environment interactions, factors that would slow down phenotypic selection. Therefore, these characters are difficult to select for and are likely to be influenced more by gene-environment interaction.

Yield is the ultimate product in which the breeder is interested. It is a highly complex quantitative character, which is governed by polygenes; its expression depends largely on the environment, as they are highly sensitive to the environment. As in many crops and plants, selection of superior genotypes based on yield is not always effective. For rational approach towards the improvement of yield, selection has to be operated through associated or secondary characters. As shown by Table 4, the secondary traits likely to contribute to selection of yield are: number of branches per plant, number of fruits per plant, male: female flower ratio, days to 50% flowering, seed moisture and oil content. In the present study, genotypic and phenotypic correlations among 14 characters of jatropha were computed (Table 4). In all the cases, the difference between phenotypic and genotypic correlations was very narrow. This indicates that either genotypic or phenotypic correlation can be used as a measure of association between different traits. Oil yield per plant exhibited highly significant positive associations with seed yield per plant both at genotypic and phenotypic levels. This implies that selection for high yield would also lead to selection for high oil content. This would be an extremely interesting selection index in jatropha.

Similarly, high correlation between number of fruits per plant and seed yield per plant at both genotypic and phenotypic levels was observed which indicates that the number of fruits per plant may be reliable indicator of seed yield as would be expected. Studies by Achten et al., 2010 made similar observation.

This clearly indicates that increased number of fruits per plant will increase seed yield and which in turn would result into increased oil yield. It may be possible to constitute a selection index for high oil yield in jatropha if all the three factors are considered. Again, this is a very important economic factor, since identification of high oil yield genotypes is the ultimate goal in Jatropha production. Number of branches per plant also showed significant positive association with seed yield. From this perspective it also means selecting for high number of fruits and branches, it indirectly means selection for high oil yield.

Genetic divergence analysis helps in assessing nature of diversity in order to identify the genetically diverse genotypes for their use in plant breeding programmes. Among the various characters studied, the most important characters contributing to the total divergence were; leaf type (22.12%), number of branches (19.24%) and seed yield (17.56%) as shown by Table 5.

The variables with lowest contribution to the genetic divergence were oil content (2.42%) and moisture content (4.12%). The above results imply that in order to select genetically diverse genotypes, the material should be screened for the important traits like leaf type, number of branches per plant and seed yield.

As indicated by the inter cluster D^2 values of the four clusters, the highest inter cluster generalized distance ($D = 53.2$) was observed between cluster II and cluster IV as well as between cluster I and II (43.2) indicating the importance of group II as source of parental material. The identification of superior genotypes based on genetic divergence is the most

appropriate strategy to start a breeding program according to Rahman et al., (2002). The lowest D² distance values were observed between groups I and IV (6.5) and II and III (8.4) indicating that the intercross of the genotypes that make up the groups may not produce superior genotypes in segregating generations.

Based on cluster wise analysis, cluster I had dwarf plants of height (78) and high oil content (37.5) while cluster III recorded genotypes with poor characters. Hybridization between genotypes in group I, II and IV is highly potential and would result into faster growing, high yielding and average height varieties. Significant correlations using principal canonical variable indicate contribution of each response variable to the genetic divergence in *Jatropha curcas*. It shows that the variables that were significant for more than one canonical variable were number of branches, leaf type and number of fruits. These variables contributed most to genetic divergence and the variables seed yield, seed moisture and oil content as those with lowest contribution to the divergence

4.1.5. Conclusions

Based on the observation made in this study for the assessment of genetic divergence in *Jatropha* germplasm found in Kenya, it can be concluded that there exist genetic divergence in the 49 genotypes studied. Based on grouping patterns cluster IV, II and I may serve as potential parents for new gene combination which will greatly contribute towards the improvement program of *Jatropha* for biodiesel production.

4.2. Evaluation of genotype-environment (G×E) interactions of *Jatropha curcas* L. in the agroecological environments of Kenya.

4.2.1. Introduction

Among species in *Jatropha* genus, only one species has been exploited in agriculture. Physic nut (*Jatropha curcas* L.) is cultivated for biodiesel oil, while its seed cake can be used as organic fertilizer and gasified to produce bioenergy. Originating in the tropical climate of Central America, *Jatropha* has been introduced into different climates such as humid and subtropics (Heller, 1996). As *Jatropha* seed is rich in hydrocarbon molecules, it is used as straight vegetable oil and or as raw material for the trans-esterification process to produce a variety of products including biodiesel, glycerine, organic fertilizer and animal feed after treatment to remove curcin (Makkar et al., 1998; Makkar et al., 2008; Makkar et al., 2009; Tiwari et al., 2007).

The potential to spur rural economy and create a state of self-sustainability is immense. Successful commercial cultivation is dependent on use of best yielding and stable genotypes which can be identified by growing the various accessions in different environments under replication. As an underutilized resource, *Jatropha* can be produced profitably on commercial scale in tropical environments. In natural conditions where *Jatropha* has been adopted in the wild in Kenya, it grows into a height of 4m with wide canopy and many branches. It is observed to resist or tolerate insect pests, diseases and drought and does not exhibit invasiveness. In this study it has been shown that growth and yield of *Jatropha* are limited by both biotic and abiotic stresses.

In Kenya, variations in climatic conditions are wide and have been divided into agro-climatic conditions. Climatic coefficient shifts are variable factors during the crop growth period which affect the yield and other characters of the crop. Hence, the yield of jatropha is affected by climatic factors and because of lack of an organized breeding program, the effect of these factors is high and yields are low. Observation of growth of the current cultivars raises questions about which ones are widely adapted across the varying agro-ecological environments and which ones should be selected for particular environment

Furthermore ranking of the mean performance of the genotypes vary from one location to another location, indicating a strong genotype x environment interaction. In analyzing genotype x environment (GxE) interactions one is able to characterize the available genotypes and deduce which ones have a minimal variance for yield across different environments and therefore can be a considered stable (Gupta et al., 1977; Sahagnia et al., 2006).

