KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF AGRICULTURE AND NATURAL RESOURCES FACULTY OF RENEWABLE NATURAL RESOURCES DEPARTMENT OF AGROFORESTRY

TRADITIONAL ECOLOGICAL KNOWLEDGE, FIELD AND MOLECULAR EVALUATION OF *Jatropha curcas* (L.) ACCESSIONS FROM GHANA

ERIC OWUSU DANQUAH MAY, 2010

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BSC. NATURAL RESOURCES MANAGEMENT

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DECLARATION

I do declare that, except references to other people's work which have been duly cited, this work submitted as a thesis to Department of Agroforestry, Faculty of Renewable Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi, for the degree of Master of Philosophy in Agroforestry, 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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(HEAD, DEPARTMENT OF AGROFORESTRY)

DEDICATION

I dedicate this thesis to my Parents- Mr and Mrs Owusu Boakye Danquah

ABSTRACT

Jatropha curcas L. production as a potential source of alternative fuel has gained popularity in Ghana. The government is collaborating with the private sector to develop about one (1) million hectares of *Jatropha* plantation throughout the country in the next 5-6 years. The questions left unanswered are: why will farmers grow *Jatropha*? What knowledge do farmers have about the plant? Where will the farmers source quality planting material from? It is likely farmers might have practical knowledge of the use of the plant which will be vital to researchers. Also it is likely there are variations in the local germplasm which can be used as a basis for improvement. The main objective of the study was to determine if there are variations or otherwise in *J. curcas* accessions collected from 10 regions of Ghana and to identify promising accessions for future genetic improvement work. To begin, a sociological survey on the Traditional Ecological Knowledge of the plant was conducted in all the ten regions of the country. It was identified that *J. curcas* was among the ten most important indigenous tree species in nine (9) out of the ten regions. It is mainly used for medicinal purposes and mostly found around homesteads. The farmers were aware that the plant can be used for bio-diesel production and showed interest to produce it for sale. Ninety (90) accessions collected from the ten regions of the country were planted in the field and evaluated for growth and yield performance. The analysis of data after ten months revealed that there were significant differences ($P \leq 0.05$) in the plant height, stem girth, number of branches, number of days to 50% flowering, number of fruits per cluster, fruit and seed yields of the accessions. These suggested variations in the germplasm used for the study. However, due to the shorter duration of the field evaluation, there was the need to employ further studies to gather additional evidence on the variations in the germplasm.

Molecular studies were therefore employed to provide an environmentally independent result to confirm the above results. Forty (40) accessions were selected based on the seed yield performance and the site (Regions) where the accessions were collected for the studies. Random Amplified Polymorphic DNA (RAPD) analysis on the forty (40) accessions with ten RAPD primers revealed an average polymorphism of 24.99%. Also the Genotype Genotype*Environmental (GGE) biplot analysis which incorporates divergence effect due to the genotype (PC1) and the divergence effect due to interactions between the genotype and field parameters (PC2) was 44.7%, which is not statistically significant (PC1 + PC2 should be more than 70% for the divergence among accessions to be statistically significant). These indicate very low genetic diversity among the accessions used in the study, indicating narrow genetic diversity of *J. curcas* germplasm in the country. It is therefore recommended that our local germplasm of *J. curcas* should be officially conserved whiles immediate efforts should be made to widen the genetic base through research. This can be done through introduction of accessions from countries such as Mexico and Central America which are the centers of origin of the plant. Also genetic resources of the plant can be introduced from India where much research and improvement on the plant has already been done. Through Inter-specific hybridization breeding of imported accessions and our local accessions the local germplasm can be improved. This will provide good quality planting material to both farmers and individuals to boost the production of the plant in the country to meet the government's aspiration of bio-diesel production from *J. curcas* seeds as an alternative source of fuel.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Humanity relies on a diverse range of cultivated (crop) species; at least 6000 of such species are used for a variety of purposes (Heller, 1996). Limited available information on many important but frequently neglected and underutilized crop species hinders their sustainable development and conservation. *J. curcas* (physic nuts) is one of these underutilized and neglected species despite its potential as source of bio-diesel (Heller, 1996).

The question that is frequently asked by even some of the elite in the field of agriculture/agroforestry is: does research on trees and tree crops for smallholders really pay off? To some, it is likely to assume it does not have a high rate of return. The evidence however shows otherwise. Alston and Pardy (2001) tabulated the rates of return to investments on various types of agricultural research and development studies especially in the field of tree and tree crop improvement. From 108 studies assembled it was observed that the average rate of return was 88%. The median rate of return was 33%. These rates are outstanding and compared favourably to the rate of return of field crops research, which is 74%. There is enough evidence to show that developing high quality germplasm of tree crops developed through research for farmers improved their product harvest and livelihoods (NAS, 1975; NAS, 1979**;** Thirtle *et al*., 2001; Place *et al*., 2002). Investment in research towards developing high quality yielding variety of *J. curcas* is likely to bring high rate of return to the farmers and the nation as a whole.

J. curcas belongs to the family Euphorbiaceae. It is thought to be native to Central

America and Mexico where it occurs naturally in the forests of coastal regions. However, *Jatropha* is pantropical now, and although toxic, it is widely planted as a medicinal plant. It is listed as a weed in Australia, India, Brazil, Fiji, Honduras,

Panama, El Salvador, Jamaica, Puerto Rico, and other parts of the Caribbean (Heller, 1996). Special interest has been shown in *J. curcas* as compared to other plants which can be used for similar purposes. This is because; it is drought resistant and thrives well on marginal lands (Heller, 1996). These advantages imply biodiesel can be produced on marginal lands with little or no effect on food production (Henning, 2002).

Until recently, in Ghana like most of the African countries, it was mostly used by the farmers as living fences around their homesteads and gardens, sometimes also around their fields, to protect crops against roaming animals. Benge (2006) observed that the recent unstable prices of petroleum product in the world market and the country, has accounted for the growing interest in *J. curcas* as a bio-diesel ―miracle tree‖ to help alleviate the energy crisis in the country and also generate alternative income for farmers in rural areas. However, there are not enough studies on *J. curcas* to guide this interest especially in Ghana.

1.2 *J. curcas* AS AN ALTERNATIVE FUEL SOURCE IN GHANA

As a measure towards developing an alternative source of fuel, the Government of Ghana is collaborating with the private sector with the aim of developing about 1 million hectares of *Jatropha* plantation in the next 5-6 years within the entire country for bio-diesel production (COMPETE, 2008). Some of the questions left unanswered are; what preference do the farmers give *Jatropha* over the other indigenous trees?

What is the indigenous knowledge and uses of *Jatropha* in the country? Also where will the farmers source their planting material? It is likely farmers have indigenous knowledge which will be vital to researchers. Also it is likely they will plant from any available source. If the genetic resources of *J. curcas* found in the country are the same in terms of yield performance then it is worth doing so. But if there are differences then by planting inferior genotypes, the nation as a whole might be missing good opportunities for not using planting materials with higher yielding potential or with more desirable characteristics. Answering the above questions is likely to provide researchers with valuable information which will not only help in developing quality planting material, it will also aid in developing suitable agroforestry technologies to meet the farmers' needs. One of the main reasons for which the potential of *J. curcas* as a bio-diesel plant is not fully utilized is that apart from agronomic, socioeconomic and institutional constraints, planned crop improvement programs are lacking in Ghana. Also there is limited information available on agronomy and genetics of *Jatropha*. There is lack of bench mark descriptors and information on genetic variability (Jongschaap *et al*., 2007; Basha *et al*., 2009). The most important inputs for successful cultivation of *J. curcas* in Ghana will be the selection of appropriate planting material. Insights into the growth performance, genetic variability in relations to provenance of collection would be a critical input for the selection of appropriate genotypes for improvement. Clearly there seems to be more questions than answers and this call for a study to answer these questions.

1.3 THE ROLE OF AGROFORESTRY

—Agroforestry is a collective name for land use systems and technologies where woody perennial (trees, shrubs, bamboo, palm etc) are deliberately kept on the same management

unit as agricultural crops and/ or animals in a spatial arrangement or temporal sequence. The woody and non-woody components significantly interact both ecologically and economically (Lundgren and Raintree, 1982)‖

The above definition implies diversity, enhancing land use system, especially in the context of interspecies diversity, as it brings together crops, shrubs, trees and in some cases, livestock on the same piece of land. Through good management practices, optimum use of the land resource can be met whiles still conserving the basic resource on which production depends for future generations (Young, 1997). Sustainability, productivity and adoptability attributes of Agroforestry make it attractive to farmers (Raintree, 1987). An agroforestry technology is an intervention usually through scientific study to address the problem of a land use system (Nair, 1993).

J. curcas is a multipurpose tree which has received a lot of publicity from the media for some time now in the country for biodiesel production. Perhaps what should have preceded the above but yet to start is an improvement on the genetic resources of the plant in the country. Benge (2006) observed that the likely problem to affect large volume *J. curcas* production in Ghana is the fact that majority of the farmers are working on small pieces of farmlands. One way to address this is to identify a high seed and oil yielding accession and develop it into an acceptable variety for land use systems, so that when the plant is even used as hedge, farmers can produce enough quantities for the biodiesel industries on their small pieces of land. This land use intervention will not only help the country to take advantage of planting materials with more desirable qualities, it will also help small scale farmers to generate income to improve their livelihood. As observed by Garrity (2004) agroforestry approach or technologies should not only make sense ecologically and environmentally, but it should be able to make money to the farmers. It is hoped that studies on *J. curcas* genetic resources in the country, will help identify

unique accessions for subsequent improvement work. This is very likely to propel the adoption and production of the plant by farmers for the government to meet her aspiration of large seed volumes for biodiesel production.

1.4 OBJECTIVES OF THE STUDY

The general aim of this study was to establish if there are variations or otherwise in the germplasm of *J. curcas* in the country. The specific objectives were:

- To study Indigenous Knowledge, uses and niches of *J. curcas* in Ghana.
- To evaluate the growth and yield performance of *J. curcas* accessions from the ten regions of Ghana.
- To assess the genetic diversity in *J. curcas* accessions collected from the ten regions of Ghana using Random Amplified Polymorphic DNA (RAPD's) technique.

1.5 HYPOTHESES OF THE STUDY

- Farmers have useful indigenous knowledge of *J. curcas* in Ghana.
- There are variations within genetic resources of *J. curcas* found in the country.

The above hypotheses were tested through sociological surveys, field trials and laboratory analysis.

CHAPTER TWO

2. 0 LITERATURE REVIEW

2.1 GENERAL DESCRIPTION

The plant belongs to the *Euphorbiaceae* family, genus *Jatropha* and species *curcas.* It is a drought-resistant bush or small tree with spreading branches, and grows to a height of about 5m high under favorable conditions. It has a smooth grey bark, which gives out a whitish watery latex when cut. The shrub has a large green to pale-green lobed leaves with a length of about 6cm to 15 cm. The leaves are positioned alternately, with petiole length of about 6mm to 23 mm. When propagated from seed five (5) roots are formed, one taproot and four lateral roots. Plants propagated from cuttings normally develop only lateral roots with one perhaps developing into a pseudo-tap root that may reach only 1/2 to 2/3 the length of a normal tap root (Heller, 1996; Henning, 2002).

2.2 DISTRIBUTION AND ECOLOGY

Jatropha is considered to be native to Central America and Mexico where it occurs naturally in the forests of coastal regions, and it is not found in these forms of vegetation in Africa and Asia, where it is found only in the cultivated form. However, *Jatropha* is almost pan tropical now, and although toxic, it is widely planted as a medicinal plant. A non-toxic variety is reported to exist in Mexico and Central America (Makkar *et al.*, 2001). A species said to be endemic to Madagascar (Madagascars), *J. mahafalensis* is reported to have promising biofuel energy production (Heller, 1996). In many parts of Africa and elsewhere, *Jatropha* is widely planted as a hedge or living fence to protect field crops since the foliage is toxic to animals. It is considered as a weed in Australia, India, Brazil, Fiji, Honduras, Panama, El Salvador, Jamaica, Puerto Rico, and other parts of the Caribbean (Benge, 2006).

J. curcas shows a wide variation in growth, production and quality characteristics but has high ecological adaptability allowing growing in a wide range of conditions (Heller, 1996: Jongschaap *et al*., 2007). A provenances trial in India indicated only modest levels of genetic variations in the local germplasm, whiles wide variation was found between the Indian and Mexican accessions (Basha *et al*., 2009). It generally grows best on well-drained soils with good aeration but performs considerably well on marginal soils where other crops fail. Ranging from Tropical very dry to moist through Subtropical thorn to wet forest life Zones, *Jatropha* grows well with a minimum of 600 mm of rainfall per year, and it withstands long drought periods. With less than 600 mm it cannot grow except in special conditions like on Cape Verde Islands, where the rainfall is only 250 mm, but the humidity of the air is very high (rain harvesting) (Heller, 1996). *Jatropha* can withstand only a very light frost that causes it to lose all of its leaves, and the seed yield will probably sharply decline (Benge, 2006).

The current distribution shows that introduction has been most successful in drier regions of the tropics with an average annual rainfall between 300 mm and 1000 mm, and occurs mainly at lower altitudes (0-500 m) in areas with average annual temperatures well above 20^0C but can grow at higher altitudes and tolerates slight frost. It is not sensitive to day length. Locally, it is grown as a boundary fence or live hedge and can be used to reclaim eroded areas (Heller, 1996; Jøker & Jepsen, 2003; World Agroforestry Center, 2003).

Jatropha is fast growing and produce seeds after approximately 1–3 years, depending on rainfall conditions and the method of propagation (cuttings or seeds, respectively).

Jatropha grows almost everywhere even on sandy and saline soils and can grow in cervices of rocks (Jones and Miller, 1992). It is a drought resistant perennial shrub, which adapts well to semi and marginal sites. It is now found in many semi-arid and arid areas in Asia and Africa (Heller, 1996; Henning, 2002).

Jatropha sheds its leaves in the dry season that is why it is best suited to arid and semiarid conditions. When the leaves are shed, it provides mulch around the base of the plant. Organic matter from the shed leaves enhances earthworm activities in the soil around the root zone of the plants, which improves soil fertility (Jones and Miller, 1991). The plant loses its leaves so as to conserve moisture in the dry season bringing about reduced growth (Heller, 1996).

2.3 FLOWERING BEHAVIOUR

Flowering occurs profusely during the rainy season. It produces male and female flowers in the same inflorescence. The flowers forms are terminal, individual with the female flowers normally slightly larger especially in the hot seasons. Each female flower is surrounded by a number of male flowers. The number of female flowers per plant is relatively low; the ratio of male to female flowers is 29:1. Both flower sexes produce nectar as a reward to pollinators. Pollen flow between male and female flowers should occur for fruit and seed set. Bees and flies mediate pollen flow between male and female flowers in the same and different individuals (Solomon Raju and Ezradanam, 2002). Seed quality depends mostly on cross-pollination. Pollinator management is an important activity for seed set by quality and quantity. Fruit set rate is related to the number of female flowers produced by the plant. Some percentage of self-pollinated (geitonogamy) fruits drops off prematurely. Cross-pollinated fruits do not drop off and all develop to maturity. Each flower has 10 stamens arranged in two distinct whorls of five (each in a single column in the androecium and in close proximity to each other). In the gynaecium, the three slender styles are connate to about two-thirds of their length, dilating to a large bifurcate stigma. Female flowers have sepals up to 18 mm long. The ovary is 3-locular, ellipsoid, 1.5–2 mm in diameter and

the style bifid. Insects pollinate the shrub; it is believed that the shrub is pollinated by moths because of its sweet heavy perfume at night, greenish white flower, versatile anthers and protruding sexual organs, copious nectar and absence of visible nectar guides. In the absence of insects, in the greenhouse, fertilization will not occur without hand pollination, self-pollination may occur in a rare hermaphrodite flower (Dehgan and Webster, 1979; Raju and Ezradanam, 2002).The fruit is an ellipsoid capsule 2.5–3 cm long, 2–3 cm in diameter, initially yellow then turning black. The inflorescences form a bunch of green trilocular ellipsoidal fruits yielding approximately 10 or more ovoid fruits. The matured fruit generally produces 3 seeds per fruit. The fruits are initially green, later yellow and finally brown/black. Early summer season is ideal for fruit/seed collection (Achten *et al.*, 2007; Solomon Raju and Ezradanam, 2002; World Agroforestry Center, 2007).

2.4 FACTORS RELATING TO *Jatropha* YIELD

The reported yields of *Jatropha* range from extremely low to high. The Anuanom Industrial Bio product Limited (AIBPL) – Ghana, estimated that the wild genetic resources in Ghana can yield seeds between 3-5 t/ha/yr and has three main fruits bearing periods; four months cycle for each year (Onua-Amoah, 2006). Openshaw (2000) observed that with one rainy season per year, there will be only one annual fruiting; for two major rainy seasons or irrigated crops, up to three times fruiting in a year can be anticipated. Also the variation in yield may be explained by differences in growth and production related factors such as; the genotype of the plant and agronomic management such as pruning and irrigation. For example Beckford (2009) observed that pruning increased terminal branching, stimulated flowering which increased fruit yield by more than 25% and provided fruiting uniformity. According to Heller, (1996) production related factors likely to influence yields are; Nutritional level of the soil and moisture

level of the soil. Therefore appropriate genotype selection and management holds some key to the maximization of production of *J. curcas*.

2.5 GENETIC VARIABILITY IN *J. curcas*

Genetic variability may be correlated with life history traits, such as reproductive system, pollination, seed dispersal mode, geographical distribution, and local abundance. There are reported variations in trait of *J. curcas* accessions, however the diversity seems to be relatively small among accessions from similar environment or the same provenance (Basha and Sujatha, 2007; Sunil *et al.*, 2008). The initial variations in fruit and seed yields of candidate plus trees of *J. curcas* were found to be insignificant when the plants were grown on common site in India, indicating low variability in the genetic resource of the plant (Basha and Sujatha, 2007). Rao *et al.* (2008) conducted a thorough and extensive wild germplasm exploration survey on 32 high yielding candidate plus trees of *J. curcas* 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. Three distinct varieties of *J. curcas* are reported so far namely; the Cape Verde variety that has spread all over the world, the Nicaraguan variety with few but large fruits and non-toxic variety devoid of phorbol esters (Henning, 2008; Basha and Sujatha, 2007; Makkar *et al.*, 2001). At present, the varieties being used to establish plantations in Africa and Asia are inedible. The cake obtained after the extraction of oil cannot, therefore be used as a source of animal feed. However there exist naturally edible varieties in Mexico (Heller, 1996; King *et al.*, 2009)

2.6 ECONOMIC IMPORTANCE OF *J. curcas*

2.6.1 Diesel fuel

Since the oil crisis of the 1970s and recognition of the limitations of world oil resources, the technology of the extraction of vegetable oil has received special attention. Another argument for the cultivation of oil crops for energy purposes is the increasing global warming/greenhouse effect associated with fossil fuel. *J. curcas* is considered to be neutral in its net addition to greenhouse gasses because the carbon dioxide released in combustion was sequestered when growing the crop (Heller, 1996).

