UNIVERSITY OF CAPE COAST

MORPHOLOGICAL AND GENETIC DIVERSITY OF *Persea americana* Mill. (AVOCADO) USING MICROSATELLITES IN THE ASHANTI AND CENTRAL REGIONS OF GHANA

JANICE DWOMOH ODURO

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BY

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Thesis Submitted to the Department of Molecular Biology and Biotechnology, School of Biological Sciences, University of Cape Coast in partial fulfillment of the requirements for the award of Master of Philosophy Degree in Botany

JUNE, 2009

DECLARATIONS

Candidate's Declaration

I hereby declare that this thesis is the results of my own original work and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature:..... Date.....

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature:..... Date:.....

Name: DR. I. K. A. GALYUON

Co- Supervisor's Signature:..... Date:.....

Name: DR. J. F. TAKRAMA

ABSTRACT

Avocado (*Persia americana* Mill) is a nutritious economic tree crop with cultivations scattered all over the country. A study was conducted to assess its distribution, uses and the morphological and genetic diversity of the crop in the Ashanti and Central Regions of Ghana.

An ethnobotanical survey was carried out in 14 districts, while morphological and genetic diversities were determined among the accessions in eight districts. Microsatellites markers were used for genetic diversity studies.

Growth of the plant was better in the Ashanti Region than in the Central Region and it thrived best in old cocoa farms. The crop was mainly cultivated on a small scale. Parts of the plant were used for various medicinal and economic purposes.

Morphologically, they were mainly of Western Indian origin. However, accessions from the Ashanti Region more diverse in the plant and fruit characters compared to the Central Region. Microsatellites analyses revealed 115 different amplification fragments, ranging from 5 to 22 alleles per locus, with an average of 11.5 alleles per locus. All the microsatellites were highly informative, with both genetic diversity and polymorphic informative content (PIC) higher than 0.5. Using the Unweighted Pair Group Method with Arithmetic Averages (UPGMA), the genotypes were clustered into seven major groups.

The wide genetic diversity among the accessions indicates that there is a wide genetic base for improvement of the crop through breeding and selection in Ghana.

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To my family, what can I possibly do without you? Thanks for your love and support; I am indeed, most obliged and grateful. John, my dear husband, thanks for your prayers, love and support. God richly bless you.

DEDICATION

To my family, my husband John and our daughter Kyerewaa.

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CHAPTER ONE

INTRODUCTION

Background

Domestication and spread of avocado

The avocado plant (*Persea americana* Mill.) is a polymorphic tree species that originated in a broad geographical area stretching from the eastern and central highlands of Mexico through Guatemala to the Pacific coast of Central America (Popenoe, 1920; Smith, 1966; Smith, 1969; Storey *et al.,* 1986). The avocado species originated from the tropics of the Western Hemisphere and has developed races, which are adaptable to a wide range of climatic conditions (Bergh, 1969 and Rhodes *et al.,* 1971).

Archaeological evidence indicates that utilization and selection of the crop have gone on in Mexico for a period of 10,000 years (Knight, 2002). Seeds found in caves in the Tehuacan Valley, Puebla State, show that during that time there was progressive selection for increased fruit size, as indicated by the increasing size of the seeds uncovered later; compared with earlier levels of excavation, and presumably also for other desired qualities (Smith, 1966; Smith, 1969).

The avocado crop first caught economic attention in the 16th century when Hernando Cortez, a Spanish soldier of fortune, set foot in Mexico City in 1519. Among the many significant events of that historic day was the discovery of the most versatile crop of the New World, the avocado. In 1526, Oviedo, a historian to the conquistadores, wrote the following description of avocado and

gave the first directions for eating it: 'In the centre of the fruit is a seed like a peeled chestnut; and between this and the rind is the part which is eaten, which is abundant, and is a paste similar to butter and of very good taste'. Hernando and Oviedo played major roles in the spread of the crop to other parts of the world outside its native country; in the sense that Hernando led the expedition that discovered the plant whilst Oviedo documented its properties and uses.

Though not discovered early enough, the avocado plant spread very fast to many parts of the world due to its nutritional value and the relish for the fruit of the plant. The spread of the plant from the South American continent has resulted in the numerous names given to the plant in many parts of the world. The most common English name for the fruit, avocado, is a modification of the Spanish name, “aguacate” or “ahuacate”; derived from the Nahuatl word “ahuacatl”. The common name for the fruit in Dutch is “advocaat” or “avocet”; in German “Abakate”, and “abacate” in Portuguese. Another name in some South American counties is “palta”. “Alligator pear” and “midshipman’s butter” are somewhat fanciful English names used occasionally (Anon., 1961; Ochse *et al.*, 1961; Morton, 1987). In West Africa, it is called “custard apple” (Gustafson, 1976). There are other traditional names of the crop in West African countries, of which Ghana is no exception. In Ghana avocado is known locally as “pɛa” or “paya” in Twi, Fante, Ga and Adangme (Irvine, 1961).

Even though the Spanish introduced avocado to the West Indies and the Atlantic Islands such as the Canaries much earlier, avocado only started to be grown in West Africa, Mauritius and India in the 1700's. It took a long time for it

to be grown as a major crop, which has been attributed to poor fruit quality (Hamish, 2004). Hamish (2004) also reported that it was difficult at that time to produce an orchard of avocado tree with consistently high fruit-quality; except by cutting out the trees that were poor fruit producers due to the problem of cross pollination.

The West Indian seedlings were introduced to the coastal areas of West Tropical Africa centuries ago; indeed, literature shows that Ghana was the first African country to have avocado introduced to by missionaries in 1750. Avocado is still cultivated in Ghana and the fruit is sold in markets; but this has not become an important industry. On the other hand avocado is cultivated in Cameroon, Angola and Kenya where it is even exported (Campbell and Malo, 1976).

Some testing in the survival rates of introduced cultivars has been conducted in Ghana and Ivory Coast, where West Indian cultivars and West Indian-Guatemalan hybrids were found to be the most promising (Campbell and Malo, 1976). Avocado is widely grown in the closed forest region in Ghana; especially, at the higher altitudes, where it appears to thrive better, hence its occasional name Mountain Pear (Irvine, 1961).

Avocado

The avocado plant (*Persea americana* Mill.) has a fruit with a high nutritive value and an unusual composition of nutrients. Though it is high in protein and fat, the outstanding compositional feature is the high fat content; this varies significantly between different cultivars (Vekiari *et al.*, 2003). There are

three general ecological groups or races of the avocado: the Mexican, the Guatemalan and the West Indian. The differences between these races are related to their maturity and oil content (Vekiari *et al.*, 2003).

A native of the Tropical Americas, which flourishes in areas with over 150 mm annual rainfall at between 55 m and 550 m altitude, the avocado fruit is pear-shaped, and the edible part is a thick layer of greenish-yellow pulp. The fruit will continue to enlarge in size while on the tree; and will only ripen after it is harvested. The oily, greenish-yellow flesh is of the consistency of firm butter, and contains good proportions of both oil and proteins.

Global cultivation of avocado

Total world avocado production increased approximately 3.3-fold over a 35-year period. The increase was from 697,869 t in 1961 to 2,303,389 t in 1996 (Knight, 2002). Annual production increased at a fairly steady rate until 1986. The total world production first passed the 2-million mark in 1996; this was the highest figure reported until 1998 (FAOSTAT Database, 2001). The lowest rate of avocado production for this period was recorded in South America. It was 205,970 t in 1961 and 382,843 t in 1996 representing an increase of only 1.9 fold as compared to the world increase of 3.3 fold. The highest rate of avocado production for this same period was recorded in Europe. It was 340 t in 1961 and 66,800 t in 1996. This represented an increase of 169 fold compared to the world rate of increase of 3.3 fold (Knight, 2002). Also, there were cultivation increases in places like Oceania, Asia, North America and Africa, during that period. Ghana

maintained a production of 4 t from 1961 to 1981; but increased to 6.3 t in 1996 (FAOSTAT Database, 2001). Cultivation continues to be dynamic in many parts of the world, such as Mexico, where avocado is a new traditional crop that is undergoing considerable expansion. On the other hand, avocado cultivation remains more or less static in many countries like Brazil; where it is not subject to demand from export markets. Cultivation in Ghana is not very encouraging; here, something should be done to enhance the economic benefit of the crop and promote export to the world market.

Diversity of avocado

Traditionally, the crop is identified using morphological characters. Since morphological traits can be influenced by environmental conditions, they are not always adequate for the identification and classification of the species. However, in some cases, morphological characters have been adequate to identify and distinguish species. The use of model genetic systems in plant and animal studies has allowed a greatly increased understanding of how genomes regulate phenotype. Methods of measuring genetic diversity have an important role within conservation programmes for genetic resources of crop plants (Newbury and Ford-Lloyd, 1997). Genetic engineering and biotechnology hold great potential for plant breeding; as they promise to expedite development of cultivars with more desirable values and traits.

The diversity of avocado inherent in the three ecological (or horticultural) races was exploited earlier on by people living where each race was native. In the case

of Mexican avocados, the native one is that which now grows in Puebla State. This exploitation started, at least, 9,000 years ago. Archaeological records support the Puebla improvement work (Smith, 1966; Smith, 1969).

Literature suggests that the diverse environmental conditions within which avocado can evolve have produced various distinct genotypes. This has made it possible to develop modern cultivars adapted to widely divergent growing conditions on six of the world's continents. Some reports also pointed out that Mexico, Guatemala and the Caribbean have contributed diverse and valuable germplasm and genotypes to cultivar development. During the first half of the 20th century, selection and breeding practices in California and Florida produced two groups of named cultivars. These have contributed materially to the 3.3-fold increase in world avocado production, from 1961 to 1996 (Knight, 2002). It is reported that since the early work in the continental United States, efforts at selection and breeding in other parts of the world, notably, Puerto Rico, Israel, Brazil and Australia, have produced new cultivars; and that this effort is still continuing (Knight, 2002).

Genetic markers are now used as diagnostic tools for identification and characterisation. Ashworth *et al*. (2004) used 25 microsatellite markers to differentiate 35 avocado cultivars and two wild relatives. Results from the research showed an average heterozygosity is high (60.7%); ranging from 32% in *P. steyermarkii* to 84% in Fuerte and Bacon. The work also showed an average heterozygosity of 63.5% for microsatellites; compared to 41.8% for restriction fragment length polymorphisms (RFLPs) in a subset of 15 cultivars used for

further work. A neighbour-joining tree, according to average shared allele distances, defined three clusters (Ashworth *et al*., 2004).

Microsatellites

Microsatellites or simple sequence repeats (SSRs) have become one of the most popular molecular markers used with applications in many different fields. High polymorphism and the relative ease of scoring represent the two major features that make microsatellites largely interesting for many genetic studies (Zane *et al.,* 2002).

SSRs are tandemly repeated motifs of 1-6 bases found in all prokaryotic and eukaryotic genomes (Zane *et al.,* 2002). They are present in both coding and noncoding regions and are usually characterized by a high degree of length polymorphism (Zane *et al.,* 2002). The origin of such polymorphism is still under debate, though, it appears most likely to be due to slippage events during DNA replication (Schlötterer and Tautz, 1992). Despite the fact that the mechanism of microsatellite evolution is still unclear, SSRs were being widely employed in many fields soon after their first description (Litt and Luty, 1989; Tautz, 1989; Weber and May, 1989) because of the high variability which makes them very powerful genetic markers. Microsatellites have proven to be an extremely valuable tool for genome mapping in many organisms (Schuler *et al.,* 1996; Knapik *et al.,* 1998), and their application spans over different areas, ranging from ancient and forensic DNA studies to population genetics and conservation/management of biological resources (Jarne and Lagoda, 1996).

The major drawback of microsatellites is that they need to be isolated *de novo* from most species being examined for the first time (Zane *et al.,* 2002). This is due to the fact that microsatellites are usually found in noncoding regions where the nucleotide substitution rate is higher than in coding regions (Zane *et al.*, 2002).

Flinders Technology Associates (FTA^{TM}**) technology**

Flinders Technology Associates **(**FTA^{TM}**)** technology is a paper-based system designed to fix and store nucleic acids directly from fresh tissues pressed into the treated paper. FTA^{TM} is a paper-based technology designed for the collection and archiving of nucleic acids, either in their purified form or within pressed samples of fresh tissue.

Proprietary chemicals impregnated into the paper act to lyse cellular material and fix and preserve DNA and RNA within the fibre matrix (Whatman, 2004). After a short drying period, pressed samples can be stored at room temperature for extended periods and processed when required. Nucleic acids are recovered by removing small punches from the pressed area and washing with simple reagents.

Genomic DNA remains attached to the paper matrix but is available for amplification by Polymerase Chain Reaching (PCR) when the paper punch is added to the PCR reaction mix. Advantages of FTA^{TM} technology have been realized for human DNA processing and forensic analysis (Zhong *et al.,* 2001), for analysing wildlife DNA (Smith and Burgoyne, 2004). FTA^{TM} is also applied

to PCR-based genotyping (Drescher and Graner, 2002; Tsukaya, 2004); however, FTA™ protocols have not been well documented for use with avocado DNA. Recognizing the potential benefits this technology could bring to sampling and molecular study of avocado cultivars, the efficacy of FTA™ for retrieval of DNA from avocado plant would enhance molecular work on the species and its cultivars.

Statement of the problem

Ghana has a lot of fruit crops. Examples of such fruit plants are mangoes (*Mangifera spp.),* oranges (*Citrus spp.*), pawpaw (*Carica papaya),* guava (*Psidium spp*.) and avocado (*Persea americana*). Avocado has a high nutritional value; especially fat, protein and vitamins (Verheij and Coronel, 1991; Hamish, 2004). In spite of this high nutritional content, malnutrition is still prevalent in most rural communities in Ghana.

Avocado plant has medicinal properties, especially in managing high blood pressure, a very common ailment in Ghana. However, this potential has not been investigated in Ghana.

Furthermore, avocado has the potential to contribute immensely to the economy of Ghana if cultivated on commercial scale, as in America, Europe and South Africa (Campbell and Malo 1976; Morton, 1987). Cultivations are scattered all over the country, but the agro-ecological distribution, morphological and genetic diversity have not been well studied.

Rationale for the study

Although there have been some research studies on avocado in some parts of the world, like California, on the taxonomy and genetic diversity, rather limited research has been done on avocado in Ghana. This work will reveal avocado's medicinal value, morphological variation, genetic diversity and its agro-ecological distribution in Ghana. As well, not much is known about the accessions of avocado introduced to Ghana. By the end of the study the parental stock of accessions in Ghana would have been identified.

Further more, the result of the research will be used as a basis for further molecular work on the plant in the country. Results on the profile of distribution of avocado plants will serve as a guide to researchers who want to work in the area. A gene library of the accessions in the regions will enhance molecular work on the plant species to be carried out in the country. In addition, it will help in the stocking of a germplasm bank for cultivation and commercialization of the crop.

Main objective

The main objective of the study was to profile the morphological and genetic diversity of avocado accessions in the Ashanti and Central Regions of Ghana using microsatellites.

Specific objectives

The concomitant objectives of the study were to:

i. assess the ethnobotany of avocado in the Ashanti and Central Regions of Ghana.

ii. determine the profiles of distribution of avocado cultivations in the two study regions.

iii. assess the morphological diversity among different accessions of avocado.

iv. define the genetic variations between avocado accessions using microsatellite.

CHAPTER TWO

LITERATURE REVIEW

History of the avocado crop plant

The avocado plant, *Persea americana* Mill., originated in the tropics of the Western Hemisphere, and has developed races, which have adapted to a wide range of climatic conditions (Bergh, 1969; Rhodes *et al.*, 1971).

Avocado is a relatively new crop of the world, outside its native range in the American Tropics (Campbell and Malo, 1976). The plant has proved to be a profitable economic crop, both for local sale and for export; to the extent that there is now a keen interest in the establishment of avocado industries in many countries. The development of superior cultivars is basic to the establishment of any horticultural industry.