The stability methods can be divided into two major groups: univariate and multivariate stability statistics (Lin et al., 1986). Knowledge on the components of the genotype environment (GxE) interaction is of great importance for genetic breeding but provides no detailed information on the performance of each cultivar under varying environmental conditions (Cruz et al., 2004). The analyses of adaptability and stability are therefore important and necessary for the identification and recommendation of superior genotypes in different environments (Eberhart and Russell, 1966; Finlay and Wilkinson, 1963; Freeman and Perkins, 1971; Perkins and Jinks, 1968; Rocha et al., 2005).

Jatropha is cultivated under variable conditions, and one aspect of domestication is the performance of each provenance under different conditions. In general, a wide genetic base is essential to prevent inbreeding depression and allow for adaptation to changing environmental conditions and to altering markets for tree species products (Dawson et al., 2009). However, published information on this aspect of *Jatropha* is very scarce, other than the Senegal and Cape Verde tests reported by Heller (1996). For Kenya to fully exploit the potential of *Jatropha curcas* L. as a biofuel there is need to evaluate the stability of performance of the current genotypes and determine their genotype x environment interactions.

The present study was intended to estimate the nature and magnitude of variability and stability of growth and yield of 49 *Jatropha* genotypes as shown by Table 1 tested in two different agro-climatic sites. Observations were recorded and the results obtained are presented under the following headings:

4.2.2. Means and coefficient of variations

The genotypes were subjected to summary statistics for the quantitative traits and a table of means was obtained. The highest mean plant height of 161 cm was recorded for Kibwezi, while Thika recorded the least mean plant height of 47.7 cm. The analysis of variance, showed significant ($P < 0.05$) differences between and within the sites. There were significant ($P < 0.05$) differences in stem girth of the genotypes between and within sites. The highest mean stem girth of 6.3 cm was recorded for Kibwezi; while Thika recorded the least mean stem girth growth of 3.2 cm. There were significant ($P < 0.05$) differences in the number of branches per plant of the within and between the sites.

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Kihwezi recorded 4.5cm while Thika 1.9cm. There were significant ($P<0.05$) differences in the type of the leaves among the genotypes. All the genotypes used in the study recorded a mean range of 3.7 and 13.4 across the two sites as shown in Table 10.

Table 10: Mean values for four common variables recorded across the two sites.

Genotypes	PH(cm)		GD(cm)		NB		LF	
	Kibwezi	Thika	Kibwezi	Thika	Kibwezi	Thika	Kibwezi	Thika
KJ1	106.9	52.4	5.9	3	5.4	1.8	9.7	9.7
KJ2	116.5	47.7	7.9	3.9	6.2	1.8	12.3	12.3
KJ3	112.5	49.5	4.9	2.3	3.1	3.4	8.1	8.1
KJ4	70.5	50.1	6.2	3.1	3.2	2	7.9	7.9
KJ5	64.5	58.1	5.8	2.4	4.9	1.9	8.2	8.2
KJ6	69.8	37.6	7.9	4	5.4	1.3	12.4	12.4
KJ7	125.2	47.4	7.8	3.9	5	2.6	11.7	11.7
KJ8	116.3	50.5	5.9	2.9	4.6	2.3	7.8	7.8
KJ9	112.4	48.1	6.6	3.3	6.7	2.2	13.4	13.4
KJ10	117.4	40.4	4.7	2.3	3.2	1.7	6.3	6.3
KJ11	113.3	47	6.9	3.4	6	2.3	12.4	12.4
KJ12	76.5	46.8	5.4	2.7	3.2	1.5	8	8
KJ13	116.9	48.4	6.4	3.2	4.3	2.1	8.1	8.1
KJ14	121.1	48.5	6	3	6	2.2	7.7	7.7
KJ15	123.4	57.6	5.5	2.7	2.7	1.7	3.7	3.7
KJ16	125.4	44.1	7.7	1.9	5	1.2	12.9	14.9
KJ17	116.5	40.3	7.9	3.9	6.2	2	9.5	9.5
KJ18	120.5	51.7	4.9	2.5	3.1	1.8	4	4
KJ19	80.5	45.7	6.2	3.1	3.2	2	12.3	12.3
KJ20	114.5	38.3	5.8	2.9	4.9	2.8	8.1	8.1
KJ21	149.8	47.3	7.9	4	5.4	1.8	7.9	7.9
KJ22	95.2	47.3	7.8	3.9	5	1.5	8.2	8.2
KJ23	78	47.9	5.9	2.9	4.6	1.5	12.4	12.4
KJ24	112.4	58.1	6.6	3.3	6.7	2.1	11.7	11.7
KJ25	117.4	37.6	4.7	2.3	3.2	2.2	7.8	7.8
KJ26	123.3	46.8	6.9	3.4	6	1.7	13.4	13.4
KJ27	146.5	48.4	5.4	2.7	3.2	1.2	6.3	6.3
KJ28	136.9	48.5	6.4	3.2	4.3	2	12.4	12.4
KJ29	76	57.6	6	3	6	1.8	8	8
KJ30	243.4	44.1	5.5	2.7	2.7	2	8.1	8.1
KJ31	285.4	49.3	7.7	3.9	5	2.8	7.7	7.7
KJ32	220.5	51.7	4.9	2.5	3.1	1.8	7.9	7.9
KJ33	170.5	45.7	6.2	3.1	3.2	2	8.2	8.2
KJ34	154.5	38.3	5.8	2.9	4.9	1.8	12.4	12.4