Special interest has been shown in the cultivation of *Jatropha* (physic nut) because the amount of oil contained in *Jatropha* seed or kernels are estimated to be ranging from 35-40% oil (Heller 1996). Also a range of 27-38% (Divakara *et al.*, 2009) and 30-35% (Foidl *et al.*, 1996; Mandpe *et al.*, 2005) of kernel weight have been reported however all gives an indication of greater output as compared to other bio-diesel crops. A study conducted by Washington State University (WSU), optimistically concluded that while many vegetable oils are used to manufacture biodiesel, a given amount of land will produce much more oil from *Jatropha* than from the common alternatives (soybeans, cotton seed, rapeseed, sunflower, groundnuts)(Benge, 2006).

Jatropha biodiesel emits about two-thirds (2/3) less in unburned hydrocarbons and almost half as much carbon monoxide and particulate matter as conventional diesel. It contains no sulfur and so emits none (http://www.jatropha.org). *Jatropha* nuts can be burnt as candlenuts, and the oil for the production of candles (Abbiw, 1990). Benge (2006) observed that, when the oil is used in place of firewood, it cuts down on the issue of deforestation and avoids the hazards caused to rural women who are subjected to indoor smoke-pollution from cooking by inefficient fuel and stoves in poorly ventilated space.

2.6.2 Medicine

Although toxic, *Jatropha* is referred to as physic or purging nut for its use as purgative/laxative, and is widely known as a source of medicine for treatment of a variety of ailments. A range of healing properties have been ascribed to leaf preparations for both topical application and ingestion (Benge, 2006; Abbiw, 1990). According to the sociological survey conducted as part of this study the medicinal uses identified included:

- Its sap serves as teeth cleaner.
- Smoke from its burnt leaves is used for treating convulsion.
- Powder developed from the bark of the plant is used for expulsion of worms.
- Its latex is applied to the gums for teething, particularly in young children.
- Lotion of crushed leaves of *J. curcas* in hot water is used in the treatment of guinea worm sores.
- Its oil is applied on itching skin.
- Mixture of seed with cereal pulp is used as purgative.
- Its sap is used to stop bleeding.
- Concoction from root and leaves are applied on sores.
- Ashes from burnt leaves are applied on sores.

2.6.3 Human Consumption

Jatropha can be toxic when consumed; however, a non-toxic variety of *Jatropha* is reported to exist in Mexico and Central America, it is said not to contain toxic Phorbol

esters (Makkar *et al.*, 2001). This variety is used for human consumption after roasting the seeds/nuts, and "the young leaves may be safely eaten, steamed or stewed." They are favored for cooking with goat meat, it is said to counteract the peculiar smell associated with it (Henning, 2006; Basha and Sujatha, 2007). As such, it is suggested by some that ―This non-toxic variety of *Jatropha* could be a potential source of oil for human consumption, and the seed cake can be a good protein source for humans as well as for livestock.‖ This non-toxic variety has not been studied as well as the toxic varieties; therefore, its properties and yields are relatively unknown and ―claims‖ unproven (Benge, 2006: Heller, 1996).

2.6.4 Pesticides

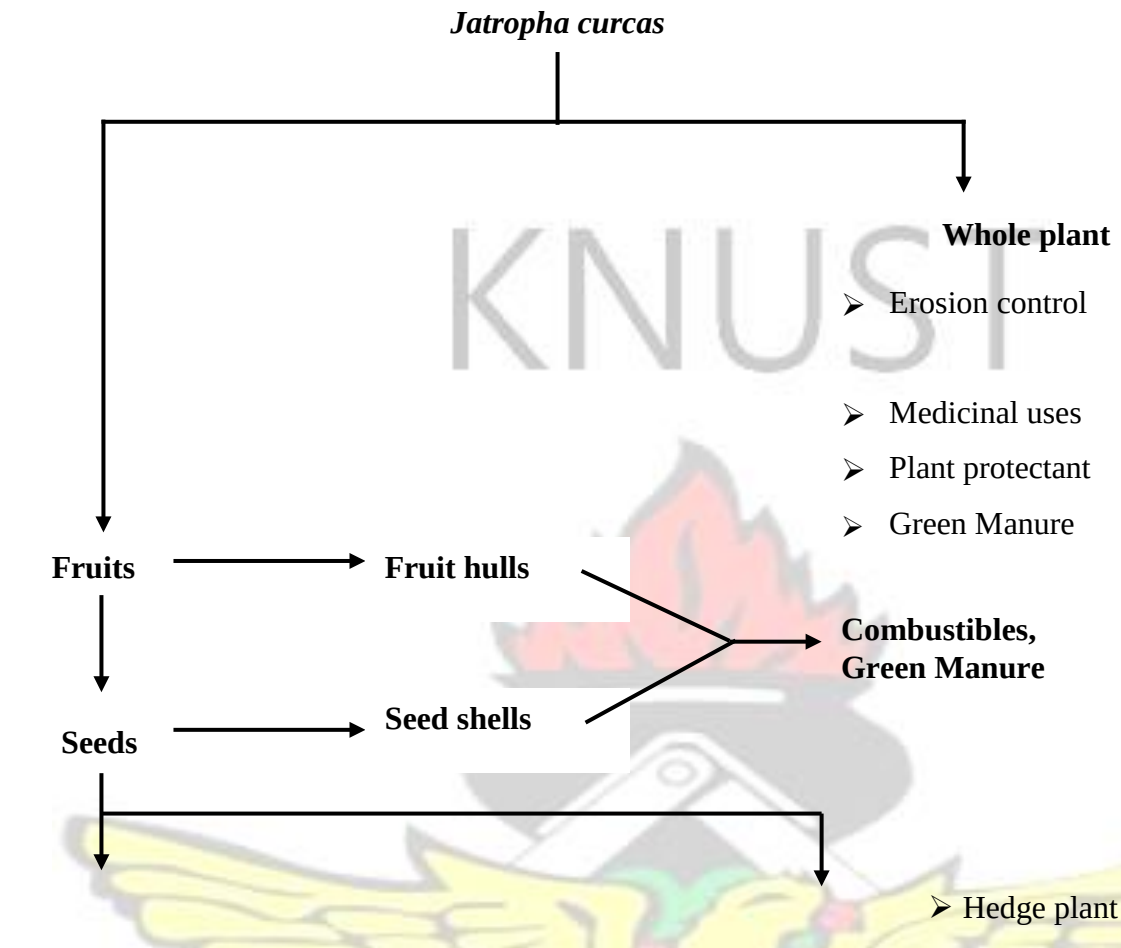
The oil and aqueous extract from oil has potential as an insecticide. For instance, it has been used in the control of insect pests of cotton including cotton bollworm, and on pests of pulses, potato, and corn. Methanol extracts from *Jatropha* seed (which contain biodegradable toxins) is undergoing test in Germany for the control of bilharziacarrying water snails. In addition, the pesticide action of the seed oil is also the subject of research of International Crops Research Institute for the Semi-Arid Tropics, (ICRISAT) in India (Benge, 2006).

2.6.5 Seed-cake

Seed-cake or press-cake is a by-product of oil extraction. *Jatropha* seed-cake contains curcin, a highly toxic protein similar to ricin in Castor, making it unsuitable for animal feed. However, it does have potential as a fertilizer. In addition, if available in large

quantities, it can also be used as a fuel for steam turbines to generate electricity. When processed as a cottage industry, the seed cake still contains approximately 15% oil, 5860 % crude protein (53-55 % true protein content) and the level of essential amino acids except lysine is higher than the Food and Agricultural Organization (FAO) reference protein (Henning, 2006; Heller, 1996). Nevertheless, without extensive processing, the seed cake is poisonous to animals, and if untreated, is only good as a source of organic fertilizer (Heller, 1996). *Jatropha* is not browsed, its leaves and stems are toxic to animals, but after treatment, the seeds or seed cake can be used as an animal feed. Being rich in nitrogen, the seed cake is also an excellent source of plant nutrient (Makkar *et al.*, 2001).

2.6.6 Different Forms of *J. curcas* Utilization



Modified from Kiefer, (1986)

Fig. 2.6.6. Different Forms of *Jatropha* Utilization

2.7 IMPORTANCE OF *J. curcas* AS A MULTIPURPOSE SPECIES

Multipurpose trees (MPT's) are trees, which make more than one substantial contribution, as products or service functions to the land use system in which they are grown. The tree component in Agroforestry can acquire environmental resources, which the crop alone will not acquire, thereby increasing the biological productivity of the system. Therefore trees in Agroforestry systems can lead to more close nutrient cycling than agriculture, and hence to more efficient use of nutrients (Young, 1997: Nair, 1993).

Jatropha is not a foliage crop, so when it is grown as living fence, it controls unwanted animals to fields and hence protect other valuable food crops. The shrubs also serve as windbreak, therefore reducing wind erosion. When *Jatroph*a is planted parallel to slopes to fix small earth or stone dam, they can control water erosion. The root of the shrub grow near to the ground surface, holding the soil particle together firmly, which slows surface run off during severe rainfall and thus water penetrates the soil and boosts harvest. *Jatropha* hedges are sources of humus and fertilizer to the soil, which improves the productivity of other agricultural crops (Jones and Miller, 1992). In West Africa, *Jatropha* is one of the common plants used as hedges. The hedges can be cut and trimmed to any height, for aesthetic purposes (Burkill, 1994).

Physic nut is well adapted to marginal areas with poor soils and low rainfall, where it grows without competing with annual food crops, thus filling an ecological niche. The species has numerous uses and in their combination lies the potential of this plant. The most important is the combination of erosion control and oil production. The use of the oil as a substitute for diesel fuel and for soap production in rural areas would improve the living conditions of the people and would offer additional income (Heller, 1996; Benge, 2006).

2.8 PESTS OF *J. curcas*

Although it is said that the toxic properties of the plant will not allow insects to attack it, many groups of insects have overcome this barrier. Especially insects of the order *Heteroptera*, which has many of its species extracting nutrients of physic nut. The stem borer *Cerambycidae* of the Coleopterious family can kill mature *Jatropha curcas* tree. Leaf - eating insects usually cause damage at the early stage of plant growth (Grimm and Maes, 1997). Millipedes can also cause total loss to young seedlings. Locust will also cause damage to leaves and seedlings. Blue bug and green stinkbug suck on the fruits. Lepidoterous larvae cause damage by creating galleries in leaves (Heller, 1996). Pests may be controlled by the use of beneficial arthropods, polyphagous predators and specialized parasitoid by conservation, which is cost efficient (Grimm and Maes, 1997). Below is a table (Table 2.7) with name of diseases, symptoms and the source of literature

Table 2.7: A table of diseases and pest of *J. curcas*

Name	Damage and symptoms	Source
Phytophthora spp., Pythium spp., Fusarium spp., etc.	damping off, root rot	Heller (1992)
Julus sp. (millipede)	total loss of seedlings	Heller (1992)
Oedaleus senegalensis (locust)	leaves, seedlings	Heller (1992)
Lepidopterae larvae	galleries in leaves	Heller (1992)
Spodoptera litura	larval feeding on leaves	Meshram and Joshi (1994)

2.9 PROPAGATION OF *J. curcas*

Several methods can be used in the cultivation of *J. curcas*, these include; direct seedling, Precultivation or nursing in polypots before transplanting, direct planting of cuttings etc. These can be grouped under two main methods, namely;

- Generative /Seed propagation
- Vegetative / Cutting propagation

The appropriate propagation method can be selected by considering factors such as labour, costs, desired type of plantation etc (Heller, 1996: Jones and Miller, 1992).

2.9.1 Generative / Seed Propagation

This is the establishment from seeds of the plant. The establishment from seeds can be by planting direct in the field with seeds or transplanting from precultivated seeds. The latter can also be done through seedbed (bare roots) or containers (Heller, 1996).

2.9.2 Vegetative / Cutting propagation

This is also the establishment of a plant from any part of the plant aside the seeds, it can be done by transplanting as in precultivation of the cuttings in containers or direct planting at the field. Planting from cuttings are influenced by character of cuttings (length, diameter, age), cutting time, storage, fungicide treatment, planting time and depth of planting (Heller, 1996). A study by Thitithanavanich (1985) to investigate the root formation of physic nut cuttings of different diameters (1, 2 and 3 cm) and lengths

(15 cm and 30 cm) in the nursery bed revealed that thicker cuttings formed more roots than the thinner ones. Cuttings of 30 cm length developed more roots and their survival rate and growth performance was higher than cuttings of 15cm length.

2.9.3 Advantages of Cuttings propagation

Cuttings are detached portions of a stem; root or leaf which when put under certain favourable environmental conditions produce new roots and shoots. The plant produced is identical in genotype with the source plant (Hartmann *et al*., 1990). Plants that are easily propagated by cuttings have many advantages, these include:

1 Many new plants can be started in a limited space with a few stock plants. 2 It is cheap, fast and easy and does not require the techniques necessary in grafting or budding.

3 There is no problem of incompatibility with rootstocks as in propagation by grafting.

4 Most often, the new independent plant produced is exactly like the parent with no genetic change, thereby increasing uniformity in the offspring's (Hartmann *et al.*, 1990).

Living fences can be established very quickly by planting cuttings directly in the field.

2.9.4 Importance of Vegetative Propagation in Agroforestry

According to Zobel and Talbert, (1984) foresters are very much interested in the use of vegetative propagation. The reasons include:

1 The preservation of the genetic composition of trees.

2 To produce more of desired genotypes to meet specific needs, such as in seed orchards.

3 Assessing of genotypes and their interaction with the environment through clonal testing.

4 To obtain maximum genetic gains when used for regeneration in operation planting programmes.

With vegetative propagation program once a desirable or suitable tree is identified or developed through breeding, it can be reproduced rapidly many times and the propagules are essentially the same genetically as the desired parent tree. Vegetative propagation allows quick and large gains because all types of genetic variation can be captured. When sexual or seed propagation is used, only a portion of the genetic variation of the trees used as parents will be passed on to the progeny (Whitlock and Fowler, 1999).

2.10 CAUSES AND KINDS OF VARIABILITY IN PLANTS

Basically, all differences in phenotypic (P) expressions of plants are the results of three things: the differing environments (E) in which the trees are found, the genetic (G) differences among trees and the interactions between the tree genotypes and the environments in which they are grown, simply P= E+G+ E*G. Some genetic variations are predictable and useful, whereas other types are random and are more difficult for the tree breeder (Zobel and Talbert, 1984: Falconer and Mackay 1996**).**

2.10.1 Environmental Variation

Some of the environmental variables include; rainfall, temperature, soil etc. some of these variables can be influenced little by humans, but these forces influence the phenotype of a tree.

Variations among trees caused by environmental differences cannot be used by plant breeding program and is often not even predictable. Environmental forces are the greatest cause of variability in some characteristics, especially those related to growth. Form and quality may be strongly affected by environmental differences, but generally, the quality characteristics in trees tend to be highly inherited and less influenced by the environment than are on growth characteristics (Bradshaw and Foster, 1992; Donald and Kent, 1993).

Zobel and Talbert (1984) have said that although foresters cannot easily control the environment, it is frequently possible to develop strains of trees that will grow satisfactorily under adverse environmental conditions. They were therefore of the view that the only method the forester possesses to overcome adverse temperature, rainfall, wind action, pest or other strong environmental influences is breeding for desirable and superior genotypes.

2.10.2 Genetic Variability

Genetic variation can be generally divided into additive and nonadditive components so that genetic variance= additive variance + nonadditive variance. In simple terms, the additive variance is due to the cumulative effects of alleles at all gene loci influencing a trait, whiles the nonadditive genetic variance can be further divided into two, namely dominance variance and epistasis. Dominance variance is due to interaction of specific alleles at a gene locus, whereas epistasis variance is due to interactions among gene loci. Genetic variation is of additive and non-additive effects (Loveless and Hamrick, 1984; Ayres and Ryan, 1999; Lewinton, 1974). When sexual reproduction is used, it is the additive component of the genetic variation that can be manipulated by the tree improvers. Whiles with asexual reproduction both additive and non-additive effects can be used to

predict the genotype of the new plant. That is, the use of vegetative propagation results in the capture and transfer to the new tree all of the genetic potential from the parent tree (Zobel and Talbert, 1984; Lande, 1998**).**

Genetic variability is complex, but if its magnitude and type are known and if it is well used, genetic variation can be manipulated to obtain good gains in some tree characteristics. Most characteristics of economic importance in forest trees are under some degree of additive genetic control. These characteristics include, wood specific gravity, bole straightness etc. They are characteristics of trees which have stronger additive variance components than growth characteristics. This is fortunate because additive variance can be successfully used in simple selection tree improvement programmes (Gram and Sork, 2001; Bradshaw and Foster, 1992). There is little that the tree improvers can do in the short term to improve the amount or kind of genetic variance available for use. The initial challenge to the tree improver is to determine the magnitude and kind of variance present from natural or unimproved populations and then to use it wisely. Zobel and Talbert (1984) observed that, through better control of the environment, it is possible to capture and use more of the genetic variance which results when intensive forest management is teamed with genetic manipulation of trees.