Three horticultural or ecological races of avocado have traditionally been recognized; namely, the Mexican, Guatemalan, and West Indian races. These races are distinguishable on the basis of morphological, physiological, and horticultural traits; and they are adapted to different climates and ecological conditions (Bergh, 1995; Bergh and Lahav, 1996). The Mexican and Guatemalan races are adapted to the cooler climates prevailing in the Mexican and Guatemalan Highlands, while the West Indian race or Lowland race (Bergh, 1995) needs a warmer climate for full development. The Mexican, Guatemalan and West Indian races correspond to *Persea americana* subspecies *drymifolia* (Schlecht. *et* Cham.) Blake, *guatemalensis* L.Wms. and *americana* Mill.

respectively (Bergh *et al.*, 1973; Bergh, 1995). These subspecies were formally referred to as varieties. This classification varies from other classifications (Kopp, 1966; Williams, 1977). The Guatemalan and West Indian races were classified together as *P. americana,* var. *americana* Mill.; and the Mexican race separately classified as *P. americana* var. *drymifolia* (Schlecht. *et* Cham.) Blake (Kopp, 1966). Another classification option separates the Guatemalan race from the other two; it classifies it as *P. nubigena* L.Wms. var. *guatemalensis* L.Wms (subgenus *Persea*); while the Mexican race (var. *drymifolia* (Schlecht. *et* Cham.) Blake) and the West Indian race (var. *americana* Mill.) have been maintained as varieties of *P. americana* (Williams, 1977).

The West Indian race thrives best in the lowland tropics, while the Mexican race is best adapted to tropical highlands and to subtropical climates (Campbell and Malo, 1976). The Guatemalan race appears to be intermediate between the two in its climatic adaptation. The races hybridize freely and hybrid cultivars have become very important in commercial cultivations.

California and Florida in the United States of America are important centres of origin of new avocado cultivars, as a result of vigorous programmes of introduction and selection. Other countries, such as Israel, are also showing promise of similar development. For various reasons, controlled breeding has not yet produced any commercial cultivars; however, it should be encouraged because of the great potential for development of superior selections (Campbell and Malo, 1976). Many of the commercial avocado cultivars nowadays are interracial

hybrids produced from seedlings from unknown sources (Alcaraz and Hormaza, 2007).

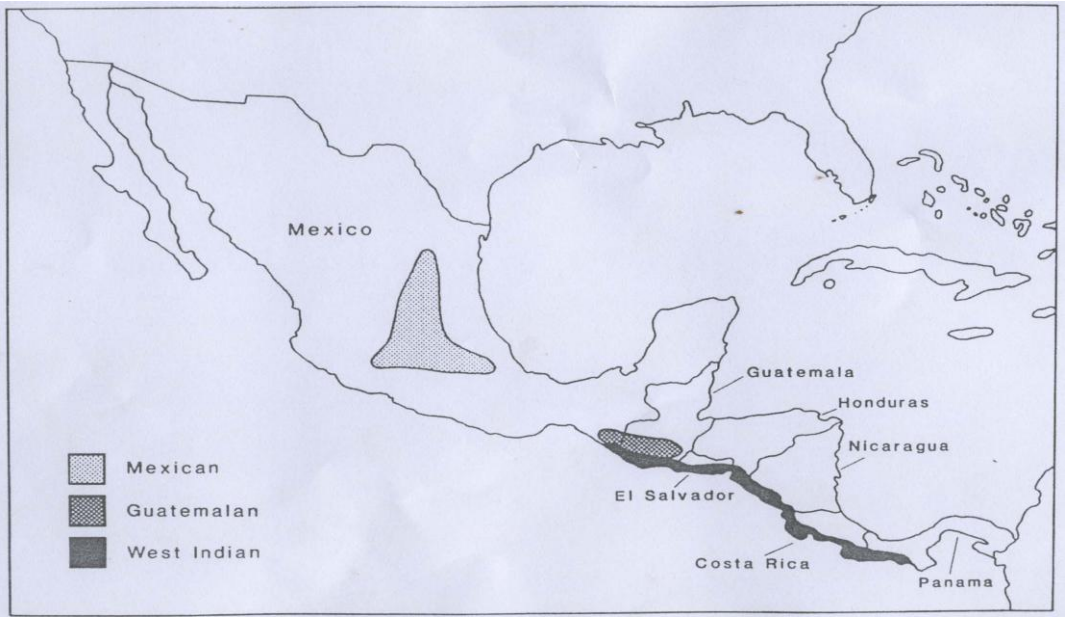

Fig. 2.1: Putative centre of origin of the Mexican, Guatemalan, and West Indian races of avocado (*Persea americana*)
Source: Smith *et al*. (1992)

Spread of the avocado

Fig. 2.1 shows the putative centre of origin of the Mexican, Guatemalan, and West Indian races of avocado (*Persea americana*). It was thought that avocado may have originated in Southern Mexico; and that it was cultivated from the Rio Grande to central Peru long before the arrival of Europeans. It was carried not only to the West Indies where it was first reported in Jamaica in 1696; but also it got to nearly all parts of the tropical and subtropical world, with suitable environmental conditions (Morton, 1987). The same authorities speculate that avocado was taken to the Philippines near the end of the 16th Century; to the

Dutch East Indies by 1750, and Mauritius in 1780. Avocado arrived in Singapore between 1830 and 1840, but Morton (1987) reports that it got to nearly all parts of the world but never became common in Malaya. It reached India in 1892, and is cultivated especially around Madras and Bangalore. However, it never became that popular, because of the preference for sweeter fruits. It was cultivated in Hawaii in 1825, and became common place throughout the islands by 1910. Henry Perrine introduced it into Florida from Mexico in 1833, and into California, also from Mexico, in 1871 (Gustafson, 1976; Rollins, 1987). Vegetative propagation began in 1890 and stimulated the importation of budwood of various types, primarily to extend the season of fruiting. Some came from Hawaii in 1904 (Morton, 1987). Presently, avocado is cultivated commercially in the United States, tropical America and the larger islands of the Caribbean. It is also cultivated in Polynesia, the Philippines, Australia, New Zealand, Madagascar, Mauritius, Madeira, the Canary Islands, Algeria, tropical Africa, South Africa, southern Spain, southern France, Sicily, Crete, Israel and Egypt (Morton, 1987).

The Spaniards took the avocado crop to Chile, probably early in the 17th Century and planted it 1,600 km southward away from the Peruvian border. Commercial cultivation started with the introduction of the California cultivars; that is, in about 1930, into two areas within 160 km of Santiago in Chile; where the industry is now centred (Morton, 1987).

The first crops was cultivated in Israel in 1908; but cultivars ‘Fuerte’ and ‘Dickinson’ were not introduced until 1924 (Morton, 1987). These aroused interest in the performance of the crop within the southern half of the coastal

plain, and within the interior valleys. Thus, development of the avocado industry has steadily proceeded; except for a period in the 1960's, when much planting stock was destroyed out of marketing problems. In 1979, Israel produced 33,000 tons and exported 28,600 tons (Morton, 1987).

Little has been recorded on early introductions of avocado into South Africa, but it is accepted that the first tree was a West Indian race seedling; planted on the coastal strip of Natal, especially, around Durban, in the late 19th century (Ludman, 1930). Fruits from these trees were of inferior quality and attracted no commercial interest (Anon., 1965).

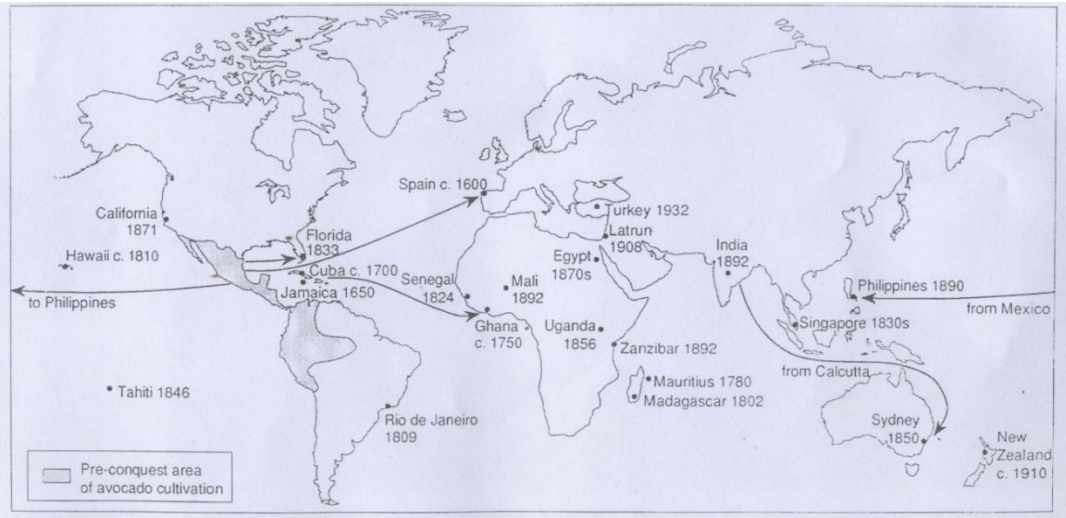

Fig. 2.2: Spread of avocado plantations from its centre of origin in Central America
Source: Smith *et al.* (1992)

Geographical distribution

The avocado plant occurs between latitudes 22°N and 12°S. It is also cultivated in various countries in Asia: India, Indonesia, Israel, and Philippines.

On the African continent, avocado is cultivated in East Africa, Madagascar, South Africa, Southern Africa and West Africa (Fig 2.2).

Commercial production status in the world

New Zealand launched a programme to expand commercial production; especially, in the Bay of Plenty, with a view of developing into a major exporter of avocados (Morton, 1987). Morton (1987) has reported that California produced 13,249.5 t in 1976 and 24,299 t in 1981. The Florida avocado crop potential is estimated to be 7,499.8 t. Cultivation in both states suffers fluctuations, because of the impact of periodic freezes, droughts, high winds or other seasonal factors.

Avocado is mainly cultivated in Mexico, the USA and in Indonesia. However, the cultivation of avocado is also expanding into some non-traditional localities; such as Sicily and Calabria in the Mediterranean area (Frega *et al*., 1990). Avocado also grows in Turkey; and plantations have rapidly expanded during the past decade.

Presently, Mexico is the leading producer of avocado with 267,300 t per annum; the Dominican Republic comes second with 144,100 t; the U.S.A. (California and Florida combined) comes third with 130,900 t; Brazil is fourth, 128,700 t. Half of California's plantings are in the San Diego County, close to Mexico (Morton, 1987).

As an exporter, Mexico again leads; followed by California, Israel, South Africa and Florida, in that order (Morton, 1987). It is interesting to note that nearly all the avocado crop produced in Brazil is consumed domestically.

The most intensive avocado production in Southern Africa is in the Republic of South Africa (Pretorius, 1972); with minor production in neighbouring countries. Most of the orchards are in Transvaal. 'Fuerte' is the most widely planted cultivar, but 'Edranol', 'Ryan' and 'Hass' are also relatively important. Other cultivars, which are common, include 'Benik', 'Carlsbad', 'Carton', 'Collinson', 'Gottfried', 'Itzamna', 'Linda' and 'Nabal' (Campbell and Malo, 1976).

The taxomonic family of avocado

Avocado belongs to the family Lauraceae. The family has several taxa of economic importance; especially, as food and spices: *Persea americana* Mill.; *Cinnamomum camphora* (L) J. Presl; *Cinnamomum zeylanicum* Blume.; *Cinnamomum cassia* (Nees) Nees and Eberm ex Blume; also ornamentals: *Laurus nobilis* L. and *Persea indica* Spreng.; and a timber genera *Ocotea* Aubl. and *Nectandra* Roland ex Rottb. The last two genera, as well as *Beilschmiedia* Nees are closely related to *Persea* (Scora and Bergh, 1990).

Ocotea and *Nectandra* are ancient genera. They existed in the New World in the Eocene flora of the Mississippi (Dilcher, 1963; Dilcher, 1973). They may have been African genera, which had dispersed to North America via South America and via islands after the Cretaceous. *Ocotea* is represented by hundreds of species in tropical America; and a few are left in Africa (Scora and Bergh, 1990).

Genus *Persea*

The genus *Persea* belong to the family Lauraceae, which is part of the primitive order Ranales (Magnoliales), consisting of more than 50 genera, which occupy primarily tropical regions, with a few genera extending into the temperate areas (Scora and Bergh, 1990).

The commercial avocado (*Persea americana* Mill.) belongs to the *Persea* subgenus of the genus *Persea*; which also contains the subgenus *P. drymifolia*. Eighty-one *Persea* species, belonging to the same subgenus have been described. Indications are that *P. schiedeana*, *P. floccosa* and *P. americana* are strongly related (Kopp, 1966). However, subsequent studies (Bergh and Ellstrand, 1986) suggested that all members, except *P. Schiedeana*, may best be classified into a single polytypic species: *P. americana* Mill. This classification was recently supported by other authorities (Furnier *et al*., 1990; Ben-Ya'acov, 1995). They argued that the various groups of plants belonging to the sub-genus *Persea* excluding *P. schiedeana* be best considered as sub-species of *P. americana* (Ben-Ya'acov, 1995). All *Persea* species examined, except *P. hintonii* which was a tetraploid (2n=4x=48) (Garcia, 1975), have a chromosome number of 2n=2x=24 (Garcia, 1975; Alcaraz and Hormaza, 2007)

The avocado species (*Persea americana*)

Avocado is a diploid member of the family Lauraceae; it belongs to one of the earliest lineages in the angiosperm phylogeny, that predates the origin of the Eudicots (Zanis *et al.*, 2002).

Avocado is indigenous to Central and South America; it was domesticated by 500 B.C. The word 'avocado' originates from the Aztec word ‘ahuacalt’; meaning, 'testicle tree' (Harper, 2001; Hamish, 2004). The avocado pear, belonging to the plant group *Persea americana*, is commonly known in Jamaica simply as “pears”. It is a native of the Tropical Americas, and flourishes in areas with over 15 cm of rainfall per annum; at between 55 and 550 meters elevation (Lawrence, http//www.radajamaica.com. jm/index.htm). Most common varieties cultivated are: Simmonds (in-season variety, ripening in the summer months; and Collinson, Lula (out-of season variety, ripening in December-February).

Wild *Persea americana* have a wide yet disjunctive distribution in Central and South America; ranging from Eastern Mexico through Central America, to the Northern Andes (Hamish, 2004). Avocado also grows on mountains in cloud forest, and on the lower slopes in rain forest, with well-drained soils. Wild fruits are 4-5 cm in diameter with some 2 cm diameter seed (Hamish, 2004). The rather large seed size is adaptation for supplying young plants with enough food, to enable them survive in the dim forest understorey until they can exploit some open gap created by fallen trees. Large seeds like this are common in other tropical species.

Avocado seeds dating to 7000 B.C. have been found at a Mexican archaeological site (Smith, 1966). Seed sizes are similar to wild varieties, indicating that the fruits were being harvested in the wild, rather than from selective cultivations. It is only in archaeological deposits, dated to about 500 B.C. that the abundance and size of avocado seeds increase; indicating cultivation

of plants from seeds selected on the basis of fruit size (Smith, 1966). However, at some other archaeological site in Mexico, small, wild-sized seeds have been found in deposits, dating to as late as 700 A.D. which indicates that avocado cultivation took quite some time to spread to the various communities (Smith, 1969).

The species has a 7 to 8 year juvenile period, high rate of flower abscission and immature fruit drop (Arumuganathan and Earle, 1991).

Botany of the avocado crop

The avocado plant is erect; usually up to 9 m, but sometimes up to 18 m or more. The diameter of the trunk is between 30 and 60 cm. It is almost evergreen but sheds leaves briefly in dry seasons at blooming time. The leaves are alternate, dark-green and glossy on the upper surface; they are whitish on the underside and have shapes such as lanceolate, elliptic, oval, ovate or obovate (Morton, 1987). The leave length can reach 7.5–40 cm long (Morton, 1987). Those of the Mexican race are strongly anise-scented. Small, pale-green or yellow-green flowers are borne profusely in racemes near the branch tips. They lack petals but have two whorls of three perianth lobes, more or less pubescent, and nine stamens with two basal orange nectar glands.