Table 10 continued

Genotypes	PH(cm)		GD(cm)		NB		LF	
	Kibwezi	Tbilisi	Kibwezi	Tbilisi	Kibwezi	Tbilisi	Kibwezi	Tbilisi
KJ35	269.8	47.3	7.9	4	5.4	2	11.7	11.7
KJ36	285.2	47.3	7.8	3.9	5	2.8	7.8	7.8
KJ37	246.3	47.9	5.9	2.9	4.6	1.8	13.4	13.4
KJ38	292.4	47.7	6.6	3.3	6.7	1.5	6.3	6.3
KJ39	217.4	49.5	4.7	2.3	3.2	1.5	12.4	12.4
KJ40	253.3	50.1	6.9	3.4	6	2.1	8	8
KJ41	236.5	58.1	5.4	2.7	3.2	2.2	8.1	8.1
KJ42	236.9	37.6	6.4	3.2	4.3	1.7	7.9	7.9
KJ43	251.1	47.4	6	3	6	1.2	8.2	8.2
KJ44	243.4	51.5	5.5	2.7	2.7	2	11.4	11.4
KJ45	225.4	48.1	7.7	3.9	5	1.8	11.7	11.7
KJ46	236.5	46.4	5.4	2.7	3.2	2	7.8	7.8
KJ47	236.9	47.2	6.4	3.2	4.3	2.1	13.4	13.4
KJ48	143.4	39.3	5.5	2.7	2.7	2.2	7.8	7.8
KJ49	285.4	49.3	7.7	3.9	5	1.7	12.4	12.4
Mean	161	47.7	6.3	3.2	4.5	1.9	9.5	9.5
Max	292.4	58.1	7.9	4	6.7	2.8	13.4	14.9
Min	64.5	37.6	4.7	2.3	2.7	1.2	3.7	3.7

4.2.3. Pooled analysis of variance (ANOVA)

In the combined analysis of variance over locations and years (Table 11), environmental effects were more highly significant than genetic ones. However, there was significant genetic variance for all traits measured. G x E interactions were highly significant for leaf type and plant height and moderately significant for days to 50% flowering, seed yield and oil content. Table 11 also shows that linear component of the G x E was significant for all the traits measured but was highly significant for leaf type and plant height. This also indicates that there is tremendous genetic variation for these traits. Broad sense

heritability based on the Kihwezi trial data only was highest for oil content (95.8) and lowest for seed yield (0.38) as shown in Table 3.

Table 11: Analysis of variance pooled across two environments for growth and yield components in 49 jatropha genotypes.

Source	d.f	Mean sum of squares				
		Height	Leaf type	Days to flowering	Seed yield	Oil content
Genotypes(G)	48	112.82**	4.47**	111.59**	24.89**	1.00*
Environment(E)	1	9330.23**	5.07**	9430.27**	0.41*	0.43*
GxE	49	245.27**	1.82*	418.14**	0.18*	0.21*
E + (GxE)	50	819.28*	2.12*	1888.32**	0.20*	0.23*
E(Linear)	1	18860.60**	10.24**	51434.66**	1596.52**	0.84*
GxE (linear)	48	434.06**	0.86*	675.86*	4.89**	0.28*
Pooled deviation	49	52.66**	2.79*	150.30**	1.24*	0.03
Pooled error	96	180.43	0.67	195.56	0.37	0.02

**,* Indicate significance at 1 and 5% level of significant respectively.

4.2.4. Variability in growth and yield components of jatropha genotypes

Figure 6 shows the mean minimum and maximum day temperatures as a deviation from 15°C for the two sites where the 49 *Jatropha curcas* accessions were sown. The two sites show diverse temperatures during plant growth. The Kibwezi site had higher mean temperatures than the Thika site with high peaks in January and December and lowest temperatures in July for the two years. The Thika site had the lowest temperatures throughout plant growth. Plant height, days to flowering, female: male flower ratio, seed yield and oil content were significantly influenced by temperature. During the cold months of June, July and August the plants shed leaves and growth was minimal (Table 12). Growth was inhibited at the Thika site when temperatures dropped below 13°C. Low mean temperatures, coupled with other factors such as low pH (5.35) makes the Thika site, unsuitable for jatropha cultivation.

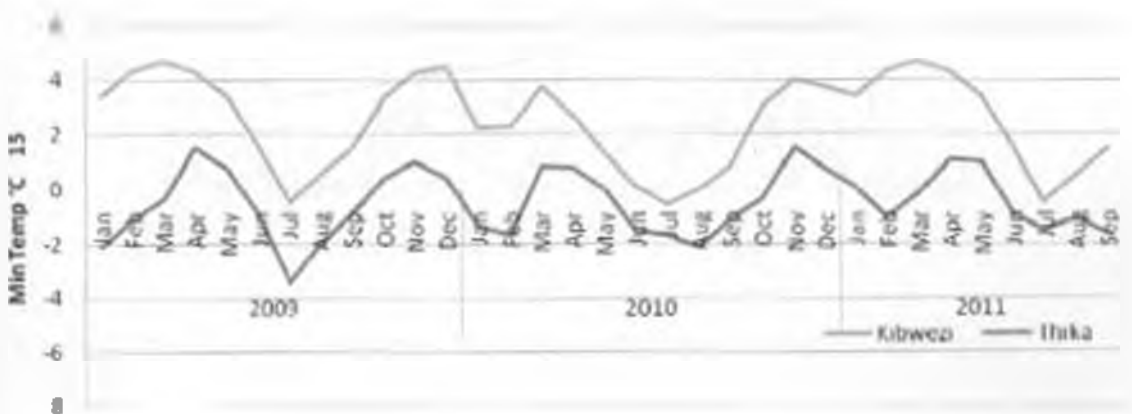


Figure 2: Maximum and minimum temperature variation trend for the period of field trials in Kibwezi and Thika.