2.10.3 Genetic x Environmental Interaction

This is a term used to describe the situation where there is a change in the performance ranking of given genotypes when grown in different environment (Zobel and Talbert, 1984). Such interactions must be known if maximum progress in breeding is to be obtained. Normally in tree improvement, a group of families/accessions are tested in a single environment and their performance extrapolated to other environment. It is

possible their relative performance might be different when grown under other conditions. Strong genotype and environmental interactions are more likely to occur when environments differ widely (Zobel and Talbert, 1984: Falconer and Mackay, 1996; Gram and Sork, 2001). A general concern in tree improvement is to recognise how the best species, sources, or even the best individual genetic selections can perform when grown in different environmental conditions. For most large-scale tree improvement programs, the major objective is to develop widely adapted strains of trees that can be used over many environments. This requires genotypes that perform well in different environments. Genotype may interact for both quality and growth characteristics. When genetic x environmental interactions are ignored, large production losses in terms of growth and quality of tree can result. There are many causes for interactions, but it is generally conceded that most of them are more closely related to edaphic than to climatic factors. Unless very small geographic areas are being considered, or where climatic variables change appreciably over short distance as in mountainous terrain, large genetic or environmental differences do not always result in genotype x environment interaction (Lynch, 1991: Mullin *et al*., 1992: Lynch, 1996)

2.10.4 Forces that shape genetic variation

All the variations in wild stands have occurred as a result of natural forces. These variations are available for use by the tree breeder/forester for recognition and packaging into individual tress in the form of improved genotypes (Linhart and Grant, 1996). The forces that cause variations work either to increase or decrease variability in a stand. Aside these forces in natural stands, humans can also interfere and help create either new variability or bring together genotypes to create new and useful genetic combinations (Lynch, 1991). Zobel and Talbert (1984) observed that although variability in our forests

today is primarily the result of natural forces over which the forester has little control, it is essential that these forces are understood. They determine the amount and kind of genetic variation found among and within populations. They therefore identified four main forces that shape variability in a natural stand; namely Mutations and Gene flow which increase variability and Natural selection and Genetic drift, which decrease variability.

- **Mutation:** it is a heritable change in the genetic constitution of an organism, usually at the level of a gene. Since the genetic makeup of a tree (its genotype) is determined by the action and interactions of thousands of genic and allelic combinations, mutations can occur somewhere in an organism with considerable frequency, but this will not happen often for any specific gene or gene complex or for a given tree characteristic. Considering the tens of thousands of genetic makeup of trees, it is not unusual for a single tree to have several mutations.

  Most are recessive and have little effect on the phenotype of tree (Lynch and Walsh, 1998: Ahloowalia, 2004). Mutations occur more or less randomly. Most mutations are deleterious and many are eliminated from the population. Some mutations are retained in the population, even though they are deleterious, because they are the recessive type and are not recognizable or detectable unless in the homozygous form. The value or importance of mutation is known when at a much later date different forces affect the environment and formerly useless mutations may actually make the tree more fit to grow and/or reproduce.

Although mutations may be rare and small, they will produce variation that can possibly make a tree more adaptable as environments change (Zobel and Talbert, 1984; Röbbelen, 1990).

- **Gene Flow/Migration:** it is the migration of alleles from one population or species into another where they may be absent or at a different frequency. Gene flow can result from several causes, but the most common is movement of pollen or seed. Occasionally, gene flow or gene transfer takes place at the species level after hybridization. The hybridization brings together two dissimilar parental genetic complexes, thus creating a ―new‖ genotype. Because the new genotype produced from hybridization or cross pollination is rare or one of a kind, it will usually exchange genes with one of the parents to produce a backcross to one of the parental species. After this process occurring several times a population of trees very similar to the original parent species results, although they will contain some genes or gene complexes that have been transferred from the one parental species to the other (Lynch, 1991: Linhart and Grant, 1996). This concept of gene flow can be utilized in breeding and tree improvement programme towards introducing desirable qualities from one species to the other. Gene flow can be important in natural populations and will cause distinct changes in patterns of variation. In conjunction with recombination it is the immediate source of increased variation patterns in many populations (Matyas, 1996: Zobel and Talbert, 1984).

- **Natural selection:** it is a strong force that usually reduces variability by determining which trees will grow and reproduce. It has directional (nonrandom) effect on the genetic makeup of trees in a population. It favours the fittest; that is, those trees with gene combinations that make them best suited to grow and reproduce in a given environment. Natural selection preserves and results in an increase in the number of those genotypes most suited to specific environments

(Palmberg, 1986; Klopfenstein and Kerl, 1995; Mason and Langenheim, 1961). Although natural selection is a process that reduces variability, it can actually preserve and results in an increase in variation if selection favours the heterozygotes. It is often difficult to assess the effects of selection because so many factors are involved in determining which tree will be best fitted to grow and reproduce. Each fitness characteristic has its own selective value and the adaptations created by one factor may either positively or adversely affect the others. In general, natural selection is considered to be a powerful force to reduce variability within population in a given direction (Zobel and Talbert, 1984; Paschke *et al*., 2005)

- **Genetic Drift:** it is a complex mechanism that operates through chance fluctuations in allele frequencies within a population. It is essentially a sampling phenomenon in which the gene frequencies in the progeny populations deviates by chance from those found in the parent populations. Such populations are almost always small and have tendency towards fixation or loss of an allele that affects a characteristic (Husband and Spencer, 1992). It is nondirectional and tends to create ―disorder‖. Which genes or alleles are fixed or lost is strictly a matter of chance. Genetic drift normally occurs in small breeding populations of perhaps 25 or fewer individuals a situation that frequently occurs in forestry due to natural catastrophies or mans influence (Zobel and Talbert, 1984; Linhart and Grant 1996).

2.10.5 Variations caused by Man

In addition to the normal variation patterns that occur in natural populations, many changes in the variation pattern in forest trees can be caused by human beings. Such things

as selection, where the best trees are removed and the poor trees are left to produce will ultimately cause a shift in gene frequencies and thus in variation patterns. These actions can cause very rapid change in variability especially when we apply intensive selection and breeding (Linhart and Grant, 1996). According to Zobel and Talbert (1984) as breeding programmes progress, it will be essential for the tree improver to purposely increase variation. There are a number of options that can be followed to do this when natural variability becomes too limited for breeding programmes. The first and the most important step, is to know and recognize variability within the species. This will help the breeder to bring useful genotypes together which will never occur in the natural conditions. Interspecific hybrids and back crosses can be produced to develop new genetic combinations. In general the activity of humans can cause relatively large changes in variance rapidly, either in a positive or in a negative way. Therefore the effort should be positive genetic gains that are needed to keep tree improvement programmes solid (Butterfield and Fisher, 1992; Linhart and Grant, 1996; Kindt, 2002).

2.10.6 The need for Tree Improvement in Agroforestry

Most people only associate tree improvement and the use of genetics with yield and quality improvement. Although the development of trees for marginal sites may be long term, it will result in substantial benefits as pressure for forest land use intensifies. As the human population increases the competition for land also increases. This is forcing tree planting on lands that were previously considered to be marginal or less suitable for timber production. As a result of the above, genetically improved tree species specifically developed to grow on these marginal lands are needed (Simons and Leakey, 2004). Zobel and Talbert (1984) observed that breeding can be used to address limiting factors such as

drought, cold, nutrient deficiency, pest and disease resistance etc. A number of apparently boron-deficiency-tolerant radiant pine tree have been found indicating the possibility of developing a special strain to grow on nutrient –deficient soils. Also research on breeding and pest resistant tree varieties have proven to be very feasible than the use of chemical control. However in agroforestry the focus has been on quantity rather than on quality of the tree species (Butterfield, 1995a; Butterfield, 1995b; Wightman, 1999). Jaenicke (1999) observed that, this quality encompasses both physiological and genetic components, but agroforestry trees have received less genetic improvement research as compared to annual crops. Therefore for agroforestry to meet its objective of successful increased productivity and ecosystem stability there is the need to improve agroforestry trees to meet the environmental land-use constraints (AttaKrah *et al.,* 2004).

2.10.7 Tree improvement and domestication initiatives

Domestication in Agroforestry involves bringing a plant into wider cultivation through a farmer–driven and market–led process. This is a science-based and iterative procedure involving the identification, production, management and adoption of high quality germplasm (Simons, 2003a). The tree species normally goes through germplasm sourcing, improvement, nursery management, planting and tree husbandry till harvesting (Simons and Leaky, 2004; Atta-Krah *et al.*, 2004). Domestication of these improved tree species would enhance the performance of trees in terms of improved tree products, such as timber, fruits, medicines and/or improved environmental services such as the amelioration of soil fertility. Above all, the farmer will be able to produce a particular tree species to meet the demand of the market, which can help the farmers to make money to better their livelihoods. Simons (1996) observed that it was this that motivated the

World Agroforestry Centre (ICRAF) in the mid-1990s to focus on poverty reduction by initiating the domestication of indigenous tree species by farmers in developing countries. To make this participatory, there was a shift from on-station formal tree improvement towards more active involvement of subsistence farmers in the selection of priority species for domestication and the implementation of the tree improvement process. Generally the farmers select indigenous fruit trees as their top five priorities. As a result of this participatory domestication approach, strategy for the domestication of indigenous trees producing high value products of traditional and cultural significance have been developed by ICRAF (Tchoundjeu *et al*., 1998; Leakey *et al*., 2003; Kwesiga *et al*., 1999). Although McNeely (2004) perceives a possible disadvantage of reduction in genetic diversity in the wild population as it is replaced by a domesticated population. However this approach to improving tree species has the following advantages:

- It has a clear poverty reduction focus, which has been endorsed by a review on behalf of UK Department for International Development (DFID) (Poulton and Poole 2001). The income derived from tree products is often of great importance to women and children, for example, to meet the demand for school fees and new uniform.
- The approach being developed is focused on simple, low-cost, appropriate technology yielding rapid improvements in planting stock quality based on selection and multiplication of superior trees in ways which create new plants, which also produce fruits within a few years and at heights which are easily harvested.
- It builds on traditional and cultural uses of tree products of domestic and local commercial importance, and meets local demand for traditional products.

- It builds on the practice of subsistence farmers to plant, select and improve indigenous fruits (Leakey *et al*., 2004; Kwesiga *et al*., 1999). Example the marula (*Sclerocarya birrea*) in South Africa, where the yields of cultivated trees increased up to 12-fold and average fruit size of 29g, while fruit sizes from trees in natural woodland were 21g (Shackleton *et al*., 2003).
- The domestication of new local cash crops provides the incentive for farmers to diversify their income and sustainability of their farming systems (Leakey, 2001a,b).

In line with the need for increased genetic diversity research in agroforestry, the World Agroforestry Centre (ICRAF) has recently established a molecular characterization laboratory as part of its program on Tree Domestication. This development has contributed in a great way to strengthen genetic diversity analysis in agroforestry tree species. Various studies have been undertaken or are underway for priority species for domestication in Africa and Latin America, including *Calycophyllum spuceanum, Irvingia gabonensis, Gliricidia sepium, Leucaena* species*, Prunus Africana, Sesbania sesban, Uapaca kirkiana, Vitex keniensis* and *Warburgia ugandensis* (Dawson and Powell, 1999; Russell *et al*., 2000; Simon and Leakey, 2004). These initiatives will help improve genetic diversity studies in agroforestry species, which has a great value for rural development.

2.10.8 Tree Improvement Techniques

Tree improvement specialists strive to improve tree species for better yield and quality. Along with better tree management practices and the reduction of time to achieve specific goals, gains in tree improvement are determined by the intensity of the inheritance and how well manipulated is the variation that is present in the population with which one is working (Linhart and Grant, 1996; Brewbaker and Sorensson, 1994).

The tree improver can however, capture more of the genetic variation that is present in a population by suitable manipulation of the environment. The tree improver's best tool to increase gains is to use the existing variations to its fullest and to help develop additional variability when needed. In order to obtain the best possible gains from tree improvement, it is necessary to understand the nature of wild populations, how they developed and how their variability can be used. The important item to remember is that forest trees are mostly wild populations that are not yet greatly changed by the action of people (Zobel and Talbert, 1984). Although the differences within and among wild tree populations have developed naturally over many years, with proper management, intensity of selection and suitable breeding techniques, improvers can bring about desirable changes very rapidly (Zobel and Talbert, 1984; Leakey *et al*., 2004). To take advantage of genetic improvement in trees once the desired character is obtained in a parent, it can be maintained over a number of generations through vegetative propagation (Leakey *et al*., 2004; Brewbaker and Sorensson, 1994).

The type of mating system within a species has a major effect on the variation pattern. Out crossing system which is common in most forest tree species, usually produce highly variable (heterozygous) genetic populations. This fortunate situation help maintain genetic variability which is very vital in selection and breeding programmes in forest trees. When pollen from a tree or a given genotype pollinates flowers on itself, it is termed selfing. This produces progeny with less genetic variability and vigour (Zobel and Talbert, 1984; Falconer and Mackay, 1996). Zobel and Talbert (1984) made the following general observations about selfed trees.

- No sound seed are formed.
- Seeds are formed but they will not germinate

- Seeds germinate but the seedlings are abnormal and often will survive only a short time before they die.
- Seedlings will survive, but they will be small, weak, often yellow in color and grow slowly.
- Seedlings grow more slowly than normal trees, but they are not poor enough to be observed easily and culled in the nursery. This result is quite common and is most dangerous because the selfed tree will be planted out and survive in plantations, but they will produce much less benefits than would be obtained from outcrossed seedlings.
- Seedlings grow as well or sometimes even better than outcrosses

2.11 TRADITIONAL ECOLOGICAL KNOWLEDGE

Traditional/indigenous Ecological Knowledge (TEK) is a term used to mean concepts, facts, perceptions, beliefs, information and values in addition to economic, social, traditional and political arrangements which have evolved with indigenous populations and human environment interactions over time (Larson, 1998).

It can also be the knowledge used by local people to make a living in a particular environment. It is a creative experiment constantly incorporating outside influences and inside innovations to meet new conditions. TEK values are generally regarded as ―superstitious‖ and ―primitive‖ (Manu, 2007). As observed by Nsiah-Gyabaah (1994), most people consider TEK as unworthy of scientific consideration and also a direct obstacle to development but they are very useful resources to industry and the world community as a whole. Therefore it is usually mistaken to think of indigenous knowledge as old-fashioned, backward, static or unchanging ideas.

Indigenous knowledge systems have many dimensions that include linguistics, medicine, clinical, psychology, botany, zoology, ethnology, ecology, climate, agriculture, animal husbandry and crafts (Chambers, 1983). It provides valuable inputs about the local environment and how to efficiently manage its natural resources. TEK has both economic and cultural values that should be given the needed recognition and incorporation into the management of natural resources (Thorne *et al*., 1995).

2.11.1 Nature of Indigenous Knowledge System

Traditional/ indigenous Knowledge forms part of several aspects of the lives of many small communities in Africa. Since time immemorial, rural communities have developed profound and detailed knowledge of local ecological systems (Musimwa, 2002). This traditional/Indigenous Knowledge is very useful and crucial for societal/cultural group survival, because they are passed on from one generation to the other which is useful in the traditional structuring and classification of their surrounding environments. Larson (1998) observed that age and gender differences influence the level of knowledge acquisition and its transmission especially among rural communities in Africa. Also the present generation learns their own skills in understanding local environments and passes it on to their children, which is important for parent-child relationships (Nsiah-Gyabaah, 1994).

2.11.2 Knowledge of Indigenous Trees

Many cultures around the world have for centuries valued plants, seeds, root or fruits as life, a vital element seamlessly woven into cultures, farming/foraging traditions, nutrition,

medicinal therapies, oral histories, religious practices, aesthetics and folk arts and science (Castillo, 2002). Indigenous knowledge of plants has played a critical role in identifying and making useful plants accessible to agriculture and industry. More so, tribal and peasant people's knowledge have encouraged and increased biological diversity. Among peasant farmers and tribal inhabitants of the tropical forest are men and women who are well versed in the diversity of biological resources (NsiahGyabaah, 1994; Larson, 1998). These knowledge and stewardship by uneducated farmers is rarely recognized. TEK has both economic and cultural values that should be given the needed recognition and incorporation into the management of natural resources (Thorne *et al*., 1995).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 TRADITIONAL ECOLOGICAL KNOWLEDGE ON *J. curcas* IN GHANA

3.1.1 Introduction and Rationale of the Study

Jatropha curcas has gained attention in tropical and sub-tropical countries and has spread beyond its centre of origin, because of its hardiness, easy propagation, drought endurance, high oil content, rapid growth, adaptation to wide agro-climatic conditions, and multiple uses of plant as a whole (Heller, 1996). However, the full potential of *J. curcas* has not been realized due to several technological and economic reasons. One of the major

reasons is the lack of high yielding varieties with high oil content (Divakara *et al*., 2010). *J. curcas* belongs to the family Euphorbiaceae. It is native to Central America and Mexico where it occurs naturally in coastal forests. However, *Jatropha* is almost pantropical now. In Ghana like most of the African countries, it is planted as live-fence around homesteads and gardens to protect crops against roaming animals (Benge, 2006).

Benge (2006) also observed that the recent unstable petroleum prices in the world market and the country, has accounted for the growing interest in *J. curcas* as a ―miracle tree‖ to help alleviate the energy crisis in the country and also generate alternative income for farmers in rural areas. According to the drafted Policy on Bio-energy (2008), the Government of Ghana is collaborating with the private sector to develop about 1 million hectares of *Jatropha* plantation in the next 5-6 years in the country (COMPETE 2008). The questions left unanswered are; why will farmers plant *Jatropha*? What are the main uses and niches of *Jatropha* in the country? Answering the above questions is likely to provide researchers with useful information which will help in initiating studies on the screening and evaluation of *J. curcas* germplasm in the country. This will help in developing quality plating material to meet the country's aspiration of large quantity of seed production for bio-diesel production. The general aim of this study was to determine the Traditional Ecological Knowledge on *J. curcas* in the country. The specific objectives were:

- To identify indigenous uses of *J. curcas* in Ghana
- To determine the major niches of *J. curcas* in Ghana.

Hypothesis

- Farmers have practical knowledge of the uses of *J. curcas* in Ghana.