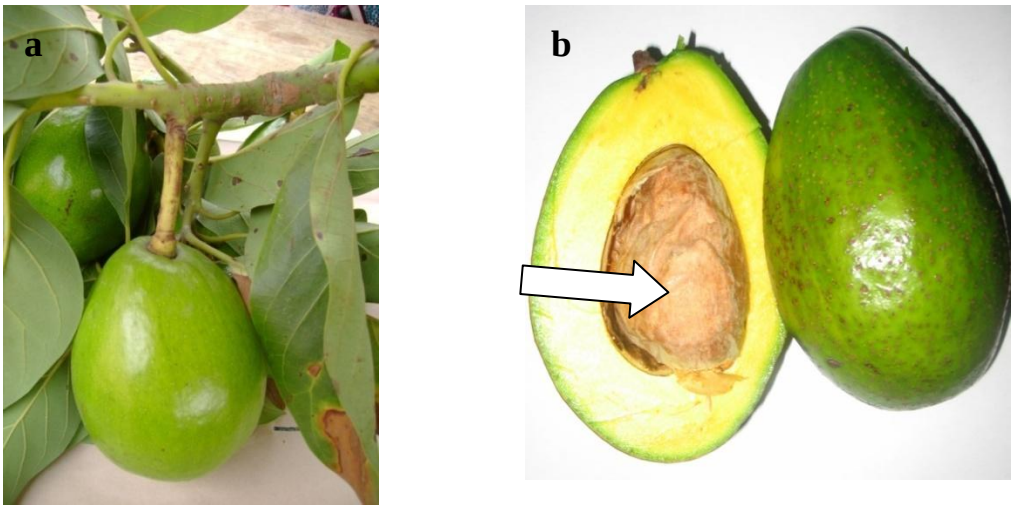

Plate 2.1: Avocado fruits showing some external and internal features. Plate (a) shows a whole fruit and plate (b) shows a fruit cut opened to show seed (arrowed).
Photos: Janice D. Oduro

The fruit, pear-shaped, often more or less necked, oval, or nearly round, may be 7.5-33 cm long and up to 15 cm wide (Morton, 1987). The skin may be yellow-green, deep-green or very dark-green, reddish-purple, or so dark a purple as to appear almost black, and is sometimes speckled with tiny yellow dots, it may be smooth or pebbled, glossy or dull, thin or leathery and up to 6 mm thick, pliable or granular and brittle (Morton, 1987). In some fruits, immediately beneath the skin there is a thin layer of soft, bright-green flesh, but generally the flesh is entirely pale to rich-yellow, buttery and bland or nutlike in flavour. The single seed is oblate, round, conical or ovoid, 5-6.4 cm long, hard and heavy, ivory in colour but enclosed in two brown, thin, papery seed coats often adhering to the flesh cavity, while the seed slips out readily (Plate 2.1). Some fruits are seedless because of lack of pollination or other factors (Morton, 1987).

Soil of the avocado plant

The avocado plant is remarkably versatile as to soil adaptability. It does well on diverse types of soil such as red clay, sand, volcanic loam, lateritic soils, or limestone. In Puerto Rico, it has been found healthier on nearly neutral or slightly alkaline soils than on moderately or highly acid soils. The avocado plant tolerates soil pH levels between six and seven. In Southern Florida however, avocado grows on limestone soils with pH ranging from 7.2 to 8.3 (Morton, 1987). The plant requires well drained soil and cannot survive in even temporarily water-logged soil. Sites with underlying hardpan must therefore be avoided. The water table should be at least 0.9 m below the surface. Avocado plants can withstand variable soil salinity. Certain cultivars such as 'Fuchs-20' and 'Maoz' have shown considerable salt-tolerance in Israel (Morton, 1987).

Importance of the avocado plant

Persea americana is a small dense evergreen tree, approximately 20 m in height. This species is popularly grown for its fruit. *Persea americana* is also suitable for improving soil in fallow regions, or controlling soil erosion on river banks (Carlowitz, 1986). It is also planted as an amenity tree for shade. The fruits have nutritional value and are rich in fat, protein and vitamins, with an energy content of 600-800 kj/100 g (Verheij and Coronel, 1991). It is usually eaten raw in salads, or in Indonesia and the Philippines it is eaten as a desert. There is not enough documentation on the economic importance in Ghana.

P. americana is able to withstand some drought, however, in severe drought during flowering, fruit set or fruit maturation, then the yield may be affected (Carlowitz, 1986; Verheij and Coronel, 1991).

Indians in tropical America add salt to the avocado fruit and eat with tortillas and coffee beverage as a complete meal (Morton, 1987). In North America, avocados are primarily served as salad vegetables, merely halved and garnished with seasonings, lime juice, lemon juice, vinegar, mayonnaise or other dressings. Often the halves are stuffed with shrimp, crab or other seafood. Avocado flesh may be sliced or diced and combined with tomatoes, cucumbers or other vegetables and served as a salad. The seasoned flesh is sometimes used as a sandwich filling. Avocado, cream cheese and pineapple juice may be blended as a creamy dressing for fruit salads (Morton, 1987).

It has been reported that the seed produces a milky fluid with the odour and taste of almond (Morton 1987). Because of its tannin content, it turns red on exposure, providing indelible red-brown or blackish ink, which was used to write many documents in the days of the Spanish conquest. These are now preserved in the archives of Popayan. The ink has also been used to mark cotton and linen textiles (Morton, 1987). In Guatemala, the bark is boiled with dyes to set the colour. The avocado fruit is an important food in South America and is nutritious with high levels of mainly unsaturated oils, minerals, vitamins and reasonable levels of protein (Hamish, 2004). Honeybees gather a moderate amount of pollen from the avocado flower for making honey (Morton, 1987). The wood has been utilized for the construction of boards and turnery. An Australian woodworker has

reported that it is suitable for carving, resembles White Beech (*Eucalyptus kirtonii*); is easy to work, and dresses and polishes beautifully. He has made it into fancy jewel boxes. A Florida experimenter made bowls of it but they cracked (Morton, 1987).

Medicinal uses

Numerous epidemiological studies suggest that a diet rich in fruits and vegetables is associated with a reduced risk of many common forms of cancer (Martinez and Giovannucci, 1997; Greenwald *et al.,* 2001; Riboli and Norat, 2003). In support of these observations, several *in vitro* and *in vivo* studies have demonstrated the anti-tumor properties of various fruit and vegetable extracts (Sauter and Wolfensberger, 1989; Shao *et al.,* 1996; He *et al.,* 1997; Riggs *et al.,* 1997; Huang *et al.,* 2002; Liu *et al.,* 2002; Roy *et al.,* 2002; Yan *et al.,* 2002; Tyagi *et al.,* 2003;). These studies have significant implications for the agricultural production of fruits and vegetables that could be geared to enhance these health promoting activities.

Avocado is an oleaginous fruit (Lewis *et al.,* 1978). This fruit has a lipid content approximating 25% of the edible portion with an energy density similar to chicken breast (Hierro *et al.,* 1992). The principal components of the lipid fraction are monounsaturated fatty acids (Swhisher, 1988). Such monounsaturates have been studied for their potential cardiovascular benefits including effects on serum lipids (Alvizouri *et al.,* 1992; Colquhoun *et al.,* 1992; Carranza *et al.,* 1995; Carranza *et al.,* 1997).

In addition to its high content of monounsaturated fats, avocados contain several bioactive phytochemicals. These include some carotenoids (Lassen *et al.,* 1944; Slater *et al.,* 1975; Heinonen *et al.,* 1989), vitamins B, vitamins C and E (Slater *et al.,* 1975), terpenoids (Moreno *et al.,* 2003), d-manno-heptulose (Shaw *et al.,* 1980), h-sitosterol (Duester, 2001), persenone A and B (Kim *et al.,* 2000), and phenols (Vinson *et al.,* 2001).

The bioactive substances in this fruit and its extract or individual components have antioxidative, radical suppressing (Kim *et al.,* 1998; Kim *et al.,* 2000; Vinson *et al.,* 2001), acetyl CoA carboxylase inhibitory (Hashimura *et al.,* 2001), and antifungal (Prusky *et al.,* 1991; Domergue *et al.,* 2000) activities. The fruit skin is antibiotic; it is employed as a vermifuge and remedy for dysentery. The leaves are chewed as a remedy for pyorrhea. Leaf poultices are applied on wounds. Heated leaves have the capacity to relief neuralgia when applied on the forehead and the juice in the leaves have antibiotic properties. The leaf decoction is useful for the treatment of diarrhoea, sore throat and haemorrhage (Morton, 1987). According to some schools of thought, the fluid from avocado leaves stimulates and regulates menstruation (Morton, 1987). In Cuba, a decoction of the new shoots is a cough remedy. If leaves or shoots of the purple-skinned type are boiled, the decoction serves as an abortifacient. Sometimes a piece of the seed is boiled with the leaves to make the decoction (Morton, 1987).

The seed is cut in pieces, roasted and pulverized and given to overcome diarrhoea and dysentery. The powdered seed is believed to cure dandruff. A piece of the seed or a bit of the decoction, put into a tooth cavity may relieve toothache.

An ointment made of the pulverized seed is rubbed on the face as a rubefacient to redden the cheeks. Oil extracted from the seed has been applied on skin eruptions (Morton, 1987).

Nutritional content and therapeutic effects of avocado fruit

Tropical fruits are important in the diet of people especially, those in developing countries. The exact manner in which avocado is used as food varies with the indigenes (Morton, 1987). The fruit is a traditional staple in Guatemala and nearby countries, the daily food of the labouring individual (Popenoe, 1920); and also used for the preparation of traditional dishes like guacamole in Mexico (Anusasamanan, 2001). It is eaten with sugar and ice cream or milk shake in some countries. The nutrient content in avocado pulp depends on ecotype (subtropical or tropical), cultivars, degree of maturity of the fruit and growing conditions (Knight, 2001).

Avocado oil is evidently similar in composition to olive oil. Avocados provide monounsaturated fats that are known to break down cholesterol in the blood. These mono-fats are thought to be one of the secrets to the health of the Mediterranean cultures (Gustafson, 2005). Avocado is rich in vitamins A, B, B6, G and E. It also contains ascorbic acid, fatty acids, β-carotene and potassium (Bergh, 1992). The fatty acid content has been reported as: palmitic, 7.0; stearic, 1.0; oleic, 79.0; and linoleic, 13.0 (Morton, 1987). Avocado oil is rich in oleate, which has a low content of saturated fatty acids, and this makes it appropriate for

direct human consumption, as well as an excellent fat in diets designed to reduce cardiovascular disease (Gurr, 1992).

A fruit of avocado of weight 100g is composed of 73.6 g of Water, 171.0 Kcal of food energy, 2.2 g of protein, 17.0 g of lipids, 1.5 g of fibre and 6.0 g of carbohydrate. It also contains 10 mg of calcium, 42.0 mg of phosphorus, 0.6 mg of iron, 4.0 mg of sodium and 602.0 mg of potassium. In addition to these are 290.0 mg of vitamin A, 0.2 mg riboflavin, 1.6 mg niacin, 0.1 mg of thiamine and 14.0 mg of ascorbic acid (Watt and Merrill, 1975).

Genetic classification

There have been several attempts to examine the classification of avocado. For example, there has been a detailed numerical taxonomic study of avocado cultivars based on morphological traits (Rhodes *et al.,* 1971). The cultivars under study tended to cluster into three groups, more or less representing the three races. Allozyme variation did not provide sufficient evidence to clarify the controversy (Torres and Bergh, 1980; Goldring *et al.,* 1985). There has been the use of restriction fragment length polymorphism (RFLP) of chloroplast DNA, ribosomal DNA, and the genes coding for the enzyme cellulase to infer a phylogeny of avocado (Furnier *et al.,* 1990). The results lent some support to the classification placing vars. *drymifolia, guatemalensis* and *americana* in a single species *P. americana* (Bergh *et al.,* 1973). Markers of minisatellite DNA and microsatellite DNA have also been used to characterize and differentiate horticultural races of avocado (Lavi *et al.,* 1991; Mhameed *et al.,* 1997). In addition, a phylogenetic

tree of nine *Persea* species of the subgenera *Eriodaphne* and *Persea* has been developed to through more light on the genetic classification of the plant (Mhameed *et al.,* 1997).

Another type of molecular marker is represented by RAPD (randomly amplified polymorphic DNA). The RAPD method is based on the amplification of random DNA sequences in low stringency polymerase chain reaction using arbitrary 10 base oligonucleotides as primers (Williams *et al.,* 1990; Welsh and McClelland, 1990). RAPD assays have been used to study genetic relationships in a number of fruit trees, including cocoa (Wilde *et al.,* 1992; Figueira *et al.,* 1994), banana (Kaemmer *et al.,* 1992), papaya (Stiles *et al.,* 1993), apple (Dunemann *et al.,* 1994), mango (Schnell *et al.,* 1995), fig (Khadari *et al.,* 1995), olive (Fabbri *et al.,* 1995), and plum (Ortiz *et al.,* 1997). In most of these cases, data of genetic similarity obtained by RAPD analysis matched classifications based on morphological and horticultural traits.

Molecular work done on avocado

In the last few years, four types of genetic markers have been used to analyse the genome of avocado (Lavi *et al.* 1994b). Isozyme markers have been used mainly to study the level of selfing and outcrossing in the cultivar Fuerte (Degani *et al.,* 1990). Two restriction fragment length polymorphism (RFLP) loci have also been used to study the relationships between the *Persea* species, and between avocado cultivars (Furnier *et al.,* 1990). DNA fingerprints (DFP), resulting from the use of multilocus minisatellite and microsatellite probes, were

used to distinguish cultivars and races (Lavi *et al.*, 1991) and for detection of linkage between a locus affecting fruit skin colour and a specific DFP band (Mhameed *et al.*, 1995). Simple sequence repeat (SSR) markers have been shown to be highly polymorphic and to generally segregate according to Mendelian laws (Lavi *et al.*, 1994a). Both DFP and SSR markers were used to estimate the heterozygosity level in the avocado genome (Mhameed *et al.*, 1996) and to define genetic relationships in the *Persea* genus (Mhameed *et al.*, 1997). None of these markers has thus far been used to construct a genetic linkage map of the avocado genome (Sharon *et al.*, 1997).

Characteristics of microsatellites

Microsatellites (simple sequence repeats, SSRs) are a form of repetitive DNA first discovered in the early 1980s (Hamada *et al.*, 1982). Their great potential as powerful genetic markers, combining the useful properties of high variability, co-dominant inheritance, and good reproducibility, was recognized simultaneously by several groups of researchers (Litt and Luty, 1989; Smeets *et al.*, 1989; Tautz, 1989; Weber and May, 1989). Over the past decade, microsatellites have assumed a central role in population-genetic studies, as well as in breeding applications. Their co-dominance makes them suitable for tracing paternity and tracking pollen movement (Queller *et al.*, 1993; Jarne and Lagoda, 1996; Goldstein and Pollock, 1997; Sunnucks, 2000). Additionally, they are abundant and evenly distributed across genomes (Hamada *et al.*, 1982; Stallings

et al., 1991; Weissenbach *et al.,* 1992), making them amenable to the study of genealogical relationships (Goldstein *et al.,* 1995).

The repetitive unit of microsatellites is generally defined as being one to five bases long. Dinucleotide repeats are the most common category of repeat in a majority of organisms (Jurka and Pethiyagoda 1995; Tóth *et al.,* 2000; Katti *et al.,* 2001) and are usually associated with non-coding regions of the genome (Young *et al.,* 2000; Temnych *et al.,* 2001). Trinucleotide repeats are often found within ORFs (Young *et al.,* 2000) due to their triplet structure. Although they do not interfere with the reading frame, they are nonetheless responsible for several human diseases, once repeated arrays exceed a threshold limit. In plants, trinucleotide microsatellites are relatively infrequent (Lagercrantz *et al.,* 1993; Ma *et al.,* 1996), compared with vertebrates and some other organisms (Ashworth *et al*. 2004).