Table 12: Coefficient of correlation between growth and yield components and monthly mean temperature during flowering and fruiting period.

	Height	No. of branches	Leaf type	D.F (50%)	F:M	F/P	Seed yield	oil Content
April	0.469**	0.123	0.177	0.343*	0.316**	0.551	0.814*	0.165**
May	-0.311*	0.132	0.141	0.338	0.141*	0.451	0.088*	0.076
June	0.021	-0.218	0.186	0.535*	-0.717*	0.452	0.582	0.345
July	-0.227	-0.162	0.283	-0.317	-0.216*	0.005	-0.41*	-0.147

NB Plant height (cm), Days to flowering (50%), Seed yield (Kg), oil content (%).

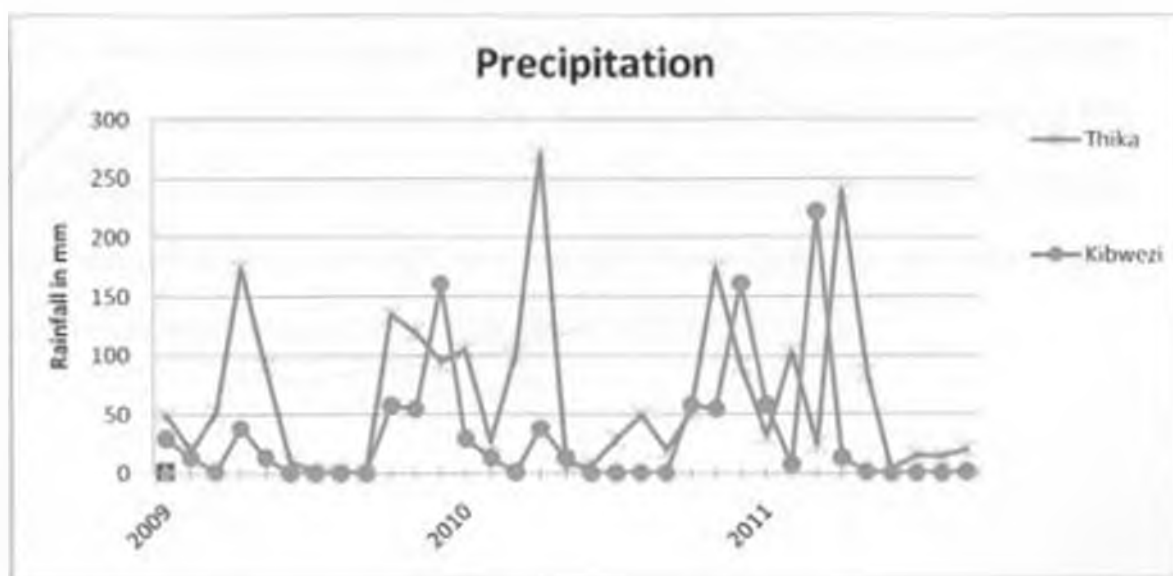


Figure 3: Precipitation distribution for the period of trials in Kibwezi and Thika.

4.2.5. Genetic parameters of growth and yield components

An overview of the total variation in genetic parameters is given in Table 3. The table shows that phenotypic coefficient of variation (PVC) was not very different from the

genotypic coefficient of variation (GCV). Female: male flower ratio and 100 seed weight gave the highest PCV and GCV. Oil content, 100 seed weight and plant height gave the highest broad-sense heritability of 95.8, 91.1 and 89 respectively, while seed yield gave the lowest. Overall, oil content and 100 seed weight were the most highly significant traits indicating that the two traits showed the highest genetic variation.

4.2.6. Coefficient of correlation between growth and yield components of jatropha genotypes.

Phenotypic coefficient of correlation between growth characters, seed yield and oil content are shown in Table 13. Seed yield was positively and significantly correlated days to 50% flowering (0.751), female: male ratio (0.662), number of fruits per plant (0.525) and number of branches (0.337) in that order. Oil content was positively significantly correlated with days to 50% flowering (0.433), female: male ratio (0.742) but had a weak insignificant positive correlation with seed yield. The number of fruits per plant was positively significantly correlated with female: male ratio and seed yield as would be expected (Kaushik et al., 2007, Rao et al., 2008).

Table 13: Coefficient of correlation between growth and yield components of *Jatropha curcas* across genotypes.

	Seed yield	No. of branches	Leaf type	D1 (50%)	F:M	F/P	Seed moisture	oil Content
Plant height	0.107							
Number of branches	0.337**	0.011	-					
Leaf type	0.037	-0.226	0.103					
Days to flowering (50%)	0.751**	0.054	0.212	0.165				
Female: Male flower ratio	0.662**	0.124**	0.235	0.194	0.36			
Number of fruits per plant	0.525*	0.348**	0.306	0.257**	0.578**	0.142*		
Seed moisture	0.432	0.376	0.198	0.419	0.494	0.239	0.752	-
Oil content	0.311	0.194	0.386	0.433**	0.112	0.742	0.128	0.018

(**, * at significant at the 0.5 and 1 % level, respectively)

NB: Plant height (cm), Girth diameter (cm), Days to flowering (50%), Seed yield (Kg), Seed Weight-100 (g), Seed moisture (%), oil content (%).

4.2.7. Stability analysis

Table 16 (Appendix 1) shows the mean regression coefficients and residual mean square deviation for regression for stability analysis for 49 genotypes. The data revealed high mean values, regression coefficients close to unity and least mean square deviations from regression.