3.1.2 Study site

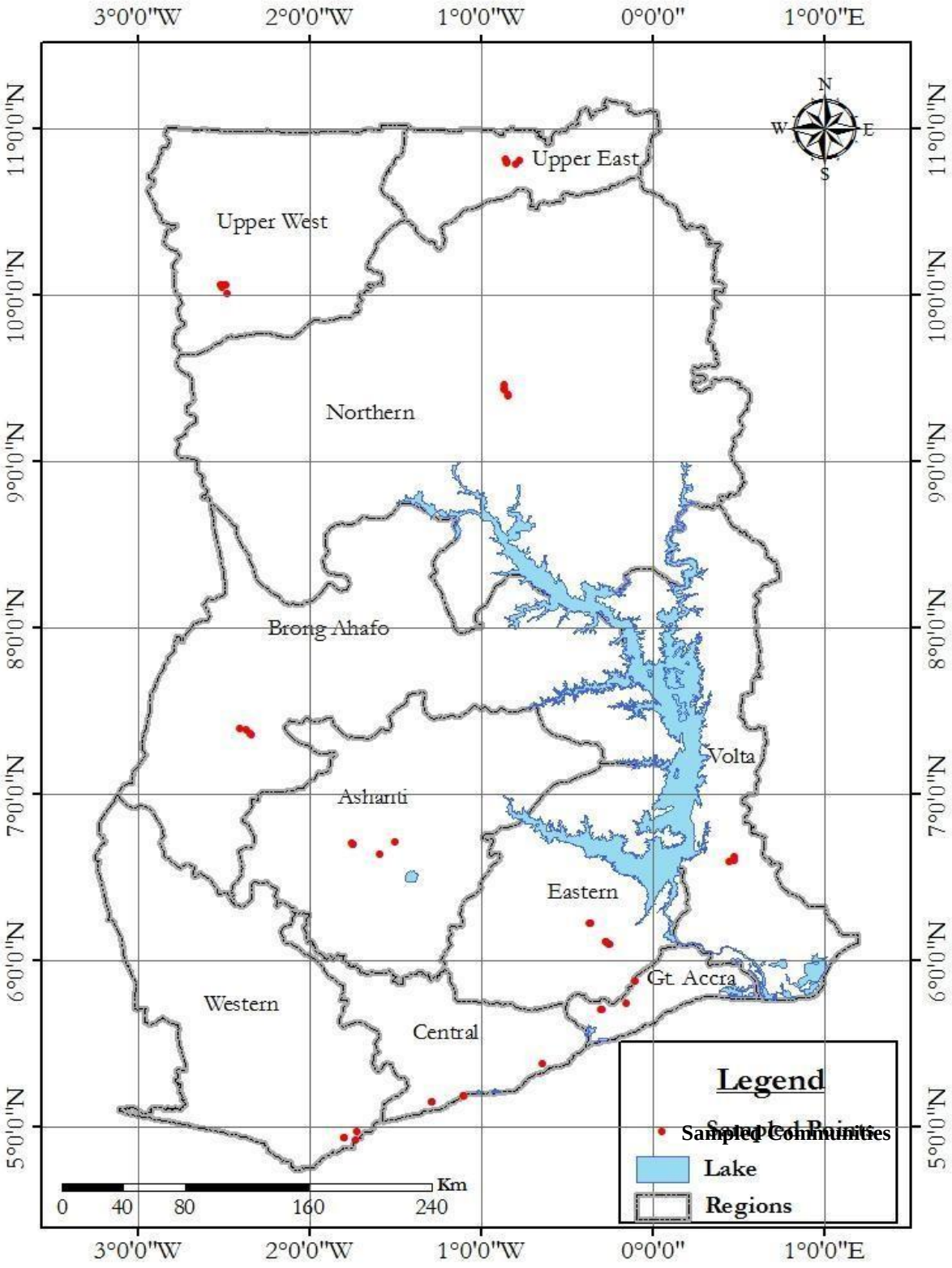
The study was conducted in all the ten regions of Ghana. In each of the regions three farming communities were selected for the study. The selected communities are listed in (Table 1 & Fig 1).

Table 3.1.2: Farming communities selected for the Traditional Ecological Knowledge on *J. curcas* in Ghana (Survey, 2009).

Region	Farming community
Upper East	Bukare, Zaare, Zuarungu

Upper West	Bamahu, Kambele, Suntaa-Nuntaa
Northern	Krugu, Wurushe, Old Kaladan
Ashanti	Kwaamo, Sepaase, Atonsu-Bokuro
Brong-Ahafo	Ayakomaso, Dumasua, Fiapre
Greater Accra	Amasaman, Dodowa, Ashiyie
Western	Bosomtwekroum, Assakai, Kodwokroum
Eastern	Kluutown, Tafo, Asokore
Volta	Sokode, Aquɛ́namawu, Havɛ́
Central	Winneba-Junction, Anto-Nsuoakye, Agyaa Number II

Figure 3.1.2a: A map of Ghana showing communities where farmers were interviewed (Source: Author).


3°0'0"W
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8°0'0"N
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W
E
S
Upper East
Upper West
Northern
Brong Ahafo
Ashanti
Volta
Eastern
Gt. Accra
Western
Central
Legend
Sampled Communities
Lake
Regions
Km
0
40
80
160
240

3.1.3 Methods

Reconnaissance survey

To select communities for the survey, a three- month reconnaissance survey was carried out from September to November 2008 throughout the ten regions of the country. Institutions such as the Ministry of Food and Agriculture (MOFA), Forest Services Division (FSD) of Forestry Commission (FC), Crop Research Institute (CRI) and Plant Genetic Resource Research Institute (PGRRI) of the Council for Scientific and Industrial Research (CSIR) and NGOs such as Anuanom Industrial Bio Products Ltd. (AIBP), BioFuel Africa Ltd Ghana, ADRA-Ghana etc were contacted throughout the country. The selected farming communities were based on recommendations from the above institutions and personal field observations.

Field survey procedure

The survey was conducted by interviewing farmers with a semi-structured questionnaire. The interview was conducted in the main local dialect of the various selected farming communities. Twenty (20) randomly selected farmers were interviewed with a semi-structured questionnaire in each farming community (Fig. 3.1.3a & Fig.3.1.3b). The questionnaire consisted of questions on the demographics of farmers, Traditional Ecological Knowledge on tree species and indigenous Knowledge on *J. curcas* (Appendix 8). As part of the questionnaire the farmers were asked to mention useful indigenous trees in their various communities. However, some wellknown cash crops such as cocoa, cashew, oil palm, Rubber etc. were exempted. Out of this list they were then asked to select ten most important useful indigenous trees amongst them. To prioritize the tree species for all the twenty (20) farmers interviewed in each community, the ten most preferred tree species by each farmer were given a score between one (1) and ten (10).

Ten (10) for the most preferred species and one (1) for the least. The scores assigned to a particular species for all the twenty (20) farmers were summed up to determine the ten most preferred species for each farming community. A similar procedure was used in the determination of the ten most preferred species for each of the ten regions. This was done by summing up the score for the ten most preferred species for all the sixty (60) farmers interviewed within the region.

Figure 3.1.3 a: A farmer being interviewed at Zuarungu, Upper East region of Ghana (2009).

Figure 3.1.3 b: A farmer being interviewed at Wrushe, Northern Region of Ghana (2009).

3.1.4 Data Analysis

The demographic data was expressed in percentages following the descriptive statistical procedure in the Statistical Package for Social Scientist (SPSS) (SPSS, 1999). The data on *J. curcas* was also expressed in percentages following the descriptive statistical procedure in the SPSS (SPSS, 1999). However, scoring and ranking procedures based on the tree species preference of the respondents were used in the determination of the ten most important tree species for each of the regions.

3.1.5 Results and Discussion

Out of the total number of respondents, 52% were females while 48% were males. More females were interviewed because during the survey females were normally met in the households as compared to males. Majority of the respondents (33%) were within the age class of 31-45 while 29%, 23% and 15% were within the age class of 18-30, 45-60 and above 60 respectively. These age classes had influence on the knowledge of indigenous uses of *J. curcas* as mentioned by the respondents. For example most of the respondents above 60 years mentioned that the seeds of the plant can be dried and arranged on stick and used as candle. None within the age class of 18-30, 31-45 and 4560 mentioned this use. Rojas *et al*. (2001) observed that gender and age class had influence on how land and forest is used. Older men and women in a community have more traditional knowledge on certain plant species as compared to the young ones in the same community. With the educational background, majority (43%) were illiterate while 37%, 16% and 4% had primary, secondary and post-secondary education respectively. Irrespective of the educational background, all the respondents demonstrated some indigenous knowledge of the plant.

Table 3.1.5a, shows the ten most preferred tree species of each region in Ghana. In the three northern regions (Upper West, Upper East and Northern) located in the savannah zones, some of the tree species that had the highest rank and therefore most preferred and common to the people were mainly; *Vitellaria paradoxum* (Shea nut), *Parkia biglobosa* (Dawadawa), *Ceiba pentandra* (Onyina), *Adansonia digitata* (Baobab), and *Tamarindus indica* (Pusiga). The most frequent reasons for these species preferences were for food, shade and medicinal use (Table 3.1.5a). For the Ashanti and BrongAhafo regions, the most preferred common tree species were; *Spathodea campanulata*

(Akuakuo-nisuo), *Alstonia boonei* (Onyamedua), *Mangifera indica* (Mango), *Triplochiton scleroxylon* (Wawa) *and Jatropha curcas* (Nkradadua). The main reason for their preference was their medicinal value (Table 3.1.5a). With the rest of the regions located mainly in the forest zones, the most preferred common species were;

Azadirachta indica (Neem tree), *Milicia excelsa* (Odum), *Triplochiton scleroxylon* (Wawa), *Khaya senegalensis* (Mahogany) and *Alstonia boonei* (Onyamedua). Their main reasons were for medicinal use and for construction (Table 3.1.4a).

It was noted that most (76%) of the trees that made the list of the ten most preferred tree species were multipurpose trees species and were planted nearer homes and in the communities for easy access by the people. Few (24%) of the tree species were found in the farmlands and forest. The knowledge and preferences given to these tree species are built based on continuous use and experiences in addressing their problems in the various communities and regions. For example in the guinea savannah zones where there is a long drought season, the preferred tree species had the potential to resist drought, provide food from their fruit, leaves etc and also provide shade. This is in line with the observation of Larson (1998) that, tree species and knowledge of trees have been built through generations living in close contact with nature and the environment which enables them to live successfully in their environment. In Africa, trees have been and will continue to be important in emergency periods such as times of drought, famines, floods, crop failure etc. where they provide food (leaves, seeds and nut, fruits, tubers and roots, gums etc) as well as products which can be gathered for sale. It is without doubt that indigenous trees contribute to the quality of rural people's diet (Le Houerous, 1986; FAO, 1986). Some of the preferred tree species were fruit tree species with the provision of food being the main reason for their preference (Table 3.1.5a).

This is in line with the observation by Quashie-Sam and Bennuah (1997) that fruit trees are among the most preferred indigenous tree species in the West Africa Sub-region.

J. curcas was found among the ten most important tree species in almost all the regions except greater Accra (Table 3.1.5a). Wood for construction and other uses influenced their preference than medicinal in the Greater Accra region, pushing the medicinal plants to the lower rank of preference in the table. This may account for why *J. curcas* being a medicinal plant, was not considered in the ten most important tree species in that region. Fifty six percent (56%) of the respondents mentioned medicinal use of the plant while the others used it for live fencing (12%) and Aesthetic/ Beautification in the community (7%) etc. (Table 3.1.5b). Irrespective of the region, majority (56%) of the respondents mentioned some medicinal use of the plant. Therefore the plant is considered as a medicinal plant with a wide range of medicinal uses (Table 3.1.5c). Abbiw (1990) also observed similar uses of the plant, thereby signifying the vital role the plant plays in the livelihood of the indigenous people. This may account for the reason why the plant is present mostly in the homestead (58%) and the community (31%) so people can easily access it (Table 3.1.5b). In the three northern regions located mostly in the guinea savannah zone of Ghana (Northern, Upper West and Upper East), the plant is used as boundary trees to demarcate their farmlands because of its ability to thrive under drought conditions. This may account for the reason why 2% of the respondents from the same zones mentioned its presence in fallowed lands in the savannah (Table 3.1.5b).

Majority (95%) of the respondents were aware the plant can be used for bio-diesel production (Table 3.1.4b). This, according to them, is due to the hype the media have given to the plant in the country for sometime now. They also added that occasionally buyers come to them to purchase seeds of *J. curcas.* This gives an indication of the

potential market for their produce. Most, (98%) of them were therefore willing to produce the seed for sale on the condition that there will be a sustained market for it. The survey also observed that farmers were worried about the marketing of their produce, citing the case of sunflower as example. They claimed they were introduced to the crop sometime ago, but had problems with marketing when the crop was ready. The government should therefore put down the necessary marketing structures to purchase the produce of the farmers to avoid repetition of the sunflower experience. Some farmers, especially in the three northern regions (Upper East, Upper West and Northern) complained that they have been told by their ancestors that the plant is an evil plant and harbours bad spirits and snakes therefore it should not be planted nearer homes. Also in the rest of the regions, the respondent mentioned that concoctions can be prepared from the plant which most of the youth use for abortion. The survey observed that these concerns are not likely to have any effect on the adoption of the plant in the country. This is because sustainable market for farmers to sell their produce will be the major motivation factor.

Table 3.1.5a: Main uses, niches and rank of ten most important indigenous tree species in the ten regions of Ghana (Survey 2009).

Tree species	Common Name	Local Name	Main Uses	Main Niches	Rank									
					UE	UW	N	BA	A	W	C	GA	V	E
Parkia biglobosa	African Locust Bean Tree	Dawadawa	F, IC	FL,CM	3	4	1	--	--	--	--	--	--	--
Vitellaria paradoxum	Shea butter tree	Shea butter tree	IC, F	CM,FL	1	1	4	--	--	--	--	--	--	--
Adansonia digitata	---	Baobab	F, IC	FL,CM	2	3	7	--	--	--	--	--	--	--
Mangifera indica	Mango tree	Amango	F, M,S	CM,FL	4	2	2	2	5			9	--	--
Azadirachta indica	Neem tree	Neem	M, WC	FL,HGC,	5	10	5	6	--	3	1	1	3	1
Jatropha curcas	**Physic nut**	**Nkradadua/Nkaneadua**	**M, FC,**	**HGC,CM**	**6**	**8**	**6**	**5**	**7**	**4**	**3**	**--**	**4**	**8**
Ceiba pentandra	Kapok tree	Onyina	WC, F	FO,FL	6	5	2	3	--	7	--	6		7
Moringa Oleifera	Moringa	Moringa	F,M	HGC	8	9	8	--	--	--	--	6	--	--
Acassia albida	Acassia	Zaana	FD	FL	9	--	--	--	--	--	--	--	--	--
Tamarindus indica	Tamarind	Pusiga	F,IC	HGC,CM	10	--	8	--	--	--	--	--	--	--
Diospyros mespiliformis	Fig tree	Gaa	S,F	HGC,FL	--	7	10	--	--	--	--	--	--	--
Blighia sapida	Akee	Akee apple	F,M	CM	--	6	--	--	--	--	--	--	--	--
Milicia excels	---	Odum	WC,M	FO,FL	--	--	--	1		4	9	5	4	5
Alstonia boonei	Emien	Onyame-dua	M,FW	FL,CM	--	--	--	8	1		6	4	6	2
Terminalia superba	Frake	Oframe	WC,M	FO,FL	--	--	--	9	--	--	--	--	--	--
Spathodea campanulata	African tulip tree	AkuaKuo-Ninsuo	M	FO,FL	--	--	--	10	4	--	--	--	--	--
Bombax buonopozense	Silk Cotton Tree	Akonkode/Akata	M	FO,FL	--	--	--	4	--	--	--	--	--	--
Triplochiton scleroxylon	Obeche	Wawa	WC,M	FO,FL	--	--	--	6	8	6	4	3	2	4
Morinda lucida	Brimstone tree	Konkroma	M	FL,CM	--	--	--	--	2	--	5	--	--	10
Persea Americana	Avocado	Pear	F,M	FL	--	--	--	--	3	--	--	--	--	--
Terminalia ivorensis	Ivory Coast almond	Emire	WC,M	FO,FL	--	--	--	--	--	--	10	--	--	--
Tectona grandis	Teak	Teak	M,P,S	CM	--	--	--	--	--	2	8	8	9	--
Senna siamea	Yellow cassia	Cassia	S,M	CM	--	--	--	--	--	--	7	--	8	--
Newbouldia laevis	Newbouldia	Sesemasa	M	HGC	--	--	--	--	--	--	--	10	--	9
Terminalia catappa	Tropical almond	Abrofokatɛ́	S	CM,HGC	--	--	--	--	9	--	--	--	--	6
Rauwolfia vomitoria	poison devil's-pepper	Kakapenpen	M,FW	FO,FL	--	--	--	--	10	--	--	--	--	--
Khaya senegalensis	African mahogany	Mahogany	M,WC	FO,FL	--	--	--	--	5	1	2	2	1	3
Entandrophragma Cylindricum	Sapeli	Sapale	WC,M	FO,FL	--	--	--	--	--	9	--	--		--
Spondias monbin	Yellow monbins	Atoa	F,FD	HGC	--	--	--	--	--	8	--	--	10	--

Cocos nucifera	Coconut Palm	Kube		F,M	HGC	--	--	--	--	--	10	--	--	7	--
Terminalia ivorensis	Ivory Coast almond	Emire		WC,M	FO,FL	--	--	--	--	--	--	10	--	--	--

MAIN USES: IC-Income F-food, M-Medicinal use, FC- Fencing, WC- Wood for construction, FD-Fodder, S-Shade, FW-Fuel wood, P-Pole. **MAIN NICHES:** FL-Farmlands, HGC-Homegardens/compound, CM- Community, FO-Forest, **REGIONS:** UE-Upper East, UW-Upper West, N-Northern, BA- Brong-Ahafo, A-Ashanti, W-Western, C48 -Central, GA-Greater Accra, V-Volta, E-Eastern

Table 3.1.5b: Major uses, niches, willingness to produce and awareness of biodiesel potential of *J. curcas* in the ten regions of Ghana (Survey 2009).

	C	W	GA	E	V	A	BA	N	UW	UE	AVG
						USES (%)					
Medicinal	64	59	50	69	64	46	56	49	53	52	56
Aesthetic/ Beautification	8	10	8	3	5	12	8	7	5	7	7
Toothpaste	3	7	12	13	15	17	20	22	20	23	15
Boundary planting	5	5	--	--	3	7	5	12	13	10	6
Others (As Candle for light)	--	2	--	3	8	10	3	5	7	8	5
Live fencing	20	17	30	12	5	8	8	5	2	--	11
						NICHES (%)					
Home gardens	57	44	67	57	81	69	60	55		60	63
Farmlands	5	3	--	--	2	3	22	21		13	8
Avenue/aesthetic in community	38	53	33	43	17	28	11	15		19	27
Fallow land/ Sec. forest	--	--	--	--	--	--	--	7	9	8	2
Primary forest	--	--	--	--	--	--	--	--	--	--	--
						AWARENESS OF BIO-DIESEL POTENTIAL (%)					
Yes	87	100	93	80	100	100	100	100		87	95
No	13	--	--	7	20	--	--	--	--	13	5
						WILLINGNESS TO PRODUCE (%)					
Yes	98	95	100	100	97	100	98	97		95	98
No	2	--	5	--	--	3	--	2	3	5	2

UE-Upper East, UW-Upper West, N-Northern, BA- Brong-Ahafo, A-Ashanti, W-Western, C-Central, GA-Greater Accra, V-Volta, E-Eastern, AVG- Average

Table 3.1.5c: Major medicinal uses and growth characteristics of *J. curcas* in Ghana as expressed by respondents (Survey, 2009).