Given their extensive usage, there has been an increase of interest in SSRs as indicated by the large number of recent publications that have involved the use of microsatellites (the word microsatellite is found in nearly 8000 records when a search of the Current Contents publication databases for the years 1995-2000 is carried out (Zane *et al.,* 2002)).The great popularity of SSRs is also demonstrated by the growing number of reports describing the isolation of these markers in many organisms, and by the creation of Molecular Ecology Notes with its associated database (Zane *et al.,* 2002).

CHAPTER THREE

MATERIALS AND METHODS

Ethnobotanical studies of *Persea americana* Mill.

Study area

An ethnobotanical survey was carried out in the Ashanti and Central Regions of Ghana. This was done to determine where in the two regions the avocado tree is predominately grown and also to determine the socio-economic importance of the tree in the study area.

Fig. 3.1: Map of Ghana showing all Regions
Source: http://www.world-geographics.com/maps/africa/map-of-regions-in-ghana/ Downloaded on 24.08.2009.

The Ashanti Region is located in the central part of Ghana. It lies between latitudes 6^0 N and 8^0 S and longitudes 0^0 and 3^0 W. It is located south of the Brong Ahafo Region, east of the Eastern Region, north of the Central Region and south-west of the Western Region (Fig 3.1). Ashanti's indigenous commercial products include *Theobroma cacao* (cocoa), timber, and mining.

The region is the third biggest in Ghana. It has an area of 24,507.29 km² (Ghana Statistical Service, 2000). The region with 21 districts (Fig 3.2) has a population of 3,612,950 (Ghana Statistical Service, 2000). This population forms 19.1% of the entire population of Ghana. It is the region with the highest population in Ghana. It has basically one ethnic group, the 'Asantes'. The major occupation of the people in this region is farming.

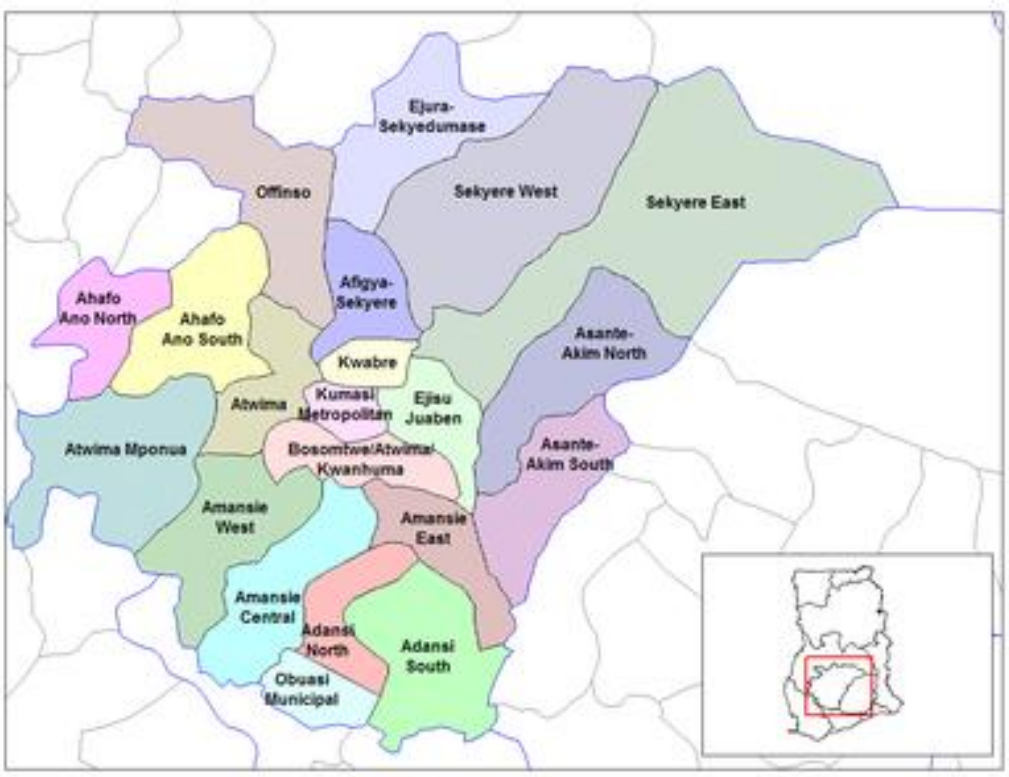

Fig 3.2: District map of the Ashanti Region of Ghana
Source: Wikipidea Encyclopedia

The region falls within the subequatorial type of climate. The average minimum temperature is about 21.5°C and average maximum temperature is about 30.7^0C with the average humidity of about 84.16% at 0900 GMT and 60% at 1500 GMT (Dickson and Benneh, 1970). The mean annual rainfall ranges between 1200 mm and 1800 mm (Swaine and Hall, 1983; Hawthorne, 1995). The region has two rainfall seasons. The first rainy season is from May to August, with the heaviest rains occurring in June. The minor rainy season occurs from September to October (Dickson and Benneh, 1970). The moderate temperature and humidity coupled with high rainfall amounts of about 165.2 mm - 214.3 mm in September have a bearing on population growth of animals and plants. Falling within the moist-deciduous ecological zone, the vegetation of Ashanti is pre-dominated by tree species (Hall and Swaine, 1976, 1981).

The Central Region is bordered by the Ashanti and Eastern Regions to the north, Western Region to the west, Greater Accra Region to the east (Fig. 3.1) and to the south by the Atlantic Ocean. It has 13 districts (Fig 3.3). The region has an area of 9,826 km² and has a population of 1,593,823 people (Ghana Statistical Service, 2000). This population makes 8.4% of the Ghanaian population.

The region is found in the coastal savannah and southern marginal forest zones of Ghana. It however has some remnants of rainforests. Much of the region's forest, especially towards the coast, has been cut and now consists more of savannah grasslands. The region is also famous for its coconut-fringed beaches, thriving fishing villages and historic towns.

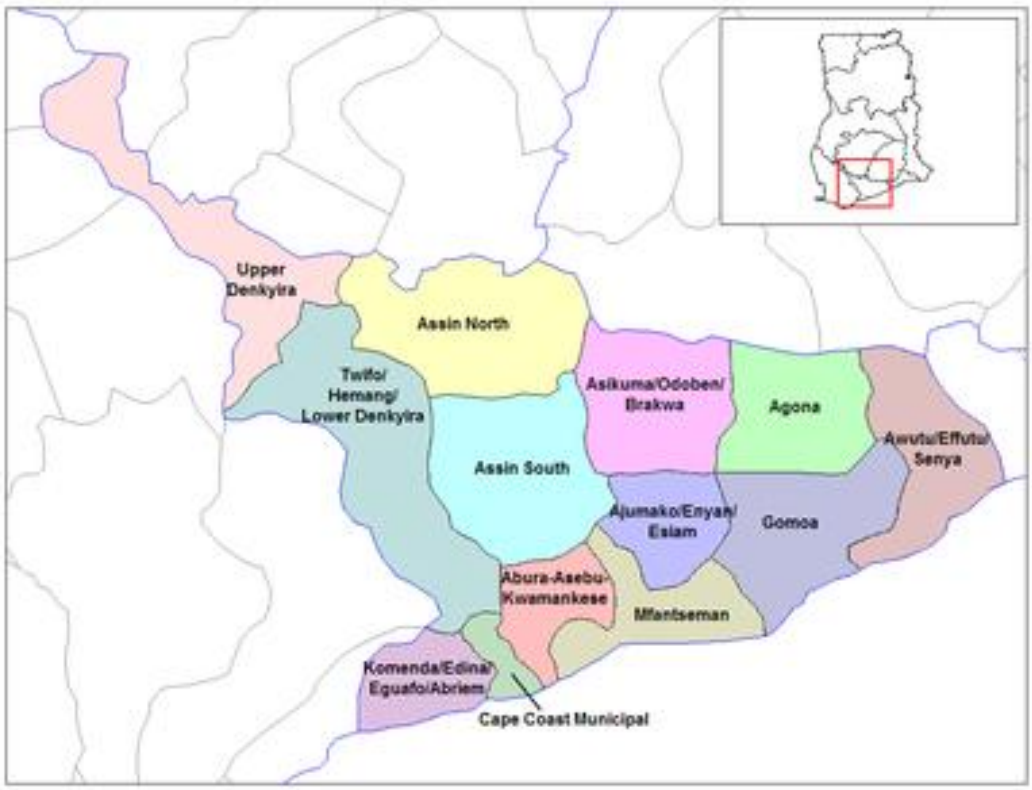

Fig 3.3: District map of the Central Region of Ghana
Source: Wikipidea Encyclopedia

Ethnobotanical survey

An ethnobotanical survey was carried out in the Ashanti and Central Regions to determine the socio-economic uses and importance of the avocado plant to the inhabitants of the regions. A total of 14 districts, comprising 12 districts from the Ashanti Region and two districts from the Central Region, were surveyed. The selection was based on systematic sampling. Five hundred and eighteen respondents were randomly selected from 94 towns, in the 14 districts; they were interviewed with the help of a structured questionnaire (Appendix 1). A maximum of 50 people were selected from the various districts for the study.

Morphological studies of *Persea americana* Mill.

A morphological study of *Persea americana* Mill. was conducted in eight out of the fourteen districts selected for the ethnobotanical studies. The selection of districts was done randomly. Each district was assigned a number; and with a random number generated from a pocket calculator, eight of the districts were selected. Seven of the districts were selected from the Ashanti Region, and one from the Central Region.

At least two avocado plants were randomly sampled for study in each study district. A total of 53 avocado trees were sampled randomly in all study districts. Morphological data (Appendix 3: Field guide to morphological studies) such as tree vigour, tree height, leaf shape *etc* were then taken from the trees. The GPS coordinates of each plant assessed was recorded and used to produce a map (Fig. 3.4). For each avocado plant selected for morphological studies, data on tree, leaf, fruit, and seed characteristics were taken. All morphological data were taken following the guide of the International Plant Genetic Resources Institute (1995).

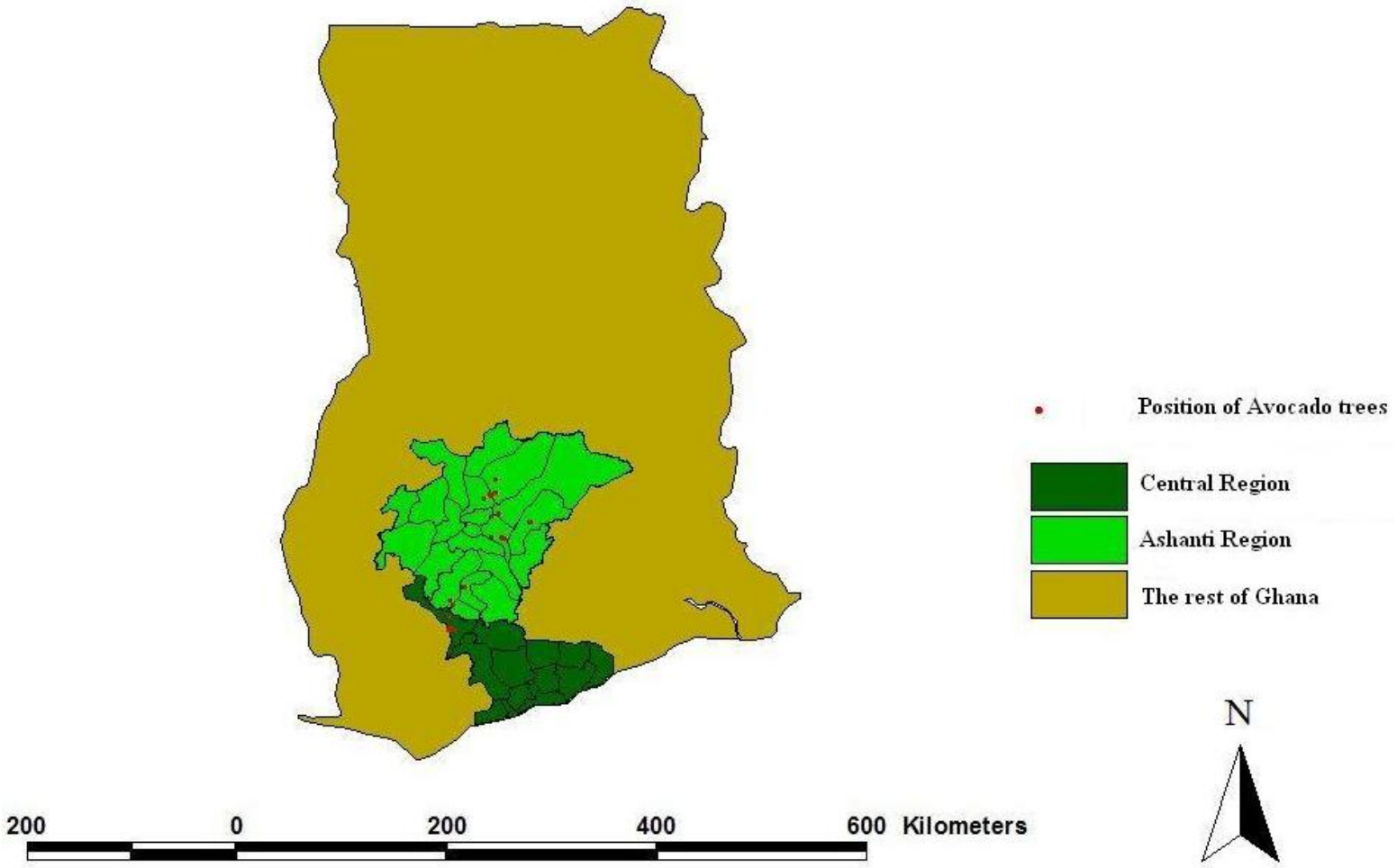

Fig. 3.4: Map of Ghana showing the geographical points of the avocado plants used for the study

Characteristics of the mature avocado tree

In this section, different morphological tree characters of the avocado plant were studied to come out with some observable morphological characters of the plants found in the area. The tree characteristics used for the study are stated below.

Tree vigour: The vigour was classified as weak, intermediate and strong.

Canopy spread: The edges of the tree crown were traced downwards with ranging poles. Two diameters perpendicular to each other were measured. The mean of these two diameters was used to estimate the canopy spread.

Tree height: Tree height was measured with the suunto clinometer PM-5 (Suunto, Valimotie, Finland) and classified into the following 1-4 m, 5-8 m, 9-12 m, 13-16 m and more than 16 m. This was to make coding and analysis more flexible.

Trunk surface: the surface of the trunk was described as smooth, rough or very rough.

Branching pattern: the branching pattern was described as extensive, intensive or both.

Other tree characteristics of which data were taken were, tree shape, distribution of branches, crotch of main branches and a measure of the truck circumference at 30 cm above ground level. The fruiting habit and characteristics of each avocado plant selected were also studied. The fruiting habit was classified into single isolated fruiting or clustered fruiting.

Leaf characteristics

Samples of leaves were taken from each tree selected for morphological studies. Leaf shape, leaf base shape, number of primary veins, primary leaf vein divergence to the main vein was measured in degrees, leaf apex shape, leaf texture and leaf margin were taken. A measure of the leaf blade length (cm) was also taken. On each tree, data on colour of mature leaf and crotch angle of leaf petiole were also taken.