The superior genotype KJ1 (higher seed yield, oil content with early in maturity) shows higher mean values, regression coefficient higher than unity (2.85) and deviation from regression near to zero (0.01) which is numerically superior than the rest of the genotypes.

4.2.7.1. Genotypes responsiveness to environmental conditions

Table 14 shows the genotypes that were responsive to environmental conditions using the stability parameters of Eberhart and Russell (1966). Genotypes KJ1, KJ2, KJ5, KJ7, KJ10, KJ11, KJ15, KJ18, KJ20, K22, KJ23, KJ24, KJ28, KJ29, KJ31, KJ34, K36, KJ39, KJ40, KJ44, K47, K48 and KJ49 would be stable and have high mean performance under high potential environments, such as the Thika site, whereas Table 15 shows genotypes KJ4, KJ12, KJ16, KJ7, KJ25, KJ29, KJ32, KJ33, KJ34 and KJ41 would be stable with high mean yields under low potential environments, such as the Kibwezi site. Further stability parameters analyses are shown in Appendix 2 (Table 17...24).

Table 14: The mean yields, regression coefficient and deviations from regression for oil content in high potential environments

Genotypes	X-i	Bi	S ² d
KJ1	35.5	5.65**	0.0
KJ2	27.99	3.39**	0.0
KJ5	33.58	2.62*	0.1
KJ7	31.7	2.63*	0.0
KJ10	29.18	1.4	0.0
KJ11	38.8	2.5	0.0
KJ15	23.66	2.72**	0.0
KJ18	27.31	2.62*	0.1
KJ20	40.93	2.63*	0.0
KJ23	36.56	1.4	0.0
KJ24	36.88	2.5	0.0
KJ28	34.39	2.72**	0.0
KJ31	27.39	2.72**	0.0
KJ34	32.33	2.62*	0.1
KJ36	27.72	2.63*	0.0
KJ40	36.7	2.5	0.0
KJ47	27.72	1.4	0.0
KJ49	37.77	2.72**	0.0

Table 15: The mean yields, regression coefficient and deviations from regression for oil content in low potential environments

Genotypes	X-i	Bi	S ² d
KJ4	36.52	0	0.0
KJ12	38.28	0.02**	0.0
KJ16	34.92	0.0	0.2
KJ17	36.06	0	0.0
KJ25	35.4	0.02**	0.0
KJ29	29.39	0.0	0.2
KJ32	31.08	0.0	0.2
KJ33	19.56	0.0	0.0
KJ41	32.74	0.02**	0.0

4.2.8. Discussion of G×E and stability analysis

Phenotypic variation showed that oil content, plant height and 100 seeds weight demonstrated high broad sense heritability of 95.8, 91.1 and 89, respectively (Table 3). This is in close agreement to results reported in *J. curcas* (Rao et al., 2008). Oil content, 100-seed weight and plant height, in that order, had the highest genetic variation and can be successfully selected for in a breeding program. Days to 50% flowering, female: male flower ratio and number of fruits per plant were highly associated with seeds yield. However, the phenotypic correlation between seed yield and oil content was low. The genetic correlations between oil content and seed yield were high (0.692). There were also significant genetic correlations between oil content, days to 50% flowering, number of fruits per plant, number of branches and female: male flower as shown in Table 13. In this study, the genetic correlations reported between seed yield and female: male flower ratio (0.411) and between seed yield and number of branches (0.413) are close to the values of 0.48 and 0.61 respectively reported by Rao et al., 2008. It is clear from the results that direct selection for high number of fruits per plant, number of branches and female: male flower ratio will lead to selection for high seed yield notwithstanding environmental influences. Selection for high oil-content would lead to selection for high yield if genetic variance is considered, since both traits are greatly influenced by environmental variance as shown in Table 11. The results suggest that it may be possible to constitute a selection index for high-oil yield in *J. curcas* that would consider seed yield, number of branches and female: male flower ratio. This is an important economic factor, since identification of high oil yield genotypes is the ultimate goal in *J. curcas* production.

The stability analysis as shown in Table 14 indicated the presence of significant G x E interactions for all the characters studied. Higher magnitude of mean squares due to environments indicates considerable differences between environments for all the characters and that these characters were greatly influenced by environments (Carels et al., 2009; Ginwal et al., 2005; Kaushik et al., 2007; Kumar et al., 2008; Rao et al., 2008,). This suggests that there were large differences between the two environments and that the greater part of the genotypic response was a linear function of environments. The partitioning of mean squares (environments + genotype x environments) (Table 11) showed that environments (linear) differed significantly and were quite diverse with respect to their effects on the performance of genotypes for the majority of yield components (Rao et al., 2008). Furthermore, the higher magnitude of mean squares due to environments (linear) as compared to genotype x environment (linear) showed that linear response of environments accounted for the major part of total variation for majority of the characters studied. G x E (linear) component was higher than genotype x environment interaction for leaf type, plant height and oil content inferring that genotypes had a predictable performance for these traits. The significance of mean squares due to genotype x environment (linear) component against pooled deviation for leaf type, plant height and oil content confirms that the genotypes were diverse for their regression response to change with the environmental fluctuations (temperature and soil pH). Similarly, the significant mean squares due to pooled deviation observed for all the characters except for oil content suggested that the deviation from linear regression also contributed substantially towards the differences in stability of genotypes. Thus, both linear (predictable) and non-linear (un-predictable) components significantly contributed

to genotype x environment interactions observed for all the traits measured (Gauch HG., 2006). Genotypes, KJ1, KJ2, KJ5, KJ7, KJ10, KJ11, KJ15, KJ18, KJ20, KJ23, KJ24, KJ28, KJ31, KJ34, KJ36, KJ39, KJ40, KJ44, KJ47 and KJ49 showed high mean values, regression coefficients close to unity and least mean square deviation from regression for oil content in high responsive environments whereas genotypes KJ4, KJ12, KJ16, KJ17, KJ25, KJ29, KJ32, KJ33 and KJ41 would be stable and have high mean oil yields under low responsive environments such as the Kihwezi site .