Major medicinal uses
Powder developed from the bark of the plant is used for expulsion of worms
Smoke from its burnt leaves is used for treating convulsion
Its sap serves as teeth cleaner and latex is applied to treat sore gums, especially in babies and children
Lotion of crushed leaves in hot water is for used for the treatment of guinea worm sores
Its oil is applied on itching skin
Mixture of seed with cereal pulp is used as a purgative
Concoctions from root and leaves and ashes from burnt leaves is used for the treatment of sores.
Its sap is used to aid blood clotting
Growth characteristics
It can grow easily from cuttings or seeds
It is a drought resistant tree species
It is a good tree species for live fencing

3.1.6 Conclusions

The main objective of the study was to determine the traditional ecological knowledge on *J. curcas* in Ghana. The survey has demonstrated that farmers have indigenous knowledge of *J. curcas* and are also aware of the bio-diesel potential of the plant. Medicinal use was one of the main indigenous uses mentioned in every community where the survey was conducted. The respondents also confirmed the ease of cultivation of the plant and they are very willing to produce it commercially if only there are markets.

3.1.7 Recommendations

To help boost the cultivation of *J. curcas* in Ghana by farmers the following are needed;

1. A Diagnostics and Design study should be conducted to identify the specific Agroforestry technology and design that will help incorporate *J. curcas* as a multipurpose tree in land use systems in the country. This will encourage combination of the plant with agricultural crops to prevent total loss should the market fail.
2. There is the need to study *J. curcas*/agricultural crop interactions so that appropriate spacing can be selected for the *J. curcas* plant and the agricultural crops to ensure efficient production and land use.
3. Screening and selecting quality planting material for farmers especially from the local *J. curcas* genetic resources will be needed to boost production of the plant.

CHAPTER FOUR 3.2 EVALUATION OF *J. curcas* ACCESSIONS FROM THE TEN REGIONS OF

3.2.1 Introduction and Rationale of the Study

As a sustainable and renewable source of energy, bioenergy will reduce the impact of rising petroleum prices, address environmental concerns about air pollution and greenhouse gases, and improve opportunities for farmers and rural communities (Nass *et al*., 2007). *J. curcas* is one of the most important bio-energy crops being considered as a source of biodiesel. The major bottleneck identified in *J. curcas* promotion programmes for biodiesel production is the use of material available by farmers (Basha and Sujatha, 2007). Therefore the success of *J. curcas* promotion programmes to boost seed production for biodiesel lies in the identification of genetically divergent material and development of genetically superior stocks or material for farmers to plant (Basha *et al*., 2009; Divakara *et al*., 2010). This calls for evaluation and identification of promising accessions among the local genetic resources in the country as a critical step in improving on the yields to achieve the desired aim. Evaluation is very essential in conservation and use of genetic resources of a plant. Germplasm evaluation, especially the local germplasm, is necessary to enhance germplasm management and utilization. This is because the local germplasm are adapted to the local conditions making the superior accessions identified easy to be adapted and accepted by framers (Basha and Sujatha, 2007; Basha *et al*., 2009). For an effective selection, there must be genetic variation in the population which are expressed in phenotypic traits of the population. Aside the qualitative traits which can be put into distinct and clear phenotypic categories, there are also quantitative trait differences in characters such as sizes, height, yield etc. which can be explored for plant improvement (Strickberger, 1976; Stransfield, 1983). Chang (1985) observed that, in assessing economic (quantitative) and other traits of potential importance in plants, mass

screening procedure under field or greenhouse or laboratory testing are prerequisites to efficient evaluation. Therefore to produce *J. curcas* seeds in large quantities for biodiesel production, there is the need for field evaluation of the local genetic resource of the plant. This will serve as a guide to sourcing good quality planting materials to farmers and individuals who want to enter into Jatropha production in the country. The general objective of this study was to evaluate the growth and yield performance of *J. curcas* accessions collected from the ten regions of Ghana. The specific objectives were:

- To evaluate the vegetative growth performance of *J. curcas* germplasm collected from the ten regions of Ghana.
- To evaluate the yield performance of *J. curcas* germplasm collected from the ten regions of Ghana.

3.2.2 Materials and Methods

3.2.2.1 Planting material collection

Planting materials were collected from promising *J. curcas* plants located within stands of *J. curcas* found within the farming communities where the sociological survey was conducted (Table 3.1.2). The criteria used included; number of branches and number of fruits per cluster per plant (Appendix 9). These are major factors reported to have effect on the yields of the plant (Beckford, 2008: Heller, 1996: Sunil *et al.*, 2008). For each criterion the plants were randomly sampled within a stand after which planting materials were collected from a selected parent plant with more number of branches as compared to the others. Planting materials were also collected from parent plants with the least number of branches. The above procedure was repeated for the number of fruits per cluster. This was to ensure that the variation within the stand can be fairly represented on the experimental field for the evaluation. Each selected *J. curcas* plant was considered as a parent plant and accession/treatment, from which cuttings were collected for the field evaluation. The various accessions and the site/region where they were collected are shown in Appendix 9.

3.2.2.2 Study site

The study was conducted at the Agroforestry Research Station of the Faculty of Renewable Natural Resources (FRNR), Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana, located at Lat 01 430 N and Long 01 360. The area falls within the moist semi-deciduous forest zone of Ghana and is characterized by a bimodal rainfall pattern, with the major wet season between May and July. This area

also experiences a short dry season in August and a long one between December and March. The annual rainfall of the area ranges between 1250mm – 1500mm. The area is characterized by a mean annual temperature of 26.6^{0}C and a mean annual humidity of 67.6%. The soil is a sandy-loam and classified as a Feriric Acrisol (FAO/UNESCO).

3.2.2.3 Experimental procedure

The cuttings from each accession/treatment were nursed in a polypot for one month before transplanting onto the field. This was to select uniform seedlings for the field trial. A randomized Complete Block Design (RCBD) with three replications was used for the study. Each plot/treatment consisted of a row of five plants with a plating distance of 1.5m within rows (Intra-rows) and 2m between the rows (Inter-rows) (Fig. 3.2.2a).

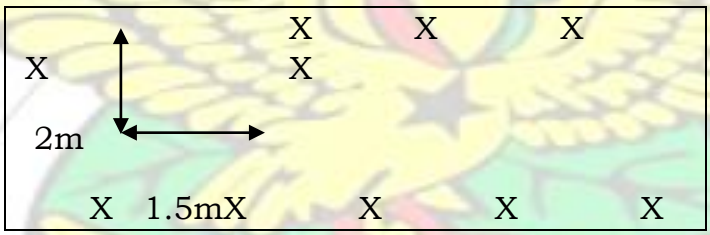

Figure 3.2.2a: Plot showing rows/treatment on the field.

Cultural practices

A total land area of 28m X 180m (5040m^2) was cleared, burnt and prepared for the field study. The cuttings were nursed in polypot for a month. The month-old seedlings were manually irrigated after planting at the field till the rains started. Weeds were controlled every four weeks on the field throughout the experimental period.

BLOCK 1		BLOCK 2		BLOCK 3	
UW1	C5	N6	UE9	UE3	N2
A10	W5	A8	V5	UW1	C5
BA2	GA10	UE4	V4	A10	W5
V8	A4	BA4	A9	BA2	GA10
UW2	W4	A5	W2	V8	A4
E8	N4	E4	UE1	UW2	W4
UE6	C6	BA6	GA5	E8	N4
BA1	BA9	N9	C2	UE6	C6
W7	UW7	E2	A6	BA1	BA9
GA1	N7	C1	W3	A8	UW7
N8	E7	UW8	UE8	GA1	N7
V7	GA3	GA8	V3	N8	E7
W6	A1	N10	GA7	V7	GA3
UW3	GA6	UW5	UE2	W6	A1
GA2	E6	BA5	E3	UW3	GA6
C7	N3	N1	C3	GA2	E6
N5	E1	V1	UW6	C7	N3
BA3	GA9	C4	W1	N5	E1
UE5	UW4	A7	V2	BA3	GA9
GA4	BA8	UE10	UE7	UE5	UW4
A2	V6	BA7	C8	GA4	BA8
E5	A3	V9	E9	A2	V6
C1	W3	UE3	N2	E5	A3

Figure 3.2.2b: Field layout showing the treatments/accessions at the field.

UE-Upper East, UW-Upper West, N-Northern, BA- Brong-Ahafo, A-Ashanti, W-Western, C-Central, GA-Greater Accra, V-Volta, E-Eastern.

BLOCK 1		**BLOCK 2**		**BLOCK 3**	
N6	UE9	UW1	C5	N6	UE9
A8	V5	A10	W5	W7	V5
UE4	V4	BA2	GA10	UE4	V4
BA4	A9	V8	A4	BA4	A9
A5	W2	UW2	W4	A5	W2
E4	UE1	E8	N4	E4	UE1
BA6	GA5	UE6	C6	BA6	GA5
N9	C2	BA1	BA9	N9	C2
E2	A6	W7	UW7	E2	A6
UE3	N2	GA1	N7	C1	W3
UW8	UE8	N8	E7	UW8	UE8
GA8	V3	V7	GA3	GA8	V3
N10	GA7	W6	A1	N10	GA7
UW5	UE2	UW3	GA6	UW5	UE2
BA5	E3	GA2	E6	BA5	E3
N1	C3	C7	N3	N1	C3
V1	UW6	N5	E1	V1	UW6
C4	W1	BA3	GA9	C4	W1

A7	V2	UE5	UW4	A7	V2
UE10	UE7	GA4	BA8	UE10	UE7
BA7	C8	A2	V6	BA7	C8
V9	E9	E5	A3	V9	E9

Figure 3.2.2b continue: Field layout showing the treatments/accessions at the field.

UE-Upper East, UW-Upper West, N-Northern, BA- Brong-Ahafo, A-Ashanti, W-Western, C-Central, GA-Greater Accra, V-Volta, E-Eastern.

3.2.2.4 Data collection and Analysis

Data on plant height, stem girth, number of days to 50% flowering, fresh fruit weight and seed weight were collected for all the accessions within the experimental period.

The data were subjected to analysis of variance using the Statistical Analysis Software (SAS 2009 version). Tables were used to show the variations in quantitative characteristics of the accessions used in the study. Forty (40) representative accessions were selected for oil content analysis. These consisted of five best and five worse accessions based on the seed yield results. In addition to these ten (10) accessions, three (3) accessions were selected at random out of the total number of accessions collected from each region, making a total of forty (40) accessions (Table 3.2.3b). Fifty grams (50g)

dry weights of seeds collected from each of these selected accessions were sent to the Department of Biochemistry and Biotechnology-KNUST Laboratory for oil extraction.

Procedure for oil extraction

The oil extraction was done following the method described by the Association of Official Analytical Chemists (AOAC). For each sample the seeds were cracked to obtain the kernel which was then ground and put on a 22X80mm paper. A small ball of cotton wool was then placed on the thimble to prevent loss of the sample. Anti bumbing granules were put in a clean and dried 250ml round bottom flask. 150ml petroleum spirit at a boiling point between 60-80^{o}C was then added and assembled. Quickfit condenser was then connected to the soxhelt extractor and refluxed for 4hrs on a high heating mantle. The flask was removed and evaporated on a stem bath. The flask now containing the oil was dried for 30min in an oven at 105^{o}C and cooled unto room temperature in a desicator. 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 were then divided by 50g and expressed as percentage to estimate the oil content of each sample.

3.2.3 Results and Discussions

3.2.3.1 Results

The highest mean plant height of 79.37cm was recorded for the accessions from BrongAhafo region, while the Upper West region's accessions recorded the least mean plant height of 75.87cm. The plant height for all the accessions used in the study ranged between 70cm and 84cm (Table 3.2.3a). The analysis of variance, showed significant ($P<0.05$) differences for mean plant height of the accessions (Appendix 1).

There were significant ($P<0.05$) differences in stem girth of the accessions (Appendix 2). The highest mean stem girth of 42.89mm was recorded for the accessions collected from Eastern region, while the Brong-Ahafo region accessions recorded the least mean stem

girth growth of 39.94mm. The mean stem girth of the accessions used in the study ranged between 37mm and 48mm (Table 3.2.3a). There were also significant (P<0.05) difference in number of branches of the accessions (Appendix 3). All the accessions used in the study recorded a mean number of 5 (Table 3.2.3a).

The analysis of variance showed that there were significant (P<0.05) differences in the number of days to 50% flowering of the accessions (Appendix 4). All the accessions flowered within 210 and 224 days after transplanting unto the field (Table 3.2.3a). Accessions from Upper West and Brong-Ahafo flowered earlier (216days) whiles accessions from Ashanti and Central regions were the latest to flower (221days). There were significant (P<0.05) differences in the number of fruits per cluster of the accessions (Appendix 5). Also, the analysis of variance of the fruit weight showed that there were significant (P<0.05) differences in the fruit yields of the accessions (Appendix 6). Accessions from Upper West Region had the highest mean fruit weight of 1972.91kg/ha while accessions from Eastern Region had the least mean fruit yield of 1958.95kg/ha. The fruit yields of all the accessions varied between 1150kg/ha and 2004kg/ha (Table 3.2.3a). The analysis of variance of the seed weight also showed significant (P<0.05) differences among the seed yields of the accessions (Appendix 7). Accessions from Upper West and Eastern Regions had the highest and lowest mean seed yields of 1435.87kg/ha and 1421.18kg/ha (Table 3.2.3a). The mean seed yields of the accessions ranged between 950kg/ha and 1475 kg/ha.

Oil extracted from 50g dry weight of seeds per accession of the 40 accessions selected fell in a range of 16.68g- 17.28g of oil. This is equivalent to 33.36 - 34.56 % of the seed weight. Accession C6 recorded the highest mass of 17.28g (34.56%) followed by A7 with

a mass of 17.25g (34.50%). The least oil content of 16.68g (33.36%) was recorded in 57.5% (23) of the accessions (Table 3.2.3b).

Table 3.2.3a: Mean, Standard deviation, Range and Coefficient of variation in quantitative characteristics of *J. curcas* germplasm collected from all the ten regions of Ghana, Ten (10) months after planting in the field.

Region	N	Height (cm)	Stem Girth (mm)	No. of branches	No. of days to 50% Flowering	Fresh weight of fruits (kg/ha)	Fresh weight of seeds (kg/ha)
Upper West	8	76.76±3.42	41.26±4.24	6±0.74	216±1.41	1972.91±10.32	1435.87±12.22
Upper East	10	75.29±3.31	42.69±3.96	5±0.82	217±2.49	1956.58±21.77	1423.02±17.78
Northern	10	76.92±3.86	41.48±3.40	5±0.63	218±2.55	1964.37±17.07	1426.78±17.22
Brong-Ahafo	9	79.26±2.93	39.83±3.63	6±0.73	216±4.74	1969.00±20.33	1431.21±19.44
Ashanti	10	77.08±3.81	41.54±4.16	6±0.70	221±2.62	1971.80±19.72	1432.31±18.97
Western	7	77.10±3.62	41.81±3.14	5±0.76	219±1.50	1963.49±24.13	1430.40±18.55
Eastern	9	76.86±3.81	42.90±5.11	5±0.67	220±1.87	1958.95±27.86	1421.18±35.38
Central	8	78.58±2.32	40.33±2.77	5±1.30	221±1.69	1962.42±25.00	1428.65±14.71
Volta	9	75.89±3.60	42.18±4.08	5±0.44	220±1.62	1962.44±28.04	1429.06±19.03
Greater-Accra	10	79.45±2.89	40.44±2.84	5±0.95	219±0.82	1965.98±26.36	1427.99±25.65
Grand mean		77.31±3.49	41.46±3.74	5±0.76	219±2.85	1964.88±22.40	1427.63±21.54
Range		70—84	37—48	4—6	210—224	1150—2004	950—1475
CV(%)		4.51	9.13	12.50	1.31	1.14	1.51

N-number of accessions

Table 3.2.3b: Estimated oil content of *J. curcas* accessions from the ten regions of Ghana. (Oil extracted from 50grams dry weight per accessions. Values in bracket are the estimated percentage of oil content).

Field Code	Collection Site/Region	Volume of oil (milliliters)	Weight of oil (g) & percentage oil content
A5	Sepaase / Ashanti	21.00	16.68 (33.36%)
V7	Havɛ / Volta	21.10	16.76 (33.52%)
W2	Bosomtwekroum/Western	21.00	16.68 (33.36%)
E4	Tafo / Eastern	21.50	17.08 (34.16%)
C7	Agyaa # 1/ Central	21.10	16.76 (33.52%)
BA1	Ayakomaso/ Brong-Ahafo	21.00	16.68 (33.36%)
GA9	Ashiyie/ Greater-Accra	21.50	17.08 (34.16%)
UE8	Zuarungu/Upper East	21.00	16.68 (33.36%)
UW8	Suntaa-Nuntaa/Upper West	21.50	17.08 (34.16%)
N6	Wrushe/ Northern	21.00	16.68 (33.36%)
W7	Kwadwokroum/ Western	21.00	16.68 (33.36%)
E6	Tafo/ Eastern	21.10	17.76 (33.52%)
C2	Winneba/ Central	21.00	16.68 (33.36%)
BA9	Fiapre/ Brong-Ahafo	21.40	17.00 (34.00%)
GA3	Amasaman/ Greater Accra	21.00	16.68 (33.36%)
UE5	Zaare / Upper East	21.50	17.08 (34.16%)
UW5	Kambele / Upper West	21.00	16.68 (33.36%)
N2	Krugu/ Northern	21.00	16.68 (33.36%)
A9	Atonsu-Bokuro/ Ashanti	21.10	16.76 (33.52%)
V5	Aquɛnamawu/ Volta	21.00	16.68 (33.36%)
BA5	Dumasua/ Brong-Ahafo	21.00	16.68 (33.36%)
GA7	Dodowa/ Greater Accra	21.00	16.68 (33.36%)
UE10	Zaare /Upper East	21.10	16.76 (33.52%)
UW3	Suntaa-Nuntaa/ Upper West	21.00	16.68 (33.36%)
N8	Old-Kaladan/ Northern	21.00	16.68 (33.36%)
W3	Bosomtwekroum/Western	21.00	16.68 (33.36%)
E9	Asokore/ Eastern	21.00	16.68 (33.36%)
C3	Winneba/ Central	21.00	16.78 (33.52%)
BA6	Dumasua/ Brong-Ahafo	21.40	17.00 (34.00%)
GA4	Dodowa/ Greater Accra	21.00	16.68 (33.36%)
UE1	Bukare / Upper East	21.50	17.08 (34.16%)
UW2	Bamahu / Upper West	21.40	17.00 (34.16%)
N7	Wrushe/ Northern	21.60	17.16 (34.32%)
A10	Atonsu-Bokuro/ Ashanti	21.00	16.68 (33.36%)
V3	Sokode/ Volta	21.00	16.68 (33.36%)
A7	Sepaase/ Ashanti	21.72	17.25 (34.50%)
V6	Aquɛnamawu/ Volta	21.00	16.68 (33.36%)

W5	Assaikai/ Western	21.10	16.76 (33.52%)
E1	Kluutown/ Eastern	21.10	16.78 (33.52%)
C6	Anto-Nsuoakye/ Central	21.75	17.28 (34.56%)

3.2.3.2 Discussions

Tree breeding strategies largely depends upon extent of variability in base population which may be measured by different parameters (Ginwal *et al*., 2004). Plant vegetative growths such as height and number of branches are important characters that can be considered as major selection indices when the objective is to incorporate *J. curcas* in an Agroforestry system. This may call for a balanced trade off in yields considering the economic potential of the understory crop in the initial years of establishment. The variation observed in reproductive characters can be useful in selecting plants for plantations (Rao *et al*., 2008; Kumar and Kumari, 2007).