Fruit characteristics

The International Plant Genetic Resources Institute (1995) prescribes the number of fruits on which data are to be taken to determine fruit characteristics. Following this prescription, data on fruit shape, fruit base shape, fruit apex shape, fruit apex position, ridges on fruit, pedicel position on fruit, pedicel shape and nailhead, pedicel apex shape were taken. Measurements taken on fruits included fruit length (cm) taken as a measure of the longest part of the fruit, average fruit diameter (cm) of five fruits measured in the mid section of each fruit, Fruit weight (g), peduncle length (cm) and peduncle diameter (mm). Fruit skin surface was classified as smooth, intermediate or rough. Using an electronic digital calliper (Powerfix®, Milomex Ltd, Bedfordshire, UK), the average fruit skin thickness of five fruits were also determined. Adherence of skin to flesh was graded as slightly, intermediate or strong. The feel of the flesh texture was also determined. Sweetness of flesh and bitterness of flesh were graded as low, intermediate or high.

Seed characteristics

Several data were taken from the seeds. The shape of the seed was recorded. Seed were weighed to determine their weights in grams. The cotyledon surfaces were classified as smooth, intermediate or rough. Attachment of cotyledons to seed was either ‘’not attached’’ or ‘’Attached’’. The length of seed cavity (cm), diameter of seed cavity (cm), length of seed (cm), diameter of seed (cm) and free space of the seed cavity were measured with an electronic digital calliper (Powerfix®, Milomex Ltd, Bedfordshire, UK). The length of seed was taken as the measure of the longest part of the seed whereas the diameter measurement was taken from the mid section of the seed with the base and tip of the seed as reference points.

Genetic diversity assessment

Genomic DNA extraction

Ten healthy young leaves from plants used for morphological studies were selected and placed in brown paper bags. The leaves were placed as flat as possible and avoided over lapping. The samples were later sent to the molecular biology laboratory at Cocoa Research Institute of Ghana (CRIG) for DNA extraction and analysis. Leaf samples of avocado collections at CRIG were also added to the samples for DNA extraction and sequencing.

A total of 71 genotypes including 18 plants from the CRIG collection were used in the investigation. The total genomic DNA of plant samples were isolated

following a modified cetyltrimethyl ammonium bromide (CTAB) extraction protocol (Aldrich and Cullis, 1993).

Using a 10 mm diameter cork borer, clean leaf discs (0.1 g) were transferred into a 2.0 ml eppendorf tube (Sorenson BioScience Inc., Utah, USA) for DNA extraction. The leaf discs were homogenized in 2.0 ml eppendorf tube, using liquid nitrogen. The homogenate was mixed with 800 µl CTAB extraction buffer [100 mM Tris-HCl (pH 8), 1.4 M NaCl, 20mM EDTA (pH 8), 2% (w/v) CTAB, 2% (w/v) PVP] and 0.5 µl (0.1% v/v) β-mercaptoethanol] by vortexing, and incubated at 65°C for 30 min with intermittent vortexing. The mixture was cooled to room temperature, and mixed with an equal volume of chloroform-isoamyl alcohol (24:1). The emulsion was centrifuged at 14,000 rpm for 15 min, the aqueous phase recovered, and the chloroform-isoamyl alcohol (24:1) extraction repeated. DNA was precipitated from the aqueous layer by adding two-thirds volume of ice-cold isopropanol (Fisher Scientific UK Ltd., Leicestershire, UK), and incubated at -20°C overnight. The DNA was pelleted by centrifuging at 14,000 rpm for 5 min, washed in 1 ml washing buffer (76% ethanol, 10 mM ammonium acetate), and again in 1 ml ethanol (80%). The DNA was again pelleted by centrifuging at 6,000 rpm for 4 min, dried at room temperature and dissolved in 40 µl TE [1mM Tris HCl, (pH 8) 0.1m M EDTA, (pH 8)] buffer. The dissolved DNA was stored at -20°C for quality assessment, quantification, PCR amplification and characterization of the accessions.

Two microliters of each sample was taken and dissolved in 3 µl of TE and 1 µl of x6 sample buffer (Bromophenol blue and xylincinol) added to it. The

DNA samples were quantified by comparing band intensities of samples to that of 0.2 µg/µL of λ DNA-*Hind III* digest ladder (Invitrogen, MD, USA), after running on 1% (w/v) DNA grade agarose (Cambrex Bio Science Rockland Inc., Rockland, USA) gel in 1 X TAE [40 mM Tris-acetate, 0.5 M EDTA (pH 8)] buffer.

Using the known concentration of the marker, the concentrations of the various DNA's were calculated with the equation: concentration (C) = mass (m)/ Volume (v) and their intensities with respect to the marker. The samples with DNA concentration above 25 ng/µl were further diluted to bring their concentration to 25 ng/µl for polymerase chain reaction (PCR). DNA samples were diluted to concentrations between 3.3 ng/µl and 25.0 ng/µl for PCR.

DNA analysis

Ten pairs of microsatellite primers designed by Sharon *et al.* (1997), and later applied by Schnell *et al.* (2003) (Table 3.1) were used for the PCR. Amplification reactions were performed with 16-50 ng of genomic DNA as template, 0.5 µM of each of the forward and reverse primers, in a 10 µl reaction volume using the AccuPower^{TM} PCR PreMix (USA Bioneer Inc., Alameda, USA) (DNA polymerase, dNTPS, a tracking dye, and reaction buffer in a premixed format). The thermal cycling was carried out on AB Applied Biosystems 2720 Thermal Cycler (Applied Biosystems, Singapore). The PCR programme consisted of an initial denaturation for 3 min at 94°C, followed by 40 cycles of denaturation at 94°C for 30 s, 1 min at appropriate primer annealing temperature, 1 min extension at 72°C. The amplification finished with an extension at 72^{o}C for 10

min, followed by maintenance of the reaction mixture at 4°C at infinity until removed for storage at -20°C.

Table 3.1: Repeat motifs and primer sequences of the microsatellite loci

Locus	Repeat Motif	Primer Sequence (5' – 3')	Anneal Temp (°C)
AVAC01	$(TG)_{15}$	F:CTGGTTGCTCTCTTGTCTACATAATA R: CGGTTTTGTAAGTTGATAG	40
AVAG03	$(TC)_{17}$	F: GCACTTCCTAAACTTGCAGGT R: CTGAACATCCAATGACAAACATCC	45
AVMIX04	$(AG)_{12}$,$(CAA)_5$,$(ACAG)_{10}$	F: CCGTTTGCTTCCTGTATC R: GTTATCCCTTCCACTTTC	50
AVAG05	$(AG)_{10}$	F: GGATCTGATGTGTGGGGGAG R:CCTGTCGGAAAAGACTATGCG	45
AVAG06	$(CT)_{18}$	F: CGACCTCTTCTTATACTC R: GTACCTCTGATAATGAGCAT	40
AVAG10	$(CT)_{22}$	F: GAATTACAAAGCACTAGAG R: GTAGAAAGTGGGCACACAT	45
AVAG13	$(CT)_{18}$	F: CTGCGATAACAACTGGAC R: AACTAGGACCTGAAACCG	50
AVAG 21	$(CT)_{22}$	F: TGTAAGTTTTAACCCCACAA R: AATCACTATTAGAGTTTTCAGTCG	50
AVAG22	$(GA)_{15}$	F: GATCATCAAGTCCTCCTTGG R: GATCTCATAGTCCAAATAATGC	55
AVAG25	$(TC)_{14}$	F: ATGGTTTTTTCCTGCCCTTT R: AACAAGCCCCCTAAAAGAA	50

Source**:** Adapted from Sharon *et al.* (1997).

Three microliters of denaturing buffer (95% formamide, 0.02 M EDTA pH 8, 1% bromophenol blue, 1% xylene cyanol, 10 mM NaOH) added to 3 µl of the PCR products. Equal volume of the denaturing buffer was added to 3 µl DNA ladder (10 bp ladder, diluted to 0.1 µg/µl in doubled distilled water). The mixture

was denatured by heating at 95°C for 5 minutes, and then immediately chilled on ice. Six microliters of the denatured PCR products (or DNA ladder in the first well) was loaded in each well of a 49-well plate (4 mm thick); the preparation was then electrophoresed on a DNA sequencing gel, containing 6% polyacrylamide, 8 M urea and 1 X TBE (Tris-Boric acid-EDTA buffer). Gels were run at 100W constant power and 2 kV for 2-2.5 h, using a BIO-RAD Sequi-Gen® GT Nucleic Acid Electrophoresis Cell (Bio-Rad, Consult EG 261, Belgium) and power pack (Bio-Rad Power Pac 300) (Bio-Rad, Consult EG 261, Belgium) and 1 X TBE as running buffer (Plate 3.3).

The products were visualised by silver staining, using the method described by Bassam *et al.* (1991). The gel was attached to the back glass plate using bind-silane; it was then fixed in 10% acetic acid for 30 min-1h, rinsed with deionised water three times (2 min in each rinse); further, it was impregnated with $AgNO_3$ (1 g/l), 37% formaldehyde (HCOH) (1.5 ml/l) for a further 30 min. After this, the gel was rinsed in deionised water for 5 s, and then developed in aqueous solution containing $NaCO_3$ (30 g/l), 1.5 ml 37% HCOH per litre, $Na_2S_2O_3.5H_2O$ (2 mg/l), until the bands were easily apparent. The development was terminated with 10% acetic acid, and the gel preserved by drying on a paper laid on a lab bench for an hour.

Bands on the developed gel were assessed as the size of the SSR bands. For each gel, the distance travelled by each marker size of the DNA ladder was measured, using a ruler. A line graph of the distance travelled by the marker size

was plotted for each gel; the equation of the relationship between them was then used to estimate the size of the unknown SSR bands of the PCR products.

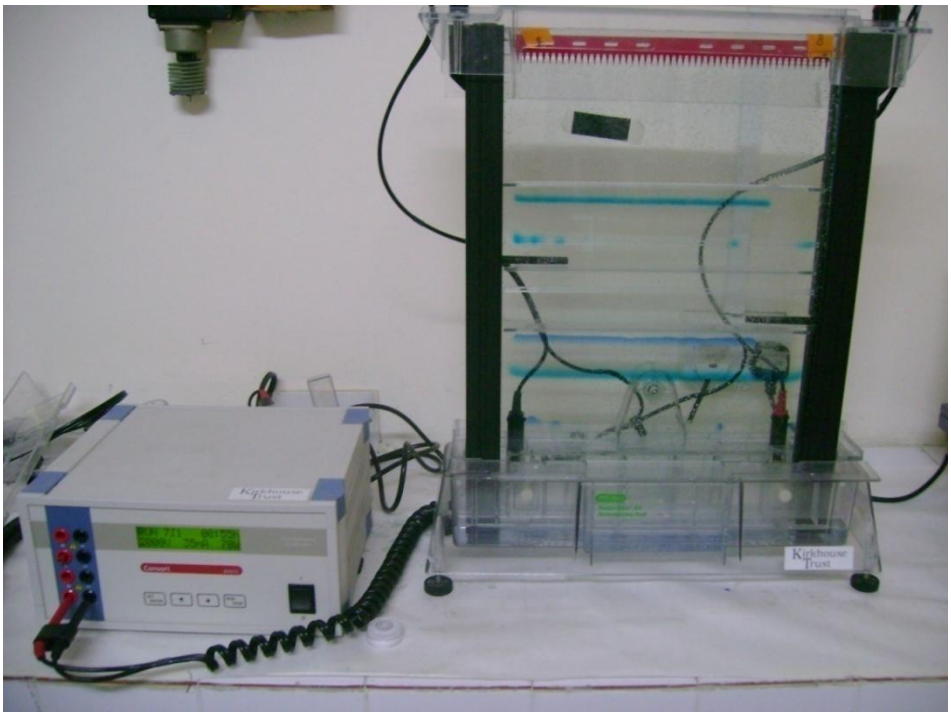

Plate 3.1: Set-up used for gel electrophoresis SSR determination of avocado genes. Photo: Janice D. Oduro

Data analysis

All ethnobotanical, morphological and genetic data were analysed using MS Excel (Microsoft Office, 2007), SPSS 12.0 (2003), Statistica version 7 (StatSoft Inc., 2004) and PowerMaker version 3.25 (Liu and Muse, 2004).

Analysis of ethnobotanical data

The results of the interviews in response to the ethnobotanical questionnaire (Appendix 1) were coded and analysed, using the descriptive

statistics protocol of SPSS version 12.0.1 (2003) statistical package. Response frequencies were calculated and plotted graphically where necessary.

Analysis of morphological data

The data on the morphological features of the plants, in response to the morphological survey questionnaire (Appendix 3), were analysed using MS Excel (Microsoft Office, 2007), Statistica version 7 (StatSoft Inc., 2004) and SPSS version 12.0.1 (2003***).*** Response frequencies were calculated and plotted graphically. Furthermore**,** cluster analysis was carried out; the hierarchical single linkage and Euclidean distance method was used to produce a dendrogram of morphological similarities, using the Statistica version 7 (StatSoft Inc., 2004).

Analysis of genetic data: genotyping and determination of genetic diversity

Allele size and the total numbers of alleles were determined for each SSR locus. Bands for same SSR locus, with different molecular weight, were scored as alleles. The scored alleles were coded using FlexiBin (Bill Amos, Cambridge, UK) analysis. Gene diversity values for each locus, and the average across all loci for all populations, were calculated using Nei's (1973) unbiased estimate. The numbers of alleles and the allelic frequencies for each SSR, and across all populations, were estimated using PowerMarker version 3.25 (Liu and Muse, 2004). The unbiased gene diversity (H_{nb}), and the observed heterozygosity (H_{obs}), for all populations, were estimated from the allele frequencies, using PowerMarker version 3.25 (Liu and Muse, 2004).

Genetic diversity for each marker was calculated according to the following equation of Nei (1973):

Genetic diversity = $1-\Sigma P_{ij}^2$

Where P_{ij} is the frequency of the jth allele for the locus summed across all the alleles of the locus. With this format, the genetic diversity comes across as the parameter Polymorphic Information Content (PIC), described by Anderson *et al*. (1993). State of Hardy-Weinberg Equilibrium HWE ($p< 0.05$), at individual loci, were tested, using PowerMarker version 3.25 (Liu and Muse, 2004).

A phylogenetic tree was constructed for all 71 individuals genotyped; this was by Unweighted Paired Group Method (UPGMA), as defined by Sneath and Sokal (1973). This method was used to produce dendrograms of genetic relationship among the genotype studied (Saitou and Nei, 1987) using PHYLIP version 3.5 (Felsenstein, 1989) and TreeView version 1.6.6. For the phylogenetic tree constructed, the bootstrap percentage was computed. Each genotyped accession was represented by a number, and all accession named (Appendix 5).

CHAPTER FOUR

RESULTS

Field and ethnobotanical survey

Age composition of respondents

The results of ethnobotanical studies and survey showed that most of the people who cultivate avocado were adults above 40 years. Table 4.1 shows that about 82% of the people who cultivate avocado were above 40 years. A significant number (36.5%) of the people who cultivate avocado are above 61years old.

It was observed that almost all the respondents cultivate avocado in either their homes or farms. The average age of the respondents was 51.5 years. Results in Table 4.1 shows that older people cultivate avocado than younger people. This is evidenced by the high number of people in the 61+ age group who cultivate avocado in the Ashanti Region.

Table 4.1: Age group composition of avocado cultivators in the Ashanti and Central Regions

Age Group (Years)	Region		Total
	Ashanti	Central	
< 21	1	1	2
21-30	16	3	19
31-40	58	13	71
41-50	98	21	119
51-60	97	21	118
61+	177	12	189
Total	447	71	G = 518

Distribution of avocado cultivators in the districts of the study area

A maximum of 50 avocados farmers were selected from each of the study districts. Selection was based on information from the traders of the avocado fruits in the study area. The results are illustrated in the graph below, Fig 4.1.