4.2.9. Conclusion

Oil content, 100 seed weight and plant height had high significant genetic variation in the two environments. Indirect selection of high number of female: male flowers, 100 seed weight and high number of fruits per plant is likely to result into selection for high oil content. There was significant environmental, genotype and G x E interactions influence for all the traits measured. Both predictable and non-predictable components of G x E influenced the performance of the genotypes for all the traits. However, although the environmental variance was higher than the genotypic variance, the genotypic variance was highly significant for all the traits measured including oil content an indication that is possible to select in a breeding program the performance of genotypes based on their mean yields at the specific locations

The genotypes are likely to produce consistent yields under the diverse growing conditions. The stability parameters indicated that the growth and yield components measured were widely adapted to changing environments. Using the Eberhart and Russell

(Eberhart SA and Russell WA, 1966) model, for oil content, genotypes KJ1, KJ2, KJ5, KJ7, KJ10, KJ11, KJ15, KJ18, KJ20, KJ23, KJ24, KJ28, KJ31, KJ34, KJ36, KJ39, KJ40, KJ44, KJ47 and KJ49 showed high mean values and would be stable in high responsive environments whereas genotypes KJ4, KJ12, KJ16, KJ17, KJ25, KJ29, KJ32, KJ33 and KJ41 would be stable under low responsive marginal environments of Kenya such as the semi-arid areas.

CHAPTER 5: GENERAL CONCLUSIONS

5.1. General conclusions

The main objective of the study was to determine if there are variations in *Jatropha curcas* germplasm available in Kenya and to identify promising accessions for future genetic improvement work; to determine genotype-environment interactions of *Jatropha curcas* on growth and yield components of the current genotypes in order to avail agronomic recommendations to farmers and plant breeders. This study has demonstrated that *Jatropha curcas* germplasm in the country exhibit genetic diversity and forms four distinct cluster (I, II, III and IV). However, phenotypic attributes alone cannot demonstrate variability in plants and therefore genotypic analysis using molecular markers may reveal more about the genetic diversity present in the studied genotypes.

The stability analysis of the genotypes showed that growth and yield components were significantly affected by genotype x location interactions. The variance analysis indicated the existence of insignificant genetic variance among the genotypes, significant difference among the environments evaluated and a significant genotype-environment interaction. The presence of significant genotype-environment interaction demonstrates a different performance of the genotypes in the environments studied, justifying the need for the use of adaptability and stability techniques from the traditional concept, which considers a genotype with high yield capacity, low response to unfavorable environments and responsive to favorable environments as the ideal.

Nonetheless, this study has paved way for investigating the genetic distinctness as well as genotype-environment interaction studies of other genotypes coming from other countries or genotypes from other geographic background. This will aid greater understanding, which can be used to create variations through hybridization techniques to improve on our local germplasm.

5.2. Recommendations

The assessment of genetic divergence in *Jatropha* germplasm found in Kenya determined diversity present in the genotypes and grouped them based on morphological characteristics. The analysis further showed that there were genotypes that matured earlier than others presenting an opportunity to develop a breeding program that would result into fast maturing varieties. However, phenotypic knowledge alone may not be enough to describe genetic diversity of a plant like *Jatropha curcas* that is greatly influenced by environment. Therefore, there is need for further study using molecular markers to dissect the genetic diversity present in the Kenyan germplasm.

The analysis of GxE interaction identified *J. curcas* genotypes that are likely to show less environmental influences and are stable across environments in Kenya. There is need, however, to include many more genotypes, replications, locations and seasons in future. Nevertheless, there was also indication that seed yield and oil content have a genetic correlation and, therefore, it will be possible in the future to select for both high seed and oil high oil yielding genotypes stable across many environments. Parental lines possessing high seed yield and high oil content and with stable genetic performance

across Kenya's diverse environments will be chosen and included in a crossing program that will develop elite *J. curcas* hybrids. The identification of these genotypes and hybrids will form the basis of large scale commercialization of *J. curcas* to meet the growing biodiesel needs of the country.

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APPENDICES

Appendix 1 (Table 16) : Mean regression coefficients and residual mean square deviation for regression for stability analysis