Significant differences at 5% were observed for each of the vegetative parameters measured (plant height, stem girth, and number of branches) (Appendix 1, 2, 3), indicating variations among the accessions used in the study. However slight differences were observed among the group of accessions collected from within the regions (Table 3.2.3a). Although these accessions were from different regions of the country with different conditions, the slight variations in vegetative growth among the accessions can be due to the highly adaptable nature of the plant to different environmental and ecological zones, thus environment had comparatively little influence on its growth performance (Kaushik *et al.*, 2007; Heller, 1996; Openshaw, 2000). Also it might partly be due to the uniform seedlings which were selected at the nursery for each of the accessions for the field evaluation. None of the accessions showed consistency in superior growth in all the vegetative growth parameters measured. Also it was observed that none of the accessions consistently recorded lower mean growth in all the vegetative growth parameters. A similar field evaluation on 34 *J. curcas* accessions from 17 states

of India revealed significant differences in plant height, stem girth, branches growth, after the 12 and 24 months in the field (Saikia *et al*., 2009). However, Sunil *et al.* (2008) observed that, identification of promising lines among perennials such as *J. curcus* would entail a concerted study over a period of time, usually 5–10 years. This indicates that, it is very likely the vegetative growth of the accessions/treatments might be uniform should the study be consistently observed for an extended period. Although the analysis of variance at 5% significance level showed differences in the vegetative growth of the accessions suggesting variations among them, there is the need for further studies before substantial conclusions on the hypothesis can be reached.

The identification of genetically divergent material plays a crucial role in breeding of high yielding and superior varieties of a tree species (Basha and Sujatha, 2007; Sharma and Sarraf, 2007).The analysis of variance showed significant ($P<0.05$) differences in the fruit and seed yields of the accessions (Appendix 6; 7). Generally the mean fruit and seed yields of accessions which produced six (6) branches were more than accessions which produced five (5) and (4) branches (Table 3.2.3a). This would be due to the number of branches which contributed to the number of flowers, which in turn contributed to the number of fruits which finally culminated in yield. Therefore a positive association between branches per plant, number of flowers and number of fruits and seeds can be drawn (Rao *et al*., 2008). Thus accessions with good branching are very likely to give good yields. Branching is one parameter which is reported to have a significant effect on the yields of *J. curcas*. Two studies have observed that the more branches developed by the plants the more flowers and fruits they are likely to have (Beckford, 2009; Gohil and Pandya, 2008).

The analysis of the oil content of the selected accessions suggested virtually no variations among the accessions (Table 3.2.3b). This suggests narrow variability in the genetic resources of *J. curcas* in the country. Indicating that, the same genotype has spread through informal planting material distribution in the country. Basha and Sujatha (2007) observed that, the initial variations in fruit and seed yields of candidate plus trees of *J. curcas* planted on a common site in India were found to be insignificant, indicating low variability in the genetic resource of the plant. Also a similar study in Thailand with forty accessions from different locations revealed no morphological differences in the intra-specific variability of the lines (Sakaguchi and Somabi, 1987). The data analyzed were collected within ten months after transplanting unto the field. These might have accounted for the variations observed in the growth and yields of the accessions.

Studies on oil content of seeds of *J. curcas* accessions from India revealed a range of (35-40) % of the seed weight (Basha and Sujatha 2007; Sunil *et al.,* 2008). The difference observed between the Indian accessions and those in this study may be due to differences in environmental conditions and the oil extraction methods. Basha and Sujatha (2007) observed that *J. curcas* was introduced into most tropical countries through the introduction and acclimatization method. Also the spread to other parts was primarily, through the vegetative propagation. Indicating the same genotype of *J. curcas* may have spread throughout the country. This may account for the narrow variations observed in the oil contents of the accessions used in the study. It is therefore likely that uniformity in the parameter may have been achieved if these growth and yield parameters had been monitored for a period of time (about 5—10years) as recommended by Sunil *et al.,* (2008).

3.2.4 Conclusions

The main objective of this study was to evaluate germplasm and find out if there were variations in the growth and yield performance of the accessions collected from the ten regions of the country. The study has shown significant differences ($P \leq 0.05$) in the germplasm used, suggesting variations in the genetic resources of *J. curcas* in the country.

3.2.5 Recommendations

Introduction of unique and distinct genetic resources of the plant from Mexico and Central America which are the centers of origin of the plant will help increase the variability in *J. curcas* germplasm in the country for breeding and improvement. To be sure of the extent of variability among the accessions, there is the need to conduct further studies to gather additional evidence on the genetic variability of the *J. curcas* germplasm in Ghana. Data must be collected over an extended period of time (5-10years), to be able to assess the diversity in the accessions. Since studies of this nature takes time, molecular studies will be an appropriate option to provide an environmentally independent evaluation of the accessions.

CHAPTER FIVE

3.3 ASSESSING THE GENETIC DIVERSITY OF *Jatropha curcas* ACCESSIONS FROM THE TEN REGIONS OF GHANA USING MOLECULAR TECHNIQUES

3.3.1 Introduction and Rationale of Study

Jatropha curcas (Euphorbiaceae) is an oil-bearing species is a multipurpose tree with considerable potential as a bioenergy crop and also fit for agroforestry and other afforestation programmes (Wood *et al.,* 1991). Basha *et al*. (2009) made the assertion that, an insight into the genetic variability of *J. curcas* collected from different regions of the world would be a critical input for the selection of appropriate genotypes for cultivation and breeding purposes. In Ghana, if farmers do not use plants from superior genetic materials, opportunities will be missed by not using planting materials with higher yield potentials and other more desirable plant characteristics. Therefore research on *J. curcas* genetic resources in the country is imperative. Little work has been done so far on germplasm collection and evaluation of genetic diversity in order to preserve this species. This is a challenging goal since knowledge on the amount of genetic variation and genetic relationship in *J. curcas* using molecular markers is sparse (Sudheer pamidiamarri *et al.,* 2006). At present, molecular markers have proven to be valuable tools in the characterization and evaluation of genetic diversity within the species (Nejia *et al*., 2007; Muchugi *et al*., 2008). Molecular markers have been successfully used in *J. curcas* for detecting genetic diversity and relationship in other countries such as India and China

(Ganesh ram *et al.,* 2008; Basha *et al*., 2007; Qi-Bao *et al*., 2008). Of these techniques, Random Amplification of Polymorphic DNA (RAPD) has several advantages, such as

simplicity of use, low cost and the use of small amount of plant material (Nejia *et al*., 2007). RAPD analysis has been used for genetic diversity assessment and for identifying germplasm in a number of plant species (Kapteyn and Simon, 2002; Welsh and McClelland, 1990). Use of such techniques for *J. curcas* germplasm characterization may facilitate the conservation and utilization of genetic resources in the country and permit the identification of unique genotypes or sources of genetically diverse genotypes. It will also help in developing quality planting material for production which will help boost *J. curcas* seed production to meet the government's dream of bio-diesel production. The general objective of this study was to assess the variations in the genetic resources of *J. curcas* in the country. The main objective was to determine the genetic diversity, of *J. curcas* germplasm in Ghana based on molecular marker techniques. Specifically to access the genetic diversity of *J. curcas* accessions collected from the ten regions of Ghana.

Hypothesis

There are variations in the genetic resources of *J. curcas* collected from the ten regions of Ghana.

3.3.3 Materials and Methods

3.3.3.1 Plant Material and Reagents

A representative set of forty (40) accessions of *J. curcas*, that included four (4) accessions from each region of the country was used for diversity analysis. The accessions were selected from ninety (90) accessions of *J. curcas* established for field trials at the Agroforestry Research Station, Faculty of Renewable Natural Resources (FRNR), KNUST. The selection was based mainly on the yield performances of the accessions. The first and last five accessions which recorded the best and least seed yields respectively were selected. Three (3) accessions were also selected at random from each of the ten regions germplasm, totaling four (4) accessions from each region (Table 3.3.3.1a). The study was conducted at the Molecular Biology Laboratory of the Crops Research Institute (CRI) - Council for Scientific and Industrial Research (CSIR), Ghana. The DNA extraction kits and the Random Amplification of Polymorphic DNA (RAPD) primers used for the study were obtained from Metabion International-Germany. The ten (10) RAPD primers (Table 3.3.3.1b) selected for the study, had been used in an earlier study and shown to have the ability to detect distinct polymorphic amplified products across *J. curcas* accessions (Subramanyam *et al*., 2009).

Table 3.3.3.1a: Laboratory Codes and Locations of the *J. curcas* accessions collected from different parts of Ghana used for the diversity assessment.

Lab Code	Field Code	Collection Site	Region
001	A5	Sepaase / Ashanti	Ashanti
002	V7	Havɛ / Volta	Volta
003	W2	Bosomtwekroum/Western	Western
004	E4	Tafo / Eastern	Eastern
005	C7	Agyaa # 1/ Central	Central
006	BA1	Ayakomaso/ Brong-Ahafo	Brong-Ahafo
007	GA9	Ashiyie/ Greater-Accra	Greater Accra
008	UE8	Zuarungu/Upper East	Upper East
009	UW8	Suntaa-Nuntaa/Upper West	Upper West
010	N6	Wrushe/ Northern	Northern
011	W7	Kwadwokroum/ Western	Western
012	E6	Tafo/ Eastern	Eastern
013	C2	Winneba/ Central	Central
014	BA9	Fiapre/ Brong-Ahafo	Brong-Ahafo
015	GA3	Amasaman/ Greater Accra	Greater Accra
016	UE5	Zaare / Upper East	Upper East
017	UW5	Kambele / Upper West	Upper West
018	N2	Krugu/ Northern	Northern
019	A9	Atonsu-Bokuro/ Ashanti	Ashanti
020	V5	Aquɛnamawu/ Volta	Volta
021	BA5	Dumasua/ Brong-Ahafo	Brong-Ahafo
022	GA7	Dodowa/ Greater Accra	Greater Accra
023	UE10	Zaare /Upper East	Upper East
024	UW3	Suntaa-Nuntaa/Upper West	Upper West
025	N8	Old-Kaladan/ Northern	Northern
026	W3	Bosomtwekroum/Western	Western
027	E9	Asokore/ Eastern	Eastern
028	C3	Winneba/ Central	Central
029	BA6	Dumasua/ Brong-Ahafo	Brong-Ahafo
030	GA4	Dodowa/ Greater Accra	Greater Accra
031	UE1	Bukare / Upper East	Upper East
032	UW2	Bamahu / Upper West	Upper West
033	N7	Wrushe/ Northern	Northern
034	A10	Atonsu-Bokuro/ Ashanti	Ashanti
035	V3	Sokode/ Volta	Volta
036	A7	Sepaase/ Ashanti	Ashanti

037	V6	Aquɛnamawu/ Volta	Volta
038	W5	Assaikai/ Western	Western
039	E1	Kluutown/ Eastern	Eastern
040	C6	Anto-Nsuoakye/ Central	Central

- Number

Table 3.3.3.1b: RAPD Primers (decamer) used for the Molecular Analysis

Primer code	**Nucleotide sequence (5'-3')**
EOD1	GGT GAC GCA G
EOD2	CTG CTG GGA C
EOD3	GTG AGG CGT C
EOD4	AAC GGT GAC C
EOD5	GGA TGA GAC C
EOD6	CCA GCT TAG G
EOD7	CAG CCC AGA G
EOD8	CAG GAT TCC C
EOD9	CTC TGC GCG T
EOD10	TGA GTG GGT G

3.3.3.2 DNA Extraction

DNA extraction from fresh leaf tissues was done using the modified DNA isolation method described by Egnin *et al.* (1998). The DNA extraction buffer contained 1M of Tris-HCl (pH 8.0), 5M NaCl, 0.5M EDTA (pH 8.0), (10,000 mwt) Poly Vinyl Pyrrolidone, 20% Sarkosine, Sodium Metabisuphite, 10% Sodium Ascorbate. Two hundred milligrams (200 mg) of leaf tissue was weighed into 2 ml eppendorf tube and ground to fine powder with liquid nitrogen. 800 µl of Buffer (extraction buffer) was added and incubated at 90^{o}C for 10 mins. The extract was vortexed every 5mins and allowed to cool at room temperature for 2 mins. 400µl of 5M Potassium Acetate was added and mixed gently by inversion 5 to 6 times and incubated on ice for 30mins with shaking and centrifuged for 10 minutes at 13,000 rpm. The supernatant was transferred into a new eppendorf tube. Equal volume of cold Isopropanol and 1/10th of 3 M Sodium Acetate were added to it by mixing it about 10 times by inversion. The DNA was precipitated at – 20^{o}C for 1 hr and centrifuged at 13,000 rpm for 10 mins to pellet the DNA. The supernatant was poured and pellets washed with 800µl of 80% Ethanol and centrifuged at 14,000 rpm for 5 mins. The alcohol was discarded and DNA pellets airdried. DNA was dissolved in 500µl of TE Tri-EDTA (10mls Tris + 2 mols 0.5M EDTA)

Buffer and treated with 4µl of RNase A at 37^{o}C for 30 mins. 250µl of 7.5M Ammonium Acetate was added and incubated on ice for 3 mins and centrifuged at 13,000 rpm for 5

mins. The supernatant was transferred into a new 1.5ml tube and 700µl of Isopropanol added and mixed by inversion and centrifuged at 13,000 rpm for 15 mins. The supernatant was discarded and pellets washed with 1ml of 80% ethanol and centrifuged at 14,000 rpm for 5 mins. The supernatant was again discarded and pellets dried at room temperature. DNA pellets were then dissolved in 200µl TE Buffer. DNA quality was checked on 0.8% Agarose gel by electrophoresis. The extracted plants DNA were subjected to molecular analysis using the random RAPD primers (Table 3.3.3.1).

3.3.3.3 RAPD Analysis

Polymerase Chain Reaction (PCR) amplification reactions were performed in 10µl volumes for all the ten markers. The reaction mixture composition was 1µl template DNA, 1µl of 10X PCR buffer, 0.9µl of 25mM $MgCl_2$, 0.4µl dNTP, 0.25µl each of primer and 0.125µl Taq polymerase, where the random primer was used, 0.5 µl was used for the 10µl PCR reaction. PCR amplification was carried out in a BIO-RAD MyclerTM Thermal cycler. The programmed temperature involved an initial denaturation step of 94ºC for 2 minutes followed by 35 cycles at 94ºC for 1 minute, annealing at 55ºC for 1 min and extension at 72ºC for 2 minutes; a final elongation cycle of 72ºC for 5 mins was included. Amplification products were maintained at 4ºC prior to electrophoresis. DNA loading dye was added to the PCR amplification products and separated by electrophoresis on 1.5% Agarose gels.

3.3.3.4 Statistical Analysis

RAPD markers across the 40 accessions were scored for their presence ―1‖ or absence ―0‖ of bands for each primer. By comparing the banding patterns of the genotypes for a specific primer, genotype-specific bands were identified while faint or unclear bands were not considered. This was used to generate a binary data for Pair wise similarity matrices, generated by the Jaccard‘s co-efficient of similarity by using the SIMQUAL format of NTSYSpc 2.1 version program (Rohlf, 2002). A dendrogram was also constructed by using the unweighted pair group method with arithmetic average (UPGMA). The same binary data were also subjected to principal component analysis (PCA) which incorporated both the phenotypic parameters measured at the field and the genotype parameters using the GGEbiplot software (Yan and Kang, 2003). In the analysis of the data using the GGEbiplot software, amplified but uninformative bands (redundant bands) were eliminated before the analysis. To estimate the percentage of polymorphism the number of polymorphic bands was divided by the total number of bands amplified for each primer (Table 3.3.4).

3.3.4 Results and Discussion

3.3.4.1 Marker Polymorphism pattern

J. curcas accessions were analyzed using ten (10) random RAPD primers of which all produced banding patterns. Six out of the ten primers used in the study showed polymorphism (Table 3.3.4). A total of 25 bands were scored, with an average of 2 bands per primer. Six (6) out of the 25 bands were polymorphic (Table 3.3.4). The number of bands amplified per primer varied from two (2) to three (3). Primers (EOD 2, 3, 5, 6 and 8) produced the maximum number of bands (3) while primer (EOD 1, 4, 7, 9 and 10) produced the least number of two (2) bands (Table 3.3.4). Similar studies by Subramanyam *et al*. (2009) with these primers on Indian accessions revealed polymorphism across the accessions used in the study. There were polymorphisms observed across the accessions for some of the primers, example EOD 2 (Figure 3.3.4b). However, it was observed that, the pictures generated from the gels generally showed that, the accessions/individuals did not exhibit clear and distinct molecular profiles for most of the primers used in the study. For example, primer EOD 5 amplified fragments of similar size from all the accessions (Figure 3.34b).

Table 3.3.4: RAPD results from 10 random primers used to screen 40 *J. curcas* accessions.