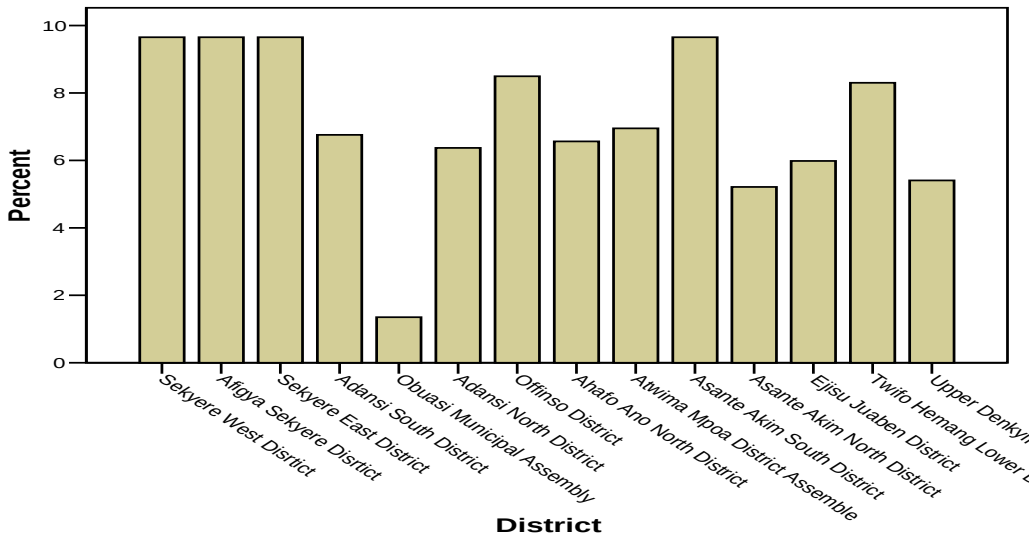

Fig. 4.1: Frequency distribution of respondents in the districts in both study regions

Vocation of avocado farmers

Table 4.2 shows that 95% of the 518 respondents were farmers. The indication here is that the prime cultivators of avocado in the study area are professional farmers; many of them specialise in cocoa farming. They cultivate the avocado plant just for the marginal benefits derived from it. Also important to the farmers is the shade avocado provides for their cocoa trees; although almost every other part of the avocado plant is also useful. The casual cultivators of avocado include traders, teachers and drivers, among others.

Table 4.2: Occupation of respondents in the two study regions

Occupation	Region		Total
	Ashanti	Central	
Farmer	426	67	493
Trader	9	1	10
Driver	4	0	4
Teacher	2	0	2
Kente Weaver	1	0	1
Carpenter	1	0	1
Missionary	1	0	1
Hair dresser	1	0	1
Queen mother	1	0	1
Civil servant	1	1	2
Accountant	0	1	1
Baker	0	1	1
Total	447	71	518

Scale of production of avocado

The majority of the farmers (85.6%) have between one and 20 avocado plants either in the compounds of their homes, or, on their farms. Out of this, 33.8% have between one and five avocado plants either in their home, farm or both. 30.5% have up to 10 avocado plants, 10.8% have up to 15 plants and 9.5% have up to 20 plants (Appendix 3a). This trend shows that the avocado plant is not cultivated on large scale in the study area. Further more, only 15.1% of the farmers had more than 20 avocado plants. Out of this, only one individual had an avocado plantation, with over 300 plants. This implies that, fewer people have or own many stands of avocado plants. This confirms earlier observation that cultivation of avocado in the study area is on small scale basis (Taah *et al.,* 2003). The explanation is that most of the cultivators grow the avocado plant just to provide shade over their cocoa trees, and also for their personal consumption. Only 0.2% of the avocado farmers have started commercial cultivation of the crop.

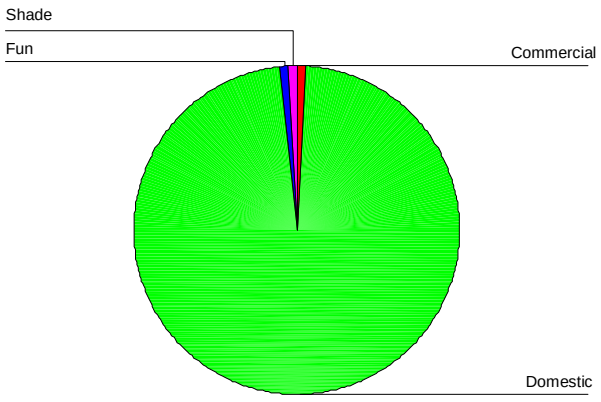

Fig. 4.2: Scale of cultivation of avocado in Ashanti and Central Regions of Ghana

Indigenous uses of avocado

The people mentioned several economic uses for the fruit of avocado. Majority of people eat the fresh fruit as food (91.3%), others use it to prepare stew (0.6%); whilst some sell it for subsistence (0.2%). Some farmers (7.5%) however sell part of their harvest and consume the rest themselves. A negligible minority had no use for the fruit (0.38%) because they just did not like the fruit.

The avocado leaves had several uses in the study area. The uses are as follows, used for soup for nursing mothers (23.6%), treatment of malaria (17.6%), treatment of heart disease (3.7%); others use it as blood tonic (2.5%), food for animals (1.5%), treatment for jaundice (1%), as a therapy for diabetes (0.77%), for typhoid fever (0.6%), stomach aches (0.6%) and for boils (0.2%). The majority (48%) of indigenes however had no use for the leaves.

The seeds were used for planting by 29% of the people; this is the most important use of the seed. Some of the people (1.5%) process the seeds into animal feed for their lifestock. A further 1.5% attested to the medicinal effects of the avocado seed to treat diabetes, stomach ache and heart disorders. Majority of the people (67%) had no use for the avocado seed.

The bark of avocado plant is also important to the people. 6% use it regularly to treat malaria, 2.7% for treatment of stomach disorders, 1% use the bark to treat typhoid fever, 0.8% as a blood tonic, 0.6% as fuelwood, 0.4% to treat hernia, 0.2% for treating toothache and another 0.2% use it for managing sickle cell. About 87.8% of the people had no use for the bark of the avocado plant.

Some people also used the roots of the avocado plant for various purposes. From the results, 2% of the people indicated that the root is used to cure diseases like, typhoid fever, cough and impotence in men. It is also used to aid babies to walk earlier than expected. The results also showed that 98% of people, however, had no use at all for the roots.

The wood of the avocado plant also has very important uses. Most rural areas with energy problems have fuelwood as an important commodity. The study revealed that 91.5% of the respondents use the avocado wood as fuelwood for their cooking. Some 8.3%, however, had no use for the wood. However, 0.19% used it as lumber for furniture either on the farm or under the shade trees in their homes.

In other economic terms, 35.9% of the people did not even obtain any income from the plant. This is obviously because they are not into commercial farming of avocados. Over 60% however make some tangible income from the avocado plant. Of this, only 1.75% of the farmers could make One Hundred Ghana Cedis (GH¢100) which is equivalent to one hundred US dollars ($100) and above from the plant. The highest revenue accrued recorded in the Central Region was GH¢ 60 which is equivalent to 60 US dollars ($60).

Morphological characterization of plants

The statistical analyses and description of the data on the morphological characters are presented in this section.

Tree characters

The crown size (canopy spread) of avocado plants ranged between 4.9 and 13.17 m. Data in Fig. 4.3 indicate that most (92.4%) of the trees had canopy spreads between 6 and 12 m.

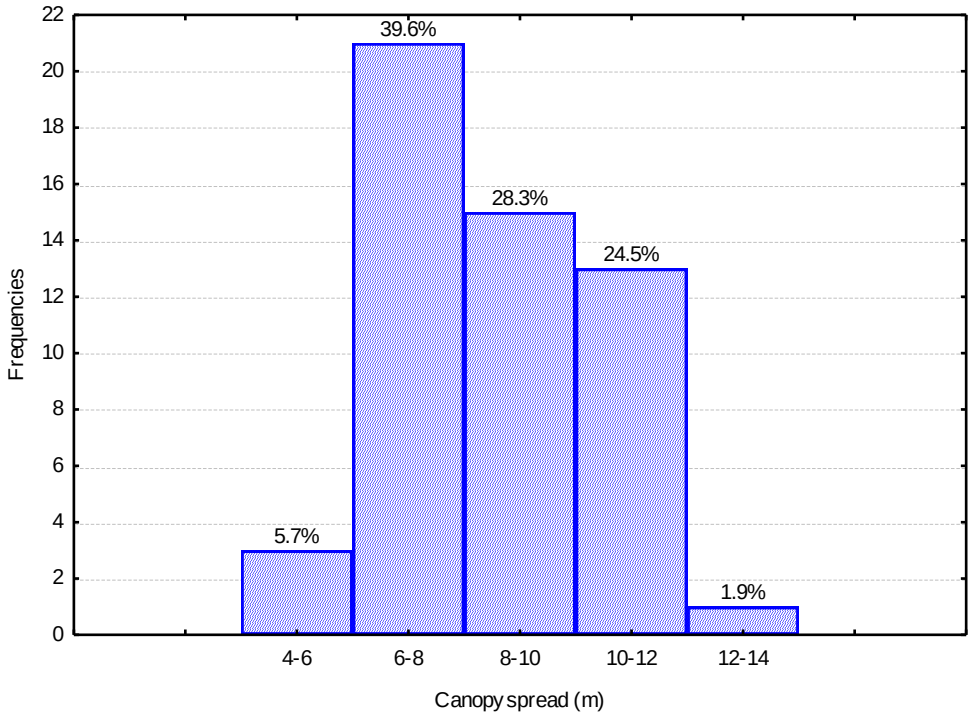

Fig. 4.3: The canopy spread (m) of avocado in the study area

Table 4.3 below contains the frequency distribution of the height and percentage distribution of avocado trees.

Table 4.3: Height (m) of avocado trees in the study area

Height (m)	Frequency	Percent
5-8	11	20.8
9-12	32	60.4
13-16	8	15.1
>16	2	3.8
Total	53	100

The trunk circumference ranged between 46.30 cm and 283.10 cm. The modal circumference was 111.0 cm. The mean tree circumference was 133.04 cm. Majority (77.4%) of the plants had circumferences between 70 cm and 160 cm.

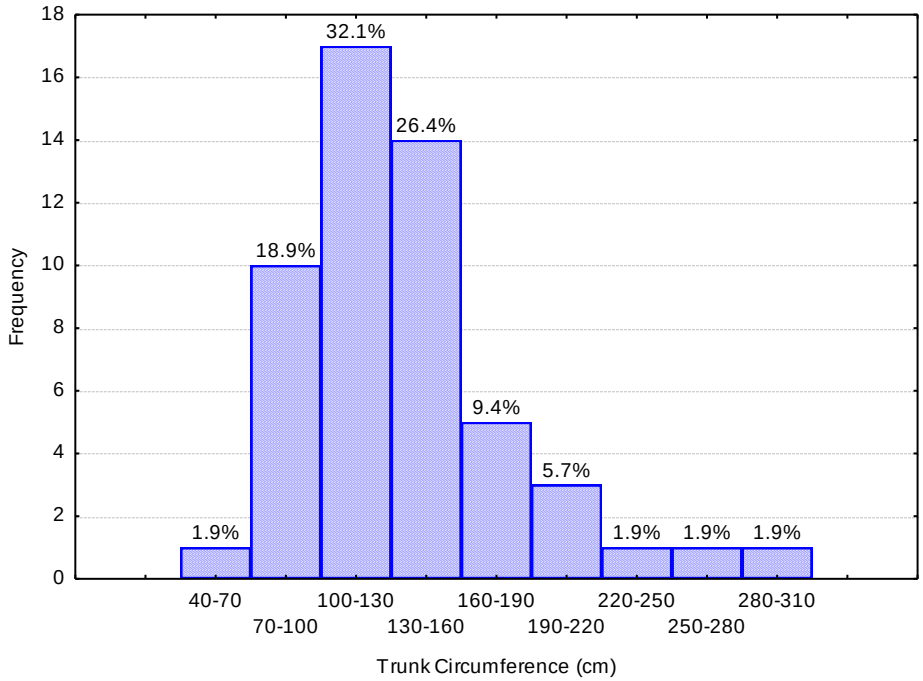

Fig. 4.4: Frequency distribution of the trunk circumference (cm) of avocado in the study area

The results showed that 49.1% of plants had intensive branching pattern and 24.5% were branching extensively. Both branching patterns were observed in 26.4% of the plants. It was also observed that 54.7% of the plants had irregular distribution of branches. Plants having ascendant distribution of branches made up 28.3% of the whole population. Other branching distributions observed were verticillate, axial and horizontal.

The least average leaf blade length recorded was 12.92 cm whilst the highest average leaf blade length recorded was 28.64 cm. The mean average leaf blade length of the samples used for the study was 19.03 cm. The leaf apex of the plants had various shapes. Almost half (49.1%) of the plants studied had acute leaf apex. The rest had very acute (1.9%), intermediate (34.0%) and obtuse (13.2%) leaf apexes.

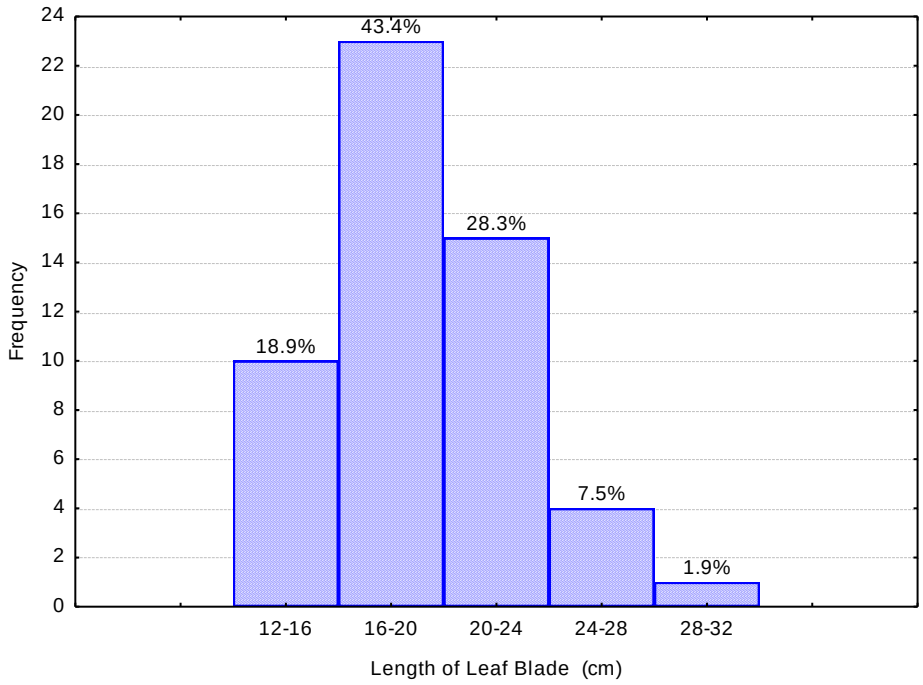

Fig. 4.5: Length of leaf blade (cm) of avocado

A large percentage (71.7%) of the plants under study had between 14 and 16 primary veins. Only 3.8% had 18 primary veins. The least number of venation being 12 was represented by 11.3% of the trees.

The lengths of the fruits studied ranged between 7 cm and 19 cm. About half (49.1%) of the fruits were between 10 cm and 13 cm long. Another 34% were up to 10 cm long. Only a small percentage (17%) was more than 13 cm but less than 19 cm long. Most (81.1%) of the fruits were in the same diameter range of 7-9 cm. Only 1.9% was in the range of 9-11 cm while a relatively small percentage (17%) was between 5 and 7 cm.