Genotypes	Height			Leaf type			Days to flowering		
	X-i	Bi	S ² d	X-i	Bi	S ² d	X-i	bi	S ² d
KJ1	106.9	1.1	-31.68	9.7	1.2	57.3	6.35	-1.1	1.7
KJ2	116.5	1.2	-12.79	12.3	-0.1	24.5	9.05	0.3	1.1
KJ3	112.5	0.6	25.98	8.1	1.5	57.3	5.75	0.4	23.4
KJ4	70.5	1.2	-56.06	7.85	1.2	39.8	5.75	1.9	2.3
KJ5	64.5	0.9	-27.89	8.2	1.2	96.5	5.35	2.0	2.1
KJ6	69.8	-0.08	-32.03	12.4	1.4	34.2	10.3	-0.1	0.2
KJ7	125.2	1.2	13.3	11.7	0.8	61.9	9	1.1	-0.2
KJ8	116.3	1.8	-27.36	7.75	1.2	11.5	7.9	-0.8	0.0
KJ9	112.4	0.8	37.98	13.4	1.0	-4.2	12.1	2.63*	-0.2
KJ10	117.4	2.55*	8.2	6.25	1.5	7.1	5.25	1.9	4.4
KJ11	113.3	0.5	-52.43	12.4	1.3	58.7	10.8	2.0	2.0
KJ12	76.5	1.3	-47.41	7.95	0.4	7.8	6.85	1.0	1.2
KJ13	116.9	0.7	99.31	8.05	1.4	57.7	6.6	2.1	-0.1
KJ14	121.1	1.3	98.65	7.65	1.2	20.5	4.35	0.2	0.3
KJ15	123.4	0.5	-59.48	3.7	0.5	52.0	4.9	2.34*	1.0
KJ16	125.4	0.6	-52.77	14.9	0.5	30.5	12.7	0.1	2.0
KJ17	116.5	1.2	-56.06	9.51	1.2	39.8	7.66	1.9	2.3
KJ18	120.5	0.9	-27.89	3.96	1.2	96.5	7.9	2.0	2.1
KJ19	80.5	-0.08	-32.03	12.3	1.4	34.2	12.1	-0.1	0.2
KJ20	114.5	1.2	13.3	8.1	0.8	61.9	5.25	1.1	-0.2
KJ21	149.8	1.8	-27.36	7.85	1.2	11.5	10.8	-0.8	0.0
KJ22	95.2	0.8	37.98	8.2	1.0	-4.2	6.85	2.63*	-0.2
KJ23	78	2.55*	8.2	12.4	1.5	7.1	6.6	1.9	4.4
KJ24	112.4	0.5	-52.43	11.7	1.3	58.7	4.35	2.0	2.0
KJ25	117.4	1.3	-47.41	7.75	0.4	7.8	4.9	1.0	1.2
KJ26	123.3	0.7	99.31	13.4	1.4	57.7	12.7	2.1	-0.1
KJ27	146.5	1.3	98.65	6.25	1.2	20.5	7.66	0.2	0.3
KJ28	136.9	0.5	-59.48	12.4	0.5	52.0	6.6	2.34*	1.0
KJ29	76	0.6	-52.77	7.95	0.5	30.5	4.35	0.1	2.0
KJ30	243.4	1.3	98.65	8.05	1.2	20.5	4.9	0.2	0.3
KJ31	285.4	0.5	-59.48	7.65	0.5	52.0	12.7	2.34*	1.0

Genotypes	Height			Leaf type			Days to flowering		
	X-I	BI	S ² d	X-I	BI	S ² d	X-I	bi	S ² d
KJ32	220.5	0.6	-52.77	7.85	0.5	30.5	7.66	0.1	2.0
KJ33	170.5	1.2	-56.06	8.2	1.2	39.8	7.6	1.9	2.3
KJ34	154.5	0.9	-27.89	12.4	1.2	96.5	12.1	2.0	2.1
KJ35	269.8	-0.08	-32.03	11.7	1.4	34.2	5.25	-0.1	0.2
KJ37	246.3	1.8	-27.36	13.4	1.2	11.5	6.84	-0.8	0.0
KJ38	292.4	0.8	37.98	6.25	1.0	-4.2	6.6	2.63*	-0.2
KI39	217.4	2.55*	8.2	12.4	1.5	7.1	4.35	1.9	4.4
KJ40	253.3	0.5	-52.43	7.95	1.3	58.7	4.9	2.0	2.0
KJ41	236.5	1.3	-47.41	8.05	0.4	7.8	11.7	1.0	1.2
KJ42	236.9	0.7	99.31	7.85	1.4	57.7	11.8	2.1	-0.1
KJ43	251.1	-0.08	-32.03	8.2	1.4	34.2	5.22	-0.1	0.2
KJ44	243.4	1.2	13.3	11.4	0.8	61.9	6.8	1.1	-0.2
KJ45	225.4	1.8	-27.36	11.7	1.2	11.5	6.85	-0.8	0.0
KJ46	236.5	0.8	37.98	7.75	1.0	-4.2	6.6	2.63*	-0.2
KJ47	236.9	2.55*	8.2	13.4	1.5	7.1	4.35	1.9	4.4
KJ48	143.4	0.8	37.98	7.75	1.0	-4.2	4.9	2.63*	-0.2
KJ49	285.4	0.5	-59.48	12.4	0.5	52.0	12.7	2.34*	1.0

Table 16 continued

Genotypes	Seed yield			oil content		
	X-1	hi	S ¹ d	X-1	bi	S ¹ d
KJ1	16.07	2.85*	0.1	35.5	5.65**	0
KJ2	15.52	0.9	0.1	27.90	3.39**	0
KJ3	16.74	1.2	0	42.84	-0.6	0
KJ4	15.22	4.58**	0	36.52	0	0
KJ5	16.05	0.4	0.3	33.58	2.62*	0.1
KJ6	14.91	0.2	0.3	32.4	-4.5	0
KJ7	16.21	0.7	0.1	31.7	2.63*	0
KJ8	16.96	-2	0.1	38.79	-0.2	0
KJ9	14.91	2.93*	0.1	29.01	-0.6	0.1
KJ10	15.72	-0.7	0.6	29.18	1.4	0
KJ11	15.62	0.5	0	38.8	2.5	0
KJ12	16.37	0	2.1	38.28	0.02**	0
KJ13	15.57	0.6	0.6	36.16	-1.5	0
KJ14	15.97	1.7	0	36.68	-0.7	0
KJ15	16.7	1.3	0	23.66	2.72**	0
KJ16	15.97	0.8	0	34.92	0	0.2
KJ17	15.22	4.58**	0	36.06	0	0
KJ18	16.05	0.4	0.3	27.31	2.62*	0.1
KJ19	14.91	0.2	0.3	38.56	-4.47	0
KJ20	16.21	0.7	0.1	40.93	2.63*	0
KJ21	16.96	-2	0.1	36.39	-0.2	0
KJ22	14.91	2.93*	0.1	19.27	-0.6	0.1
KJ23	15.72	-0.7	0.6	36.56	1.4	0
KJ24	15.62	0.5	0	36.88	2.5	0
KJ25	16.37	0	2.1	35.4	0.02**	0
KJ26	15.57	0.6	0.6	36.18	-1.5	0
KJ27	15.97	1.7	0	31.75	-0.7	0
KJ28	16.7	1.3	0	34.39	2.72**	0
KJ29	15.97	0.8	0	29.39	0	0.2
KJ30	15.97	1.7	0	27.53	-0.7	0
KJ31	16.7	1.3	0	27.39	2.72**	0
KJ32	15.97	0.8	0	31.08	0	0.2
KJ33	15.22	4.58**	0	19.56	0	0
KJ34	16.05	0.4	0.3	32.33	2.62*	0.1
KJ35	14.91	0.2	0.3	31.41	-4.5	0
KJ36	16.21	0.7	0.1	27.72	2.63*	0