Primer code	Nucleotide sequence(5'-3')	# of individuals/ accessions amplified	#of bands amplified	# of polymorphic bands	Percentage polymorphism
EOD1	GGT GAC GCA G	40	2	1	50
EOD2	CTG CTG GGA C	40	3	1	33.33
EOD3	GTG AGG CGT C	40	3	1	33.33
EOD4	AAC GGT GAC C	40	2	0	0
EOD5	GGA TGA GAC C	36	3	0	0
EOD6	CCA GCT TAG G	40	3	1	33.33
EOD7	CAG CCC AGA G	40	2	1	50
EOD8	CAG GAT TCC C	40	3	0	0
EOD9	CTC TGC GCG T	40	2	1	50
EOD10	TGA GTG GGT G	40	2	0	0
	Average	40	3	1	24.99

- Number

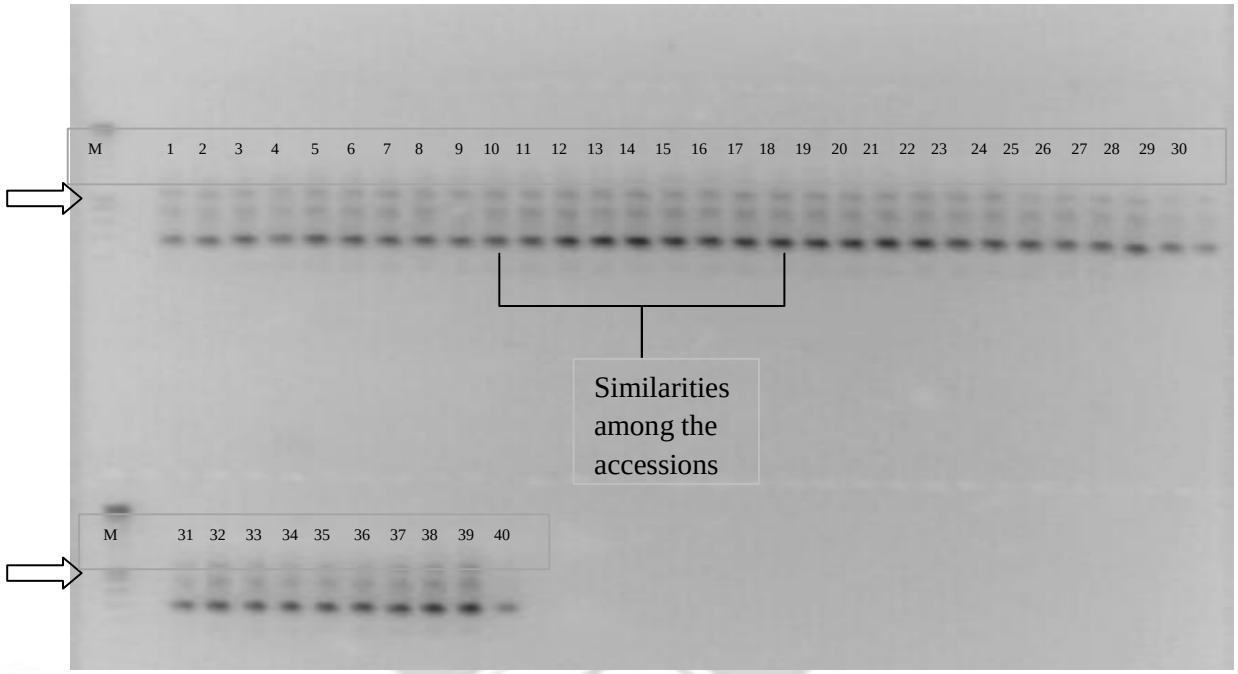

Figure 3.3.4a: Electrophoretic analysis of DNA amplification produced using RAPD primer (EOD 5). (The numbering order follows the numbering order in Table 3.3.3.1a).

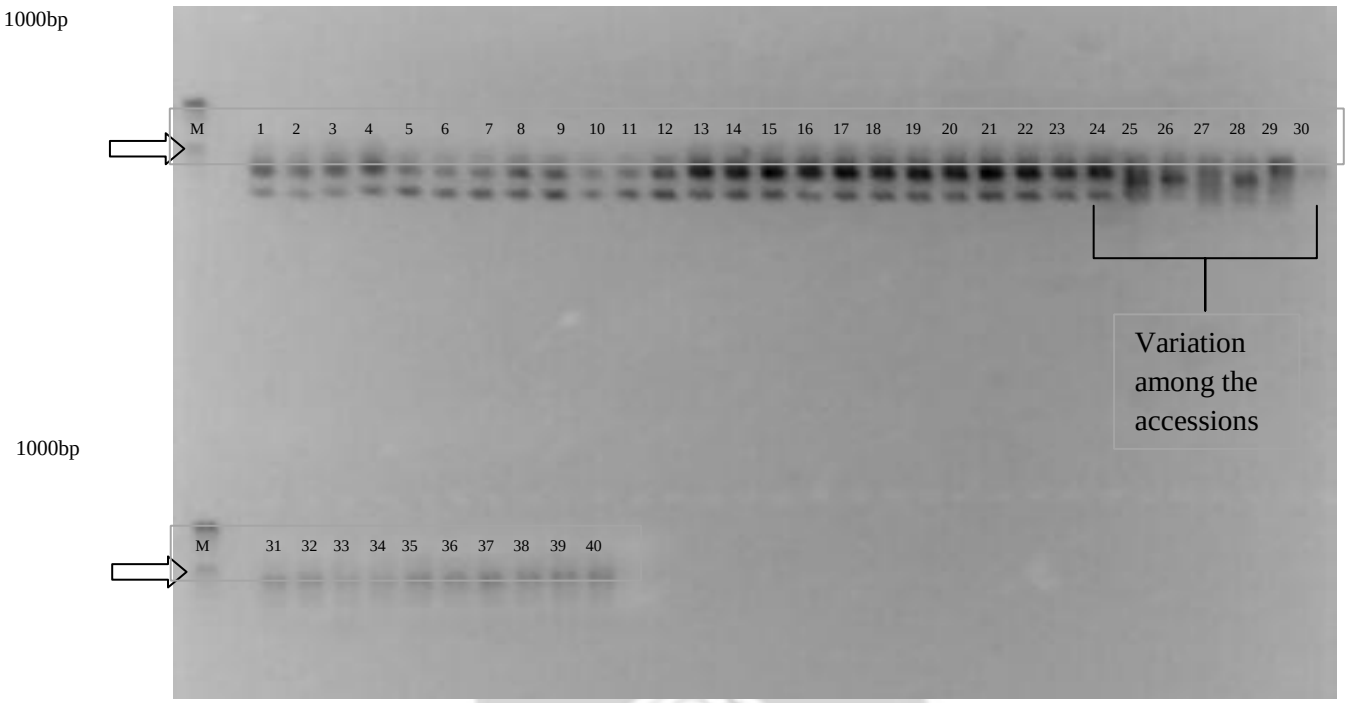

Figure 3.3.4b: Electrophoretic analysis of DNA amplification produced using RAPD primer (EOD 2). (The numbering order follows the numbering order in Table 3.3.3.1a).

3.3.4.2 Genetic Diversity

Random Amplified Polymorphic DNA (RAPD) analysis provides information that can help define the distinctiveness of species and phylogenetic relationships at the molecular level. This technique if used for germplasm characterization will also assist in identifying unique genotypes or identify genetically diverse genotypes for plant improvement (Ganesh Ram *et al.*, 2008; Kapteyn and Simon, 2002; Welsh and McClelland, 1990). Table 3.3.4 shows the accessions and the score for each of the 25 bands. The Jaccard's genetic similarity coefficient varied from 0.78 to 1.00 (Table 3.3.4.2b). Thus the pair wise comparisons recorded values between a highest of 1.00 and lowest of 0.78. Many pairs of accessions had the highest Jaccard's similarity coefficient of 1.00 between them as shown in Table 3.3.4.2b. UPGMA cluster analysis of the Jaccard's similarity coefficient generated a dendrogram (Figure 3.3.4.2a) which illustrated the overall genetic relationship among the accessions used in the

study. The amplification of the accessions followed similar patterns on nine (9) of the ten (10) primers, except EOD 2 which showed much polymorphism

(Figure 3.3.4b). Band CC7 was present in group one but absent in group two whiles band CC8 and was absent in group one but present in group two (Table 3.3.4.2a) accounting for the two main groups observed on the dendrogram (Figure 3.3.4.2a). The two main groups (1 and 2) were further sub grouped, group 1 has two more subgroups (1a and 1b) consisting of 30 and 1 accessions respectively. Group 2 also consisted of 2 subgroups (2a and 2b) consisting of 5 and 4 accessions respectively (Figure 3.3.4.2a). Subgroup 1a was further grouped into 1a-1 and 1a-2 consisting of 27 accessions and 3 accessions respectively (Figure 3.3.4.2a). From the dendrogram, the *J. curcas* germplasm can be group into five (5) main subgroups (1a-1, 1a-2, 1b, 2a and 2b) (Figure 3.3.4.2a). The range of the dendrogram (0.93 – 1.00) (Figure 3.3.4.2a) and the number of accessions with 1.00 Jaccard's coefficient of variation gives an indication of very low genetic diversity among the accessions used in the study. Also the dendrogram clusters of the accessions did not reflect the regions or the three main ecological zones (Savannah, Transitional and Forest zones) of the country, from which the planting materials were collected. These suggest that the accessions are of similar genotypes irrespective of the region or ecological zones from which the planting materials were collected. This might be due to the easy and wide environmentally adaptability nature of the plant (Heller, 1996; Henning, 2006).

The principal component analysis (Figure 3.3.4.2b) was derived by a biplot, which incorporated both the phenotypic parameters measured at the field and the genotypic parameters estimated from the primers. Using the Genotype, Genotype* Environmental biplot (GGEbiplot) software (Yan and Kang, 2003), four main groups can be observed.

Group I, II, III, IV are made of 25, 9, 2 and 4 accessions respectively. However, the sum of the divergence of the two principal components, divergence effect due to the genotype (PC1) and the divergence effect due to interactions between the genotype and field parameters (PC2) was 44.7%, which is not statistically significant (PC1 + PC2 ≥ 70% for the divergence among accessions to be statistically significant). This indicates that the groups are not significantly different from each other (Figure 3.3.4.2a). Thus suggests virtually no genetic diversity/variation among the genetic resources of *J. curcas* in the country. These might be the reason why the study observed no clear and distinct morphological differences in the group of the accessions. A number of studies elsewhere have also made similar observations. Using RAPD and ISSR markers, Basha and Sujatha

(2007) found that many of the Indian samples of *J. curcas* accessions did not exhibit distinct molecular profiles, signifying low variations among the Indian germplasm. Also accessions of *J. curcas* collected from China, exhibited low levels of genetic variation when both SSR and AFLP makers were used to access their genetic diversity (Qi-Bao *et al*., 2008). This implies that planting materials from any part of the country is very likely to have seed virtually of the same yields. Although improving agronomic practices might be needed, developing quality planting material will be a requirement for yield improvement if the country wants to meet her aspiration of bio-diesel production as an alternative energy source. *J. curcas* was introduced from Mexico and Central America (believed to be its center of origin) into Africa and Asia probably through dispersal by Portuguese seafarers via Guinea Bissau (World Agroforestry Center, 2009; Ganesh Ram *et al*., 2008; Qi-Bao Sun *et al*., 2008). It got acclimatized and spread to other parts mainly through vegetative propagation (Burkill, 1994; Heller, 1996; Basha and Sujatha, 2007).

The sociological survey of this study observed that, farmer borrow *J. curcas* planting materials from one another leading to informal distribution of the germplasm among farmers and individuals in the country. Therefore, the genetic resource of *J. curcas* in Ghana may be only part of this introduced accession which has spread to other parts through vegetative propagation. Thus almost all the germplasm are true to type and comes from common parent plants. This may have accounted for the narrow genetic base of the germplasm in Ghana. There is therefore an immediate need for research towards widening the genetic resource base of the plant in the country. Genetic resources can be introduced from India and other countries which are noted for their advances in improvement of the Jatropha plant (Divakara *et al*., 2009; Basha and Sujatha, 2007) and through hybridization techniques, improve upon our local gemplasm. Basha *et al*. (2009) and Bhutta *et al*. (2006) observed that successful identification of genotype(s) for cultivar up-grading also help to develop strategies for the establishment of effective conservation programmes. Improving the local genetic resources will help the government and farmers to successfully meet their aspiration of large volume of Jatropha seeds for bio-fuel and income respectively.

Table 3.3.4.2a: Band scores for 40 *Jatropha curcas* accessions.

Acc/Bands	A5	V7	W2	E4	C7	BA1	GA9	UE8	UW8	N6	W7	E6	C2	BA9	GA3	UE5	UW5	N2	A9	V5	BA5	GA7	UE10	UW3	N8	W3	E9	C3	BA6	GA4	UE1	UW2	N7	A10	V3	A7	V6	W5	E1	C6
CC1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CC2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	9	9	9	9	0	1	1	1
CC3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CC4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	9	1	9	0	9	9	9	9	9	9	9	9	9	9
CC5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	9	9	9	9	9	9	9	9	9	9	9	9
CC6	1	1	9	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CC7	1	1	9	1	1	9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	9	9	9	1	9	9	9	9	9	0	0	0	0
CC8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
CC9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CC10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CC11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	9
CC12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	9
CC13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CC14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	9
CC15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	9
CC16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	9
CC17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
CC18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
CC19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1
CC20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CC21	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	9	9	9
CC22	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
CC23	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	1	0	0	0
CC24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
CC25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9

Acc- Accessions, 1—Present, 0—Absent, 9—Missing data

Table 3.3.4.2b: Jaccard's similarity coefficient of 40 *Jatropha curcas* accessions.

	A5	V7	W2	E4	C7	BA1	GA9	UE8	UW8	N6	W7	E6	C2	BA9	GA3	UE5	UW5	N2	A9	V5	BA5	GA7	UE10	UW3	N8	W3	E9	C3	BA6	GA4	UE1	UW2	N7	A 10	V3	A7	V6	W5	E1	C6
A5	1.00																																						
V7	1.00	1.00																																					
W2	1.00	1.00	1.00																																				
E4	1.00	1.00	1.00	1.00																																			
C7	1.00	1.00	1.00	1.00	1.00																																		
BA1	1.00	1.00	1.00	1.00	1.00	1.00																																	
GA9	1.00	1.00	1.00	1.00	1.00	1.00	1.00																																
UE8	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																															
UW8	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																														
N6	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																													
W7	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																												
E6	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																											
C2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																										
BA9	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																									
GA3	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																								
UE5	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																							
UW5	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																						
N2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																					
A9	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																				
V5	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																			

Table 3.3.4.2b continue: Jaccard's similarity coefficient of 40 *Jatropha curcas* accessions

	A5	V7	W2	E4	C7	BA1	GA9	UE8	UW8	N6	W7	E6	C2	BA9	GA3	UE5	UW5	N2	A9	V5	BA5	GA7	UE10	UW3	N8	W3	E9	C3	BA6	GA4	UE1	UW2	N7	A10	V3	A7	V6	W5	E1	C6
BA5	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																		
GA7	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																	
UE10	0.95	0.95	0.94	0.95	0.95	0.94	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	1.00																
UW3	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	1.00															
N8	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.94	1.00														
W3	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.94	1.00	1.00													
E9	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.94	1.00	1.00	1.00												
C3	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.84	0.89	0.89	1.00	1.00											
BA6	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.94	1.00	1.00	1.00	1.00	1.00										
GA4	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.94	1.00	1.00	1.00	0.89	1.00	1.00									
UE1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.94	1.00	1.00	1.00	1.00	1.00	1.00	1.00								
UW2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.94	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00							
N7	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.93	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00						
A10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.93	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00					
V3	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.93	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
A7	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.93	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			

V6	0.83	0.83	0.88	0.83	0.83	0.88	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.78	0.83	0.83	0.88	0.94	0.94	0.88	0.88	0.94	1.00	1.00	1.00	1.00	1.00
W5	0.88	0.88	0.93	0.88	0.88	0.93	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.82	0.88	0.88	0.94	1.00	1.00	0.94	0.94	1.00	1.00	1.00	1.00	1.00	0.89	1.00	
E1	0.88	0.88	0.93	0.88	0.88	0.93	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.82	0.88	0.88	0.94	1.00	1.00	0.94	0.94	1.00	1.00	1.00	1.00	1.00	0.89	1.00	1.00
C6	0.83	0.83	0.90	0.83	0.83	0.90	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.91	1.00	1.00	0.91	0.91	1.00	1.00	1.00	1.00	1.00	0.83	1.00	1.00	1.00

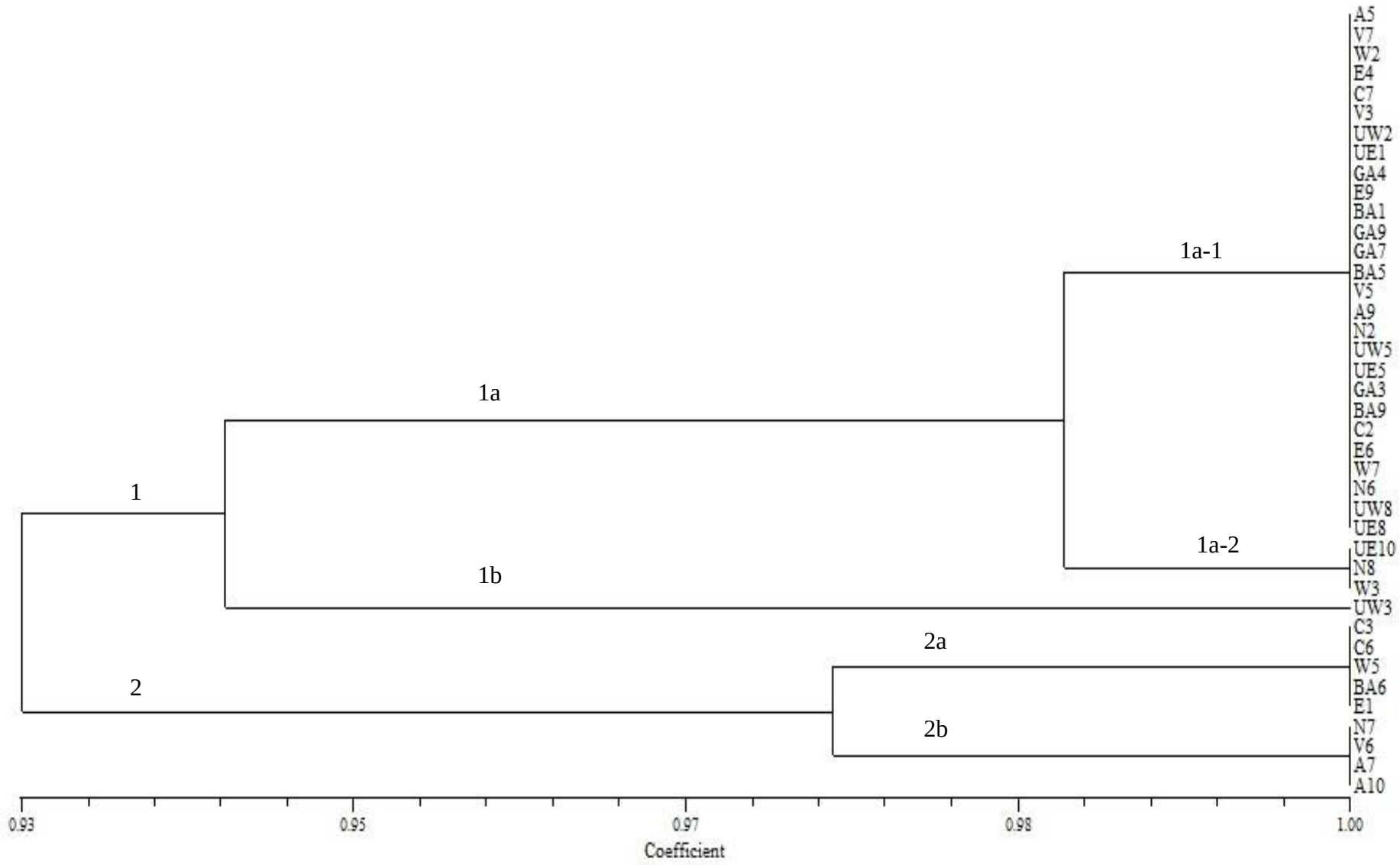

Figure 3.3.4.2a: A dendrogram of 40 *J. curcas* accessions based on Jaccard's similarity coefficient.