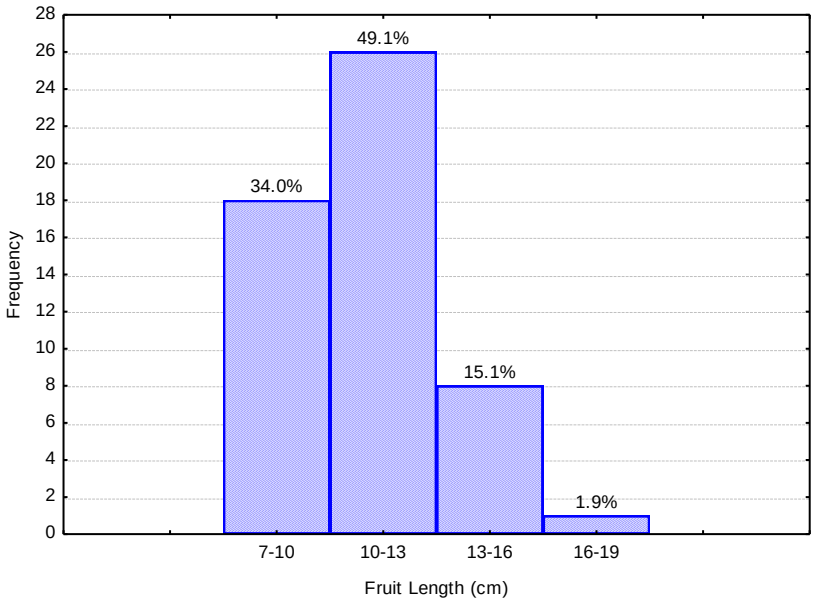

Fig. 4.6: Fruit length (cm) of avocado

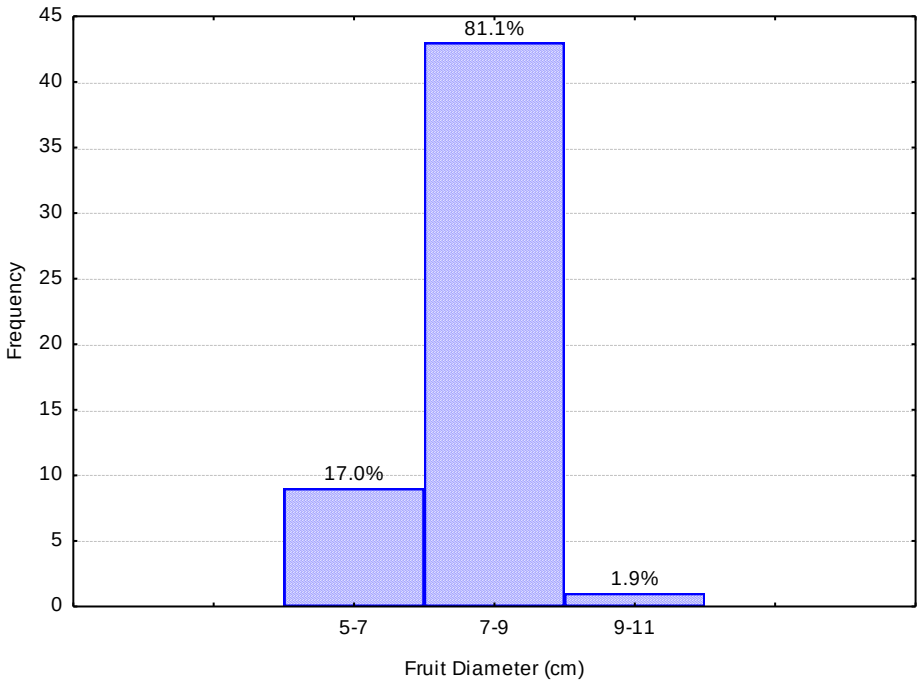

Fig. 4.7: Fruit diameter (cm) of avocado

Fruit weight of avocado studied was variable, however, more than half (58.6%) of them weighed between 220 g and 370 g. Only 18.9% weighed more than 420 g while 11.3% weighed between 170 g and 220 g. Another 11.3% weighed between 370 g and 420 g.

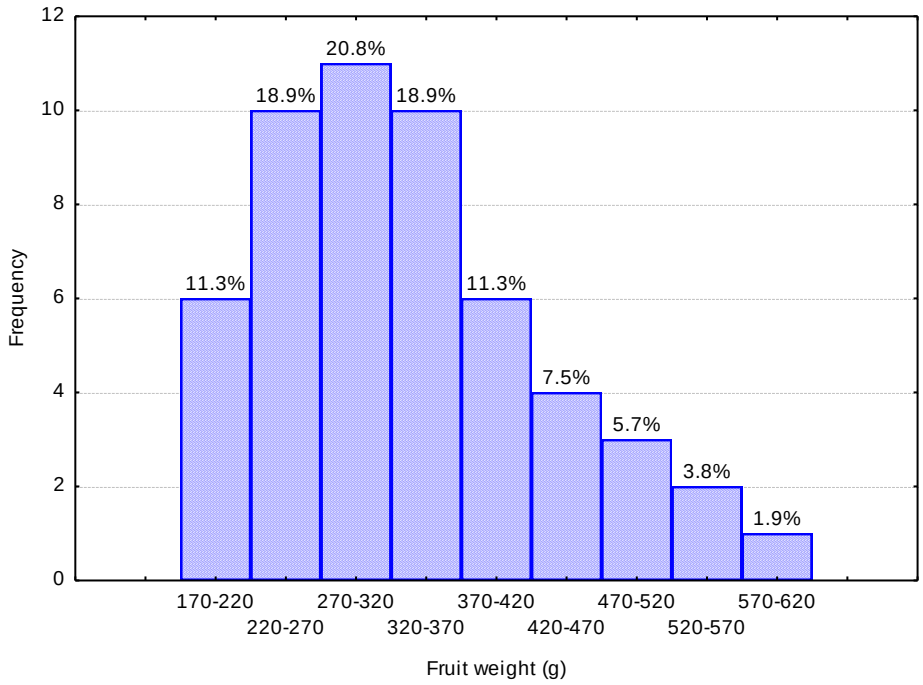

Fig. 4.8: Weight (g) of avocado fruits

The shapes of fruit studied included oblate, spheroid (Plate 4.1B), high spheroid, ellipsoid, narrowly obovate (Plate 4.1A), obovate (Plate 4.1D), pyriform (Plate 4.1C), clavate and rhomboid. Of these, 39.6% of the fruits were narrowly obovate, 20.8% were rhomboid, 13.2% were pyriform, 9.4% were clavate, and 5.7% each were highly spheroid and ellipsoid. One each (1.9%) of the 53 trees studied had fruits of oblate, spheroid and obovate shapes. More than half of the plants studied (54.7%) had partial ridges on their fruits whilst 17% were entirely covered with ridges (Plate 4.1D). There were no ridges (Plates 4.1 A & B) on fruits of 28.3% of the plans. From the results, 35.8% of the fruits had a strong glossy skin (Plate 4.1 A). Another 35.8% of the plants had medium glossy skins.

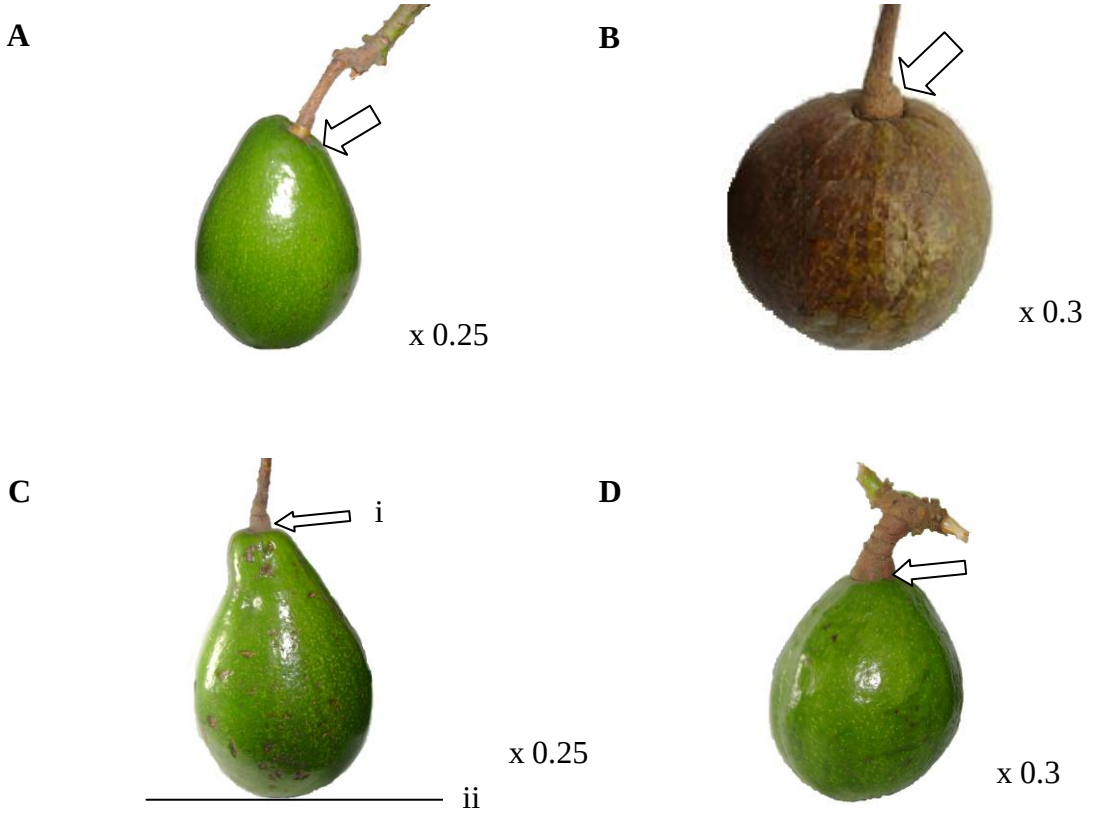

Plate 4.1: Avocado fruits showing different morphological characters. (A) fruit with a very asymmetrical pedicel position and a strong glossy skin; (B) a rounded pedicel shape fruit with a weak glossy skin; (C) a fruit with (i) a central fruit apex position and (ii) a symmetric fruit apex position; (D) a fruit with a conical pedicel shape fruit. Photos: Janice D. Oduro

Plants having fruits with weak glossy skin were 28.3%. Also, 37.7% had smooth skin surface while 49.1% and 13.2% had intermediate and rough skin surfaces respectively. The fruits assumed different colours upon ripening. The colours of the ripe fruits were, red (28.3%), green (24.5%), speckled (22.6%), purple (11.3%), light green (3.8%), dark green (3.8%). The colour of fruit next to skin was light green in 67.9% of the fruit, green in 30.2% and yellow in 1.9% of the fruits. The skin of the fruit had thickness ranging between 2 mm and 8 mm, however, almost all (96.2%) of the fruits had their fruit thickness between 2 mm and 6 mm. More than half (50.9%) of all fruits had their fruit thickness within 2 mm and 4 mm.

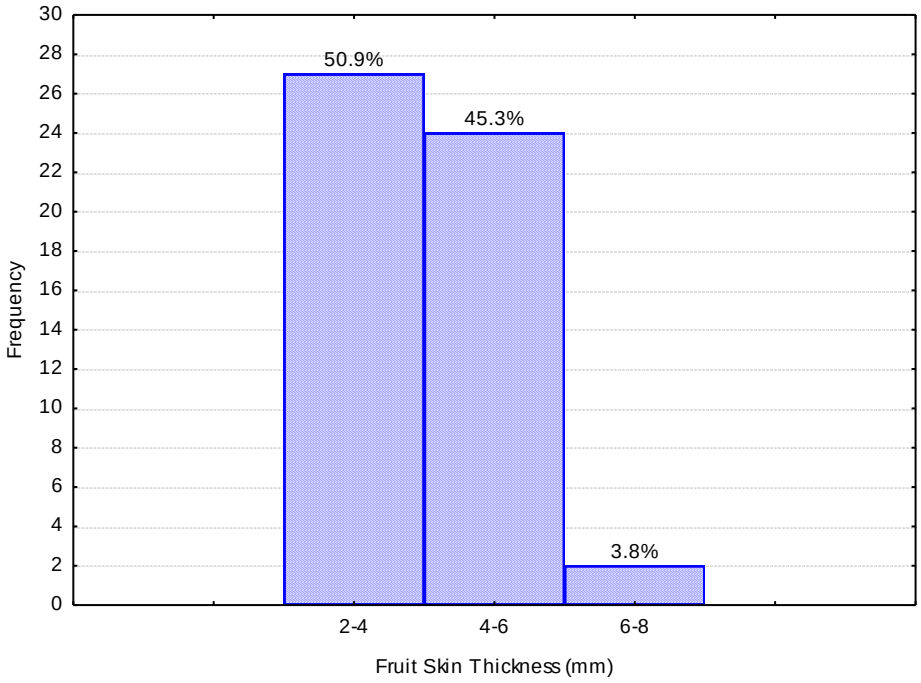

Fig.4.9: Fruit skin thickness (mm) of avocado

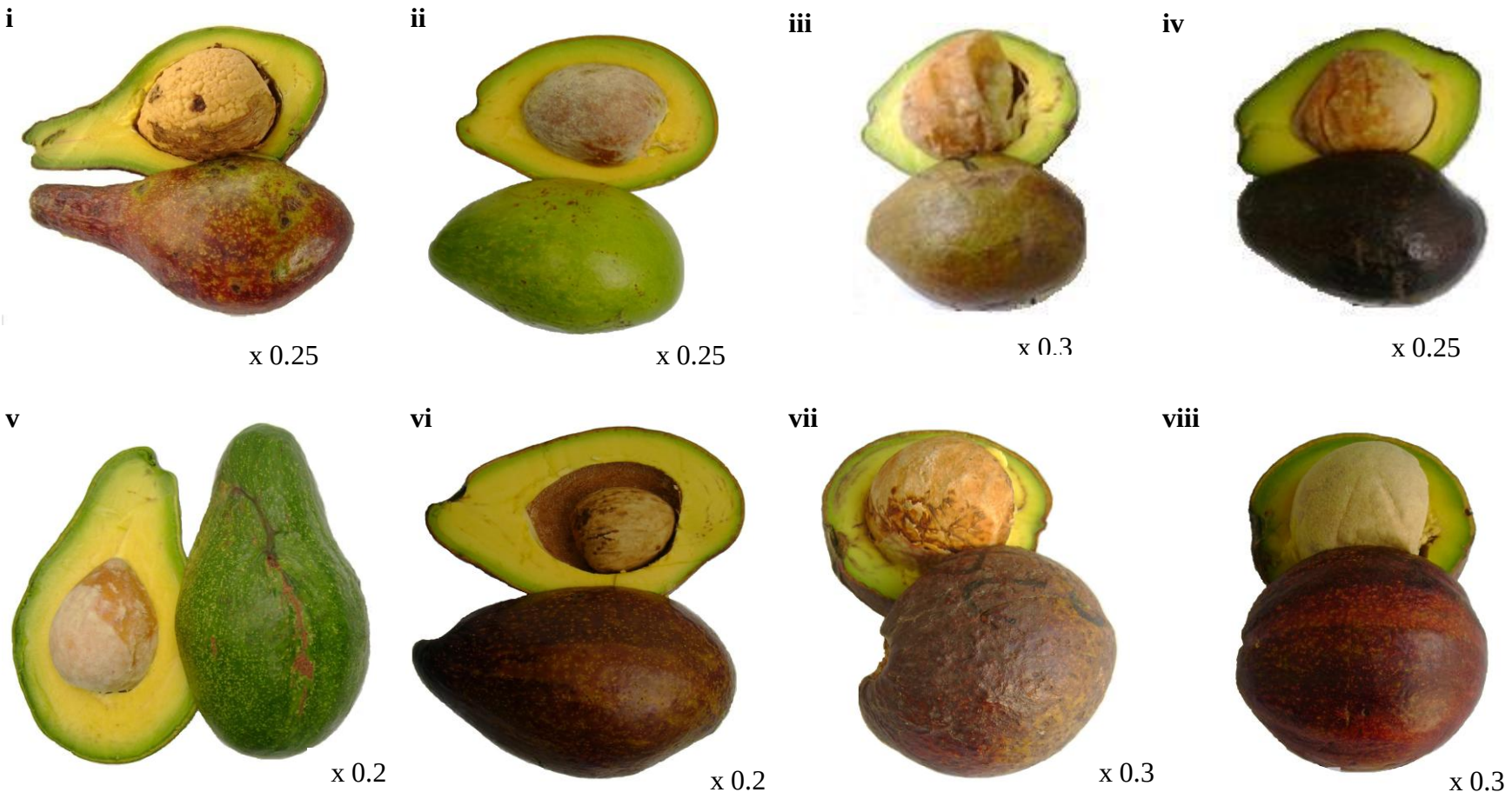

Plate 4.2: Avocado fruits showing various morphological characters. (i) Pyriform shape, broadly ovate seed, cotyledon not attached to seed (ii) Narrowly obovate shape (iii) Ellipsoid shape, free space on seed base (iv) Narrowly obovate (v) Clavate shape (vi) Rhomboidal shape, small seed, cotyledon not attached to seed (vii) Oblate shape, base flattened apex rounded seed (viii) Spheroid shape
Photos: Janice D. Oduro

The strength of attachment of flesh to skin of avocado studied varied. There was a strong attachment in 13.2% of the fruits. 67.9% had a slight attachment while 18.9% had an intermediate strength of attachment.