Genotypes	Seed yield			oil content		
	X-i	bl	S ² d	X-i	bl	S ² d
KJ37	16.96	-2	0.1	37	-0.2	0
KJ38	14.91	2.93*	0.1	37.77	-0.6	0.1
KJ39	15.72	-0.7	0.6	29.34	1.4	0
KJ40	15.62	0.5	0	36.7	2.5	0
KJ41	16.37	0	2.1	32.74	0.02**	0
KJ42	15.57	0.6	0.6	31.16	-1.5	0
KJ43	14.91	0.2	0.3	27.29	-4.5	0
KJ44	16.21	0.7	0.1	31.26	2.63*	0
KJ45	16.96	-2	0.1	32.33	-0.2	0
KJ46	14.91	2.93*	0.1	24.41	-0.6	0.1
KJ47	15.72	-0.68	0.6	27.72	1.4	0
KJ48	14.91	2.93*	0.1	37	-0.6	0.1
KJ49	16.7	1.3	0	37.77	2.72**	0

Appendix 2: Stability parameters analysis tables

The parameters investigated to establish the genotype stability included height, leaf type, days to flowering, seed yield and oil content (Tables 17a..17h). They revealed the following results:

(i) Height

- ($\beta_i > 1$) -genotypes responsive to the improvement of environmental conditions and recommended for favorable environments included:- KJ2, KJ4, KJ7, KJ8, KJ10, KJ12, KJ14, KJ17, KJ20, KJ21, KJ23, KJ25, KJ27, KJ30, KJ33, KJ36, KJ37, KJ39, KJ41, KJ44, KJ45 and KJ47

Table 17: Genotypes responsive to the improvement of environmental conditions and recommended for favorable environments based on height.

Genotypes	X-I	BI
KJ2	116.5	1.2
KJ4	70.5	1.2
KJ7	125.2	1.2
KJ8	116.3	1.8
KJ10	117.4	2.55*
KJ12	76.5	1.3
KJ14	121.1	1.3
KJ17	116.5	1.2
KJ20	114.5	1.2
KJ21	149.8	1.8
KJ23	78	2.55*
KJ25	117.4	1.3
KJ27	146.5	1.3
KJ30	243.4	1.3
KJ33	170.5	1.2
KJ36	285.2	1.2
KJ37	246.3	1.8

Genotypes	X-i	Bi
KJ41	236.5	1.3
KJ44	243.4	1.2
KJ45	225.4	1.8
KJ47	236.9	2.55*

- ($\beta_i < 1$) genotypes less responsive to the improvement of environmental conditions and recommended for unfavorable environments included: - KJ3, KJ11, KJ13, KJ15, KJ16, KJ24, KJ26, KJ28, KJ29, KJ31, KJ32, KJ40, KJ42 and KJ49.

Table 18: Genotypes less responsive to the improvement of environmental conditions and recommended for unfavorable environments based on height.

Genotypes	X-i	Bi
KJ3	112.5	0.6
KJ11	113.3	0.5
KJ13	116.9	0.7
KJ15	123.4	0.5
KJ16	125.4	0.6
KJ24	112.4	0.5
KJ26	123.3	0.7
KJ28	136.9	0.5
KJ29	76	0.6
KJ31	285.4	0.5
KJ32	220.5	0.6
KJ40	253.3	0.5
KJ42	236.9	0.7
KJ49	285.4	0.5

- ($\beta_i = 1$ - broad stable genotypes recommended for most environments included KJ1, KJ5, KJ9, KJ18, KJ 22, KJ 34, KJ 38, KJ 46.

Table 19: Broad stable genotypes recommended for most environments based on height.

Genotypes	X-i	Bi
KJ1	106.9	1.1
KJ5	64.5	0.9
KJ9	112.4	0.8
KJ18	120.5	0.9
KJ22	95.2	0.8
KJ34	154.5	0.9
KJ38	292.4	0.8
KJ46	236.5	0.8
KJ48	143.4	0.8

- $S^2d = 0$ showed that KJ10, KJ23, KJ39 and KJ40 were stable genotypes.

(ii) Seed yield

- ($\beta_i > 1$) and ($S^2d = 0$) - genotypes responsive to the improvement of environmental conditions and recommended for favorable environments include KJ1, KJ3, KJ4, KJ9, KJ14, KJ15, KJ17, KJ22, KJ27, KJ28, KJ30, KJ31, KJ33, KJ38, KJ46, KJ48 and KJ48.