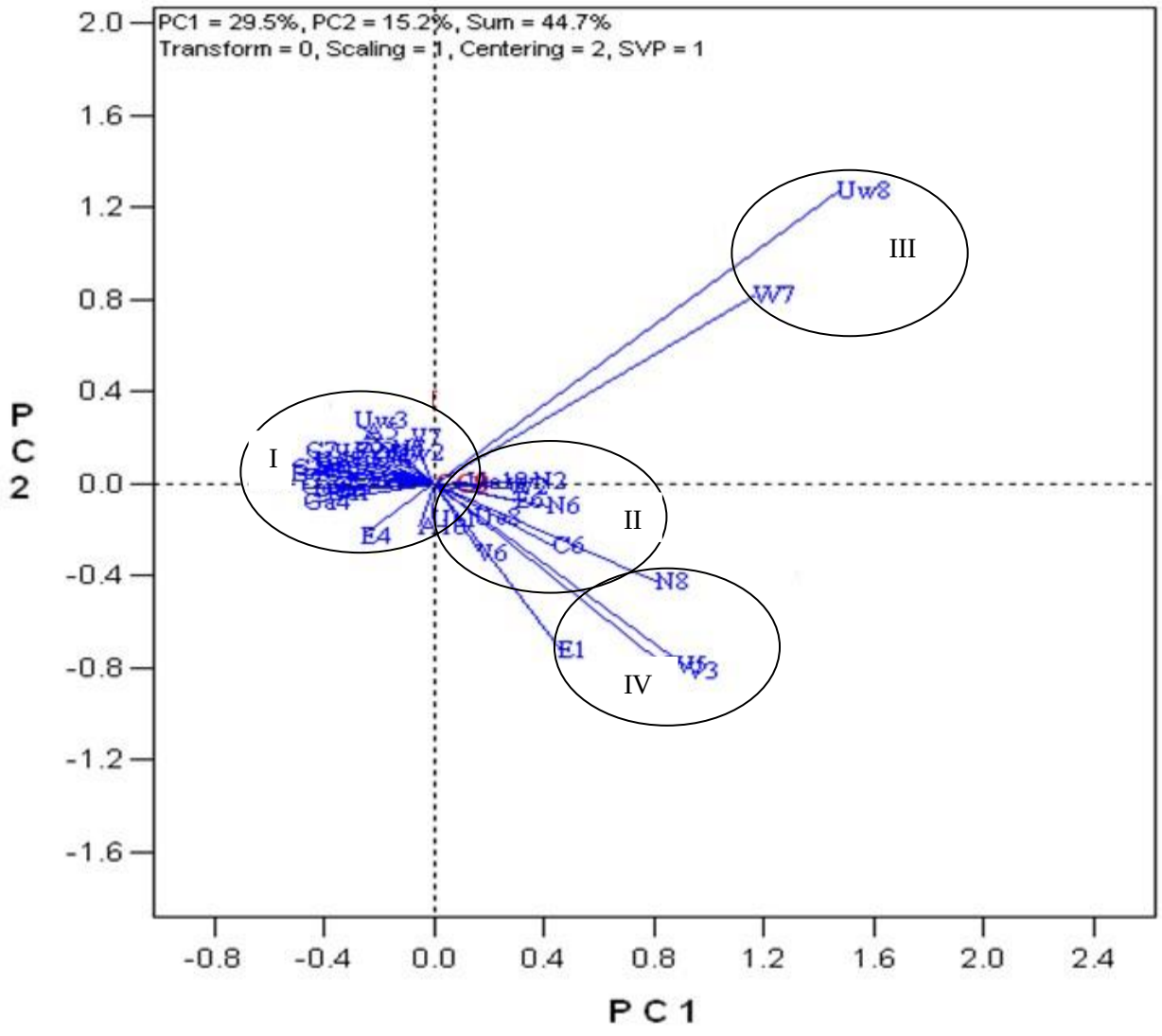

Figure 3.3.4.2b: GGE biplot of 40 *J. curcas* accessions based on phenotypic parameters (field data) and genotypic parameter (primers). PC1 and PC2 are first and second principal components respectively.

3.3.5 Conclusions

The main objective was to determine the genetic diversity, based on morpho-agronomic attributes and molecular markers (RAPD), so that the variations in the genetic resources of *J. curcas* accessions collected from the ten regions of Ghana could be ascertained. This study has demonstrated that the *J. curcas* germplasm in the country is of very low genetic diversity. Low levels of genetic variation in the local accession calls for exploring through research to increase the genetic base of our germplasm. This study has paved way for investigating the genetic distinctness of other accessions coming from other countries or accessions from other geographic background so that plant improvement can be initiated as soon as possible. The genetic and morphological relatedness of accessions from other countries such as Mexico, Central America, India etc where superior genotypes is known to exist can be compared with the genotypes in Ghana. This will aid greater understanding, which can be used to create variations through hybridization techniques to improve on our local germplasm.

3.3.6 Recommendations

- To initiate improvement on the local germplasm, there should be a formal conservation of the germplasm with reliable institution such as Plant Genetic Resources Research Institute (PGRRI) of the Center for Scientific and Industrial Research (CSIR). One accession can be selected from each of the five groups (1a-1, 1a-2, 1b, 2a and 2b) as shown by the dendrogram (Figure 3.3.4.2a). This is because all the accessions in each group are genetically similar.

- Accessions from the two extreme groups of the dendrogram (eg. V6 and A5) can be crossed and their F1 generation monitored for growth and yields vigour as compared to the performance of their parents

- Genetic resources of the plant from the countries of origin, which are Mexico and Central America can be introduced and compared with our local germplasm. Many researchers have reported considerable variations between the accessions from other parts of the world and the accessions from the countries of origin. Therefore germplasm for genetic improvement programs in the country should be introduced from its country of origin rather than from other introduced populations.

- Also plant research institutions in India can also be of help since they are noted for their advancement in the breeding and improvement of *J. curcas.* Genetic resources can be introduced from India to improve the local germplasm through hybridization techniques

- Although selection based on fruit, seed and other yield components are needed for improving the productivity of the plant, knowledge of pest resistance, drought resistance and other yield attributes of both the local and introduced germplasm are also needed in improving on our genetic resources.

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LIST OF APPENDIXES

Appendix 1: Analysis of variance at 5% significant level for the height (cm) growth of the accessions at maturity (10 months growth at the field).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	91	1480.855833	16.273141	1.52	0.0095
Error	178	1909.683353	10.728558		
Corrected Total	269	3390.539187			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Blocks	2	5.269647	2.634823	0.25	0.7825
Accessions	89	1475.586187	16.579620	1.55	0.0074

Appendix 2: Analysis of variance at 5% significant level for the Stem girth (mm)

growth of the accessions at maturity (10 months at the field).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	91	1096.229905	12.046482	1.55	0.0069
Error	178	1384.323284	7.777097		
Corrected Total	269	2480.553189			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Blocks	2	10.530183	5.265091	0.68	0.5094
Accessions	89	1085.699722	12.198873	1.57	0.0059

Appendix 3: Analysis of variance at 5% significant level for the number of branches of the accessions at maturity (10 months at the field).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	91	167.9333333	1.8454212	1.62	0.0033
Error	178	202.7333333	1.1389513		
Corrected Total	269	370.6666667			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Blocks	2	35.2666667	17.6333333	15.48	0.0001
Accessions	89	132.6666667	1.4906367	1.31	0.0445

Appendix 4: Analysis of variance at 5% significant level for number of days to 50% flowering of the accessions (10 months at the field).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	91	1095.881481	12.042654	1.46	0.0170
Error	178	1470.859259	8.263254		
Corrected Total	269	2566.740741			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Blocks	2	103.1407407	51.5703704	6.24	0.0024
Accessions	89	992.7407407	11.1543903	1.35	0.0449

Appendix 5: Analysis of variance at 5% significant level for the average number of fruits per cluster for the accessions (10 months at the field).

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
Model	91	152.9888889	1.6811966	1.72	0.0012
Error	178	174.3777778	0.9796504		
Corrected Total	269	327.3666667			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Blocks	2	1.6222222	0.8111111	0.83	0.4386
Accessions	89	151.3666667	1.7007491	1.74	0.0010

Appendix 6: Analysis of variance at 5% significant level of fresh Fruit weight (kg/ha) of the accessions (10 months at the field).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	91	277033.0915	3044.3197	2.54	0.0001
Error	178	213442.6680	1199.1161		
Corrected Total	269	490475.7595			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Blocks	2	1386.6369	693.3184	0.58	0.5620
Accessions	89	275646.4546	3097.1512	2.58	0.0001

Appendix 7: Analysis of variance of fresh Seed Weight (kg/ha) of the accessions at 5% significant level.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	91	192384091813	2114110899	2.54	0.0001
Error	178	148224074895	832719521.88		
Corrected Total	269	340608166709			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Blocks	2	962942255.51	481471127.75	0.58	0.5620
Accessions	89	191421149558	2150799433.2	2.58	0.0001

Appendix 8: Sample of Questionnaire used for the Traditional Ecological Knowledge on *J. curcas* in Ghana.

Sociological Survey on Traditional Ecological Knowledge of *Jatropha curcas* in Ghana

1. DETAILS OF RESPONDENT

1.1 Name of respondent:

……

1.2 Age class 18 – 30 [ ] = 1 31 – 45 [ ] = 2

45 – 60 [ ] = 3 Above 60 [ ] = 4

1.3 Sex Male [ ] = 1 Female [ ] = 2

1.4 Highest level of education Primary [ ] = 1 Secondary [ ] = 2

Tertiary [ ] = 3 Illiterate [ ] = 4

1.5 Marital status Single [ ] = 1 Married [ ] = 2

2. TRADITIONAL ECOLOGICAL KNOWLEDGE OF INDIGENOUS TREE SPECIES

2.1 mention some useful indigenous tree species which are of importance to you? (Leave out the traditional trees like pawpaw, orange, oil palm, cocoa)

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

2.2 Out of the list above (2.1) mention the ten (10) most important indigenous tree species, starting from the most important to the least.

1……2……

3……4……

5……6……

7……8……..

9……10……

2.3 What are the uses of the above (2.2) tree species? (Could be more than one use)

1……

……

2……..……

……

3……..……

……

4……

……

5……..……

……

6……

……

7……

……

8……

……

9……

……

10……

……

2.4 Where are the above (2.3) tree species mostly found? (For all the ten species in the order of 2.2) Eg. Farmlands [a] 1, 2

- Farmlands [a]
- Homegardens [b]
- Secondary Forest [c]
- Primary Forest [d]
- Avenue/aesthetic planting in community [e]
- Others (please specify) [f]

3. TRADITIONAL ECOLOGICAL KNOWLEDGE ON *Jatropha curcas*

3.1 Have you seen or heard about the *Jatropha* plant before? (This will be asked with a sample at hand). (Use local name)

Yes [ ] = 1 No [ ] = 2

3. 2 If seen, where?

……

……

3. 3 If heard, what?

……

……

……

3. 4 If yes to one or all (see question 2. 1), can you give its local name? What does it mean ……

3. 5 Have you used or seen someone using the plant for anything?

Yes [ ] = 1 No [ ] = 2

3.6 If yes to (2.5), what was it used for?

Live fencing [1] Medicinal [2] Toothpaste [3] Boundary [4] Planting [5] Aesthetic/beautification [5] Others state..... [6]

3.7 How often do you see the plant?

Very often/common [1]
Often/common [2]
Rare [3]
Very rare [4]

3.7 If yes to one or all (see question 3.5), is there any beliefs associated with the use of *Jatropha.*

……

……

……..…

……..…..

3. 8 Do you know it can be used for Bio-diesel energy?

Yes [ ] = 1 No [ ] = 2

3. 9 If yes, how did you know?

……

……

3.10 State why you want to produce or not produce *J. curcas* for sale?

……

……....……

……

QUESTIONNAIRE FOR INSTITUTIONS

4. DETAILS OF INSTITUTION

4.1Name of institution: …….

4.2 Is your institution involved in any *Jatropha curcas* Project?

Yes [ ] = 1 No [ ] = 2

4.3 If yes to (4.2) when was the project started?

……

……

……

4.4 If yes to (4.2) what are the objectives?

……

……

……

……

4.5 Do you know of any other institution working on *Jatropha*.

……

……

…

4.6 Where can stands of *Jatropha curcas* be located in this town?

……

……

……

4.7 Where do you source the Planting Materials for the project?

……

……

4.8 Do you employ farmers on the project?

Yes [ ] = 1 No [ ] = 2

4.9 If yes to (3.6) do you offer any technical training them? Please, specify

……

…..……

…….....

.....

4.10 Does the project consider any integrated land-use systems (e.g. agroforestry)? If yes specify (*detailing type, major components and reason*).

……

……

…….....

.....

4.11 Do you foresee some constraints with *Jatropha curcas* production in Ghana? Yes [ ] No [ ]. If yes list some of them.

……

……

……

…….....

4.11 What do you think should be done? (4.10)

……

……

……

4.12 If all the money to do research on the plant is available, what would you want done?

(Tick, you can tick more than one)

- Research on growth and yield performance [1]
- Research on planting material development [2]
- Research on optimum yield planting distance [3]

Others,state……

……

Appendix 9: Regions, towns, code and characteristics of Accessions used for the study.

UPPER WEST REGION	UPPER EAST REGION	NORTHERN REGION	BRONGAHAFO REGION	GREATER-ACCRA REG.	ASHANTI REGION	CENTRAL REGION	VOLTA REGION	EASTERN REGION	WESTERN REGION
Bamahu	**Bukare**	**Krugu**	**Ayakomaso**	**Amasaman**	**Kwaamo**	**Winneba**	**Sokode**	**Kluutown**	**Bosomtwekroum**
UW1 (MSB)	UE1 (MSB)	N1 (MSB)	BA1 (MSB)	GA1 (MSB)	A1 (MSB)	C1 (MSB)	V1 (MSB)	E1 (MSB)	W1 (LSB)
UW2 (LSB)	UE2 (LSB)	N2 (LSB)	BA2 (LSB)	GA2 (LSB)	A2 (LSB)	C2 (LSB)	V2 (LSB)	E2 (LSB)	W2 (MSB)
Kambele	UE3 (MNF/C)	N3 (MNF/C)	BA3 (MNF/C)	GA3 (LNF/C)	A3 (MNF/C)	C3 (MNF/C)	V3 (MNF/C)	E3 (BFS)	W3 (BFS)
UW3 (MSB)	**Zaare**	**Wrushe**	**Dumasua**	**Dodowa**	**Sepaase**	**Anto-Nsuoakye**	**Aqucnamawu**	**Tafo**	**Assaikai**
UW4 (LSB)	UE4 (MSB)	N4 (MSB)	BA4 (MSB)	GA4 (MSB)	A4 (MSB)	C4 (MSB)	V4 (MSB)	E4 (LSB)	W4 (MSB)
UW5 (LNF/C)	UE5 (LSB)	N5 (LSB)	BA5 (LSB)	GA5 (LSB)	A5 (LSB)	C5 (LSB)	V5 (LSB)	E5 (MSB)	W5 (SFS)
Suntaa-Nuntaa	UE6 (MNF/C)	N6 (MNF/C)	BA6 (LNF/C)	GA6 (LNF/C)	A6 (MNL/C)	C6 (LNF/C)	V6 (LNF/C)	E6 (SFS)	**Kwadwokroum**
UW6 (MSB)	UE7 (LNF/C)	N7 (LNF/C)	**Fiapre**	GA7 (MNF/C)	A7 (LNF/C)	**Agyaa No 1**	**Havɛ**	**Asokore**	W6 (MSB)
UW7 (LSB)	**Zuarungu**	**Old-Kaladan**	BA7 (MSB)	**Ashiyie**	**Atonso-Bokuro**	C7 (MSB)	V7 (MSB)	E7 (MSB)	W7 (LSB)
UW8 (MNF/C)	UE8 (MNF/C)	N8 (MSB)	BA8 (LSB)	GA8 (MSB)	A8 (LSB)	C8 (LSB)	V8 (LSB)	E8 (LSB)	
	UE9 (LSB)	N9 (LSB)	BA9 (MNF/C)	GA9 (LSB)	A9 (MSB)		V9 (MNF/C)	E9 (BFS)	
	UE10 (MSB)	N10 (MNF/C)		GA10 (MNF/C)	A10 (MNF/C)				

MSB- More Stem Branches (4-6), **LSB-** Least Stem Branches (<3)**, LNF/C-** Least Average Number of Fruits per Cluster (5-7)**, MNF/C-** More Average Number of Fruit per Cluster (8-10)