There was a wide range of peduncle length of fruits studied. Most (83%) of the fruits however had peduncle lengths between 2 cm and 6 cm. About forty seven percent of the fruits had peduncle lengths of 4-6 cm. Very few (7.6%) had their peduncle lengths over 8 cm.

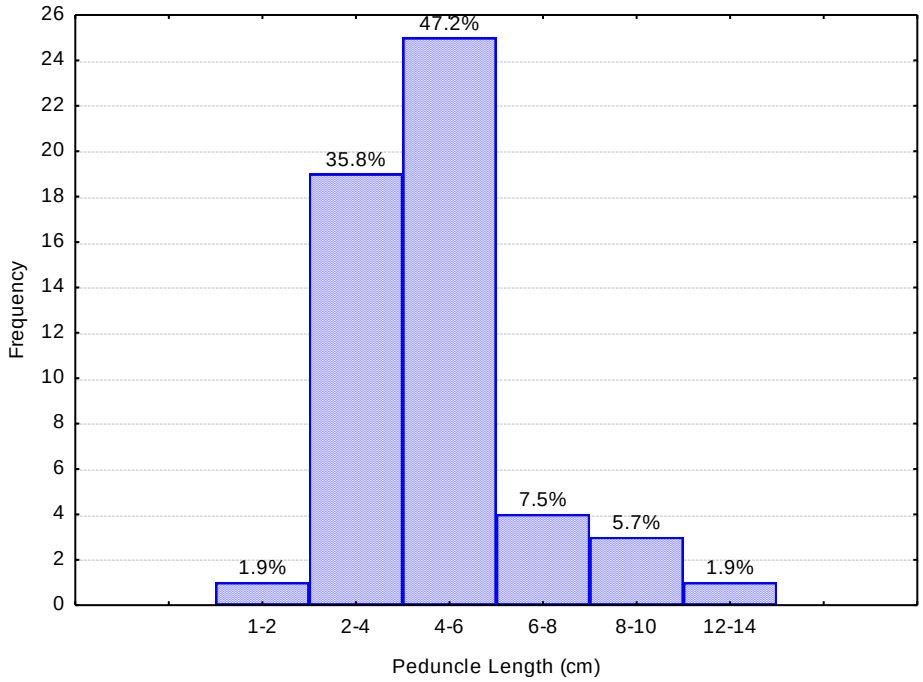

Fig. 4.10: Peduncle length (cm) of avocado

A much larger percentage (92.5%) of fruits had their peduncle diameters between 4 mm and 8 mm. Only 7.6% of the fruits had peduncle diameters between 8 mm and 12 mm.

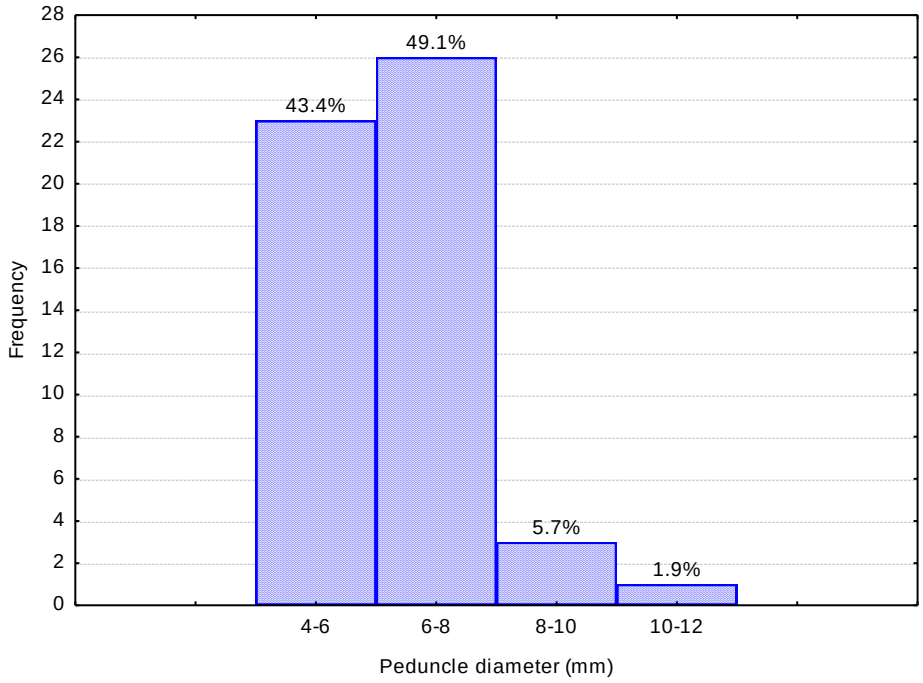

Fig. 4.11: Peduncle diameter (mm) of avocado

Pedicel lengths were between 0.5 cm and 2.0 cm. Mostly, the fruits had pedicel lengths between 0.5 cm and 1.5 cm. 96.2% of fruits had pedicles lengths in the range of 0.5 cm and 1.5 cm.

Pedicles were either centrally positioned or asymmetrically positioned on fruits. The results showed that 50.9% of pedicels were centrally positioned on fruits whiles 49.1% were asymmetrically positioned. Nailhead pedicel were also either present or absent. In 39.6% of fruits, nailhead pedicel were present but were absent in 60.4% of fruits.

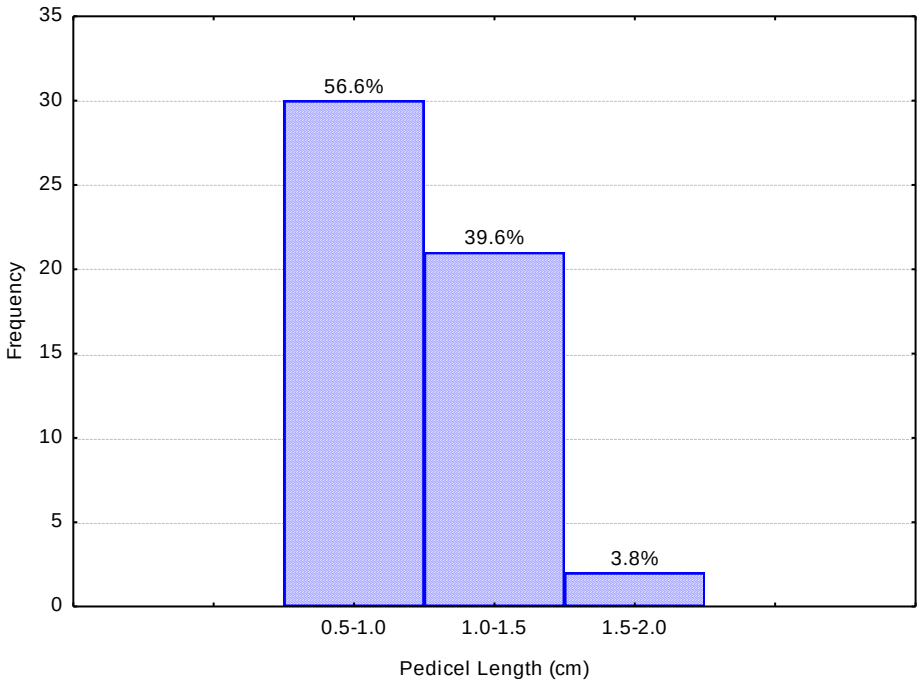

Fig. 4.12: Pedicle length (cm) of avocado fruits

The avocado fruits studied had five different seed shapes. Twenty seeds representing 37.7% were broadly ovate, 35.8% had their base flattened and conical apexes, 18.9% had base flattened and rounded apexes, 5.7% were cordiform shaped and only 1.9% were ellipsoid in shape.

x 0.7

Broadly ovate shaped seed

x 0.5

Ellipsoid shaped seed

x 0.6

Base flattened, apex conical shaped seed

x 0.3

Base fattened, apex rounded shaped seed

Plate 4.3: Various seed shapes of avocado. Photos: Janice D. Oduro

Seeds were between 2 cm and 8 cm in length. Only 1.9% had very short seed length of between 2 cm and 4 cm. Majority (84.9%) however had seed lengths between 4 cm and 6 cm. Another small percentage (13.2%) had relatively large seed lengths measuring between 6 cm and 8 cm.

The seeds of the avocado covered cavities almost the same lengths as those of the seeds. Most (92.4%) of the seed cavities were between 4 cm and 8 cm long. Only 7.5% were above 8 cm but were also less than 10 cm long.

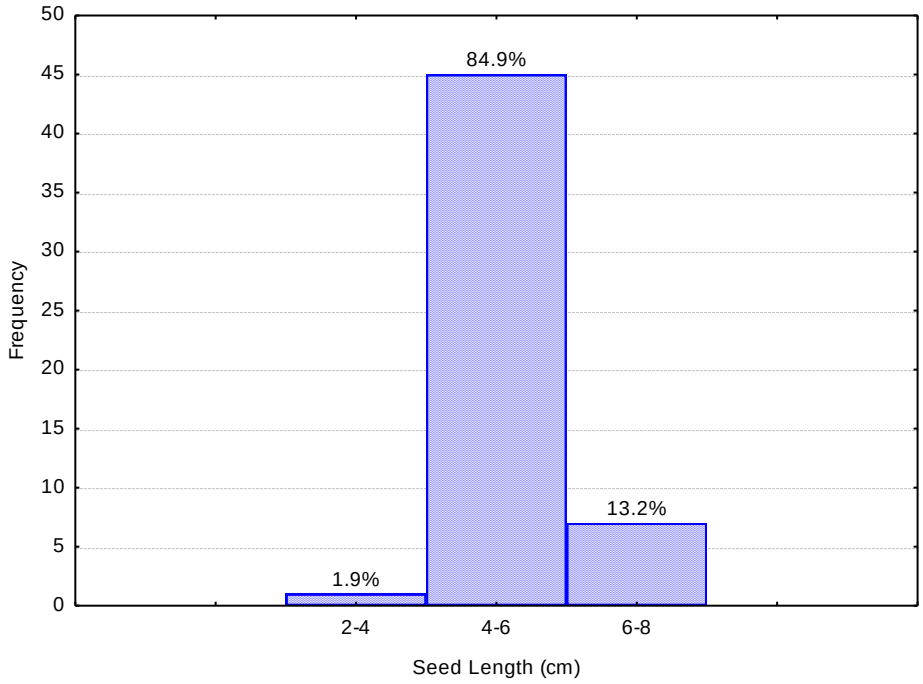

Fig. 4.13: Length of seeds (cm) of avocado fruits

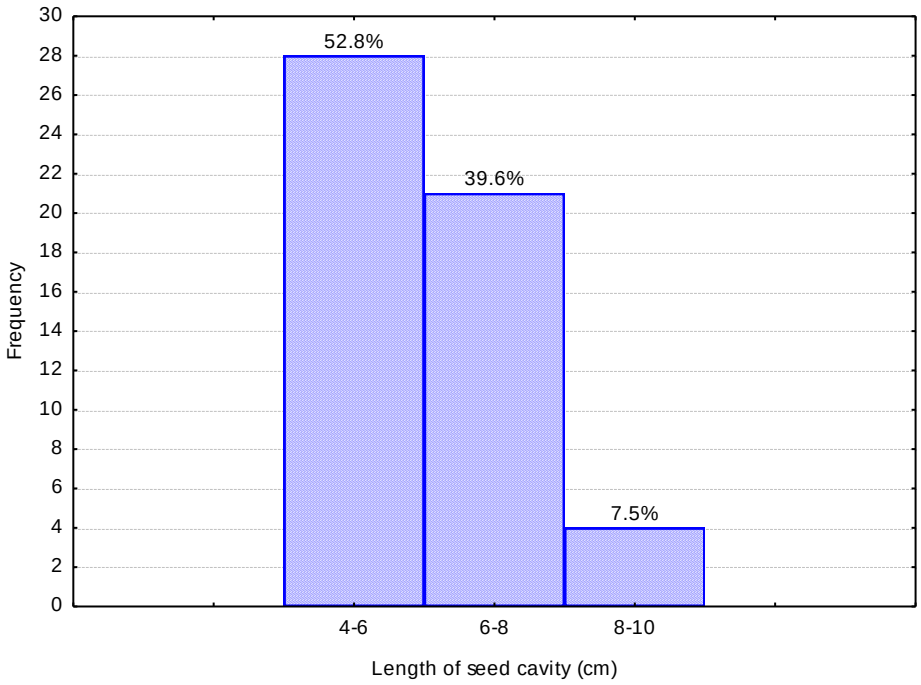

Fig. 4.14: Seed cavity lengths (cm) of avocado fruits

Seed diameters between 4.0 cm and 5 cm were more (64.2%) than those between 3.0 cm and 4.0 cm (9.4%), and 5.0 cm and 6.0 cm (26.4%).

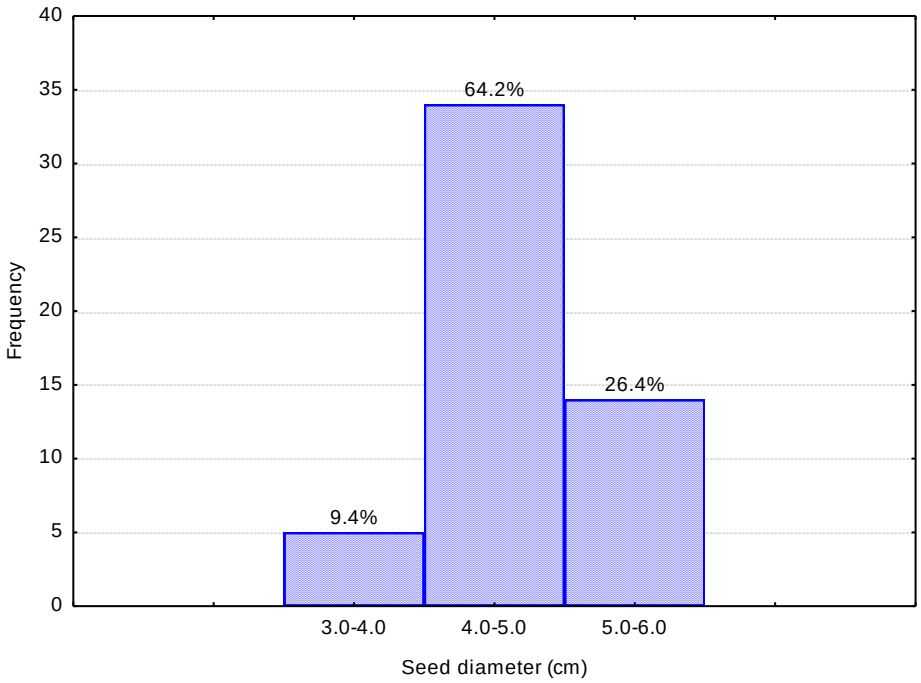

Fig. 4.15: Diameters (cm) of avocado seeds

The diameter of seed cavity is comparable to the diameter of seeds. Most (92.5%) of the seed cavity diameters ranged between 4 cm and 6 cm. Only 1.9% had a seed cavity diameter between 3 cm and 4 cm. Another small percentage (5.7%) was between 6 cm and 7 cm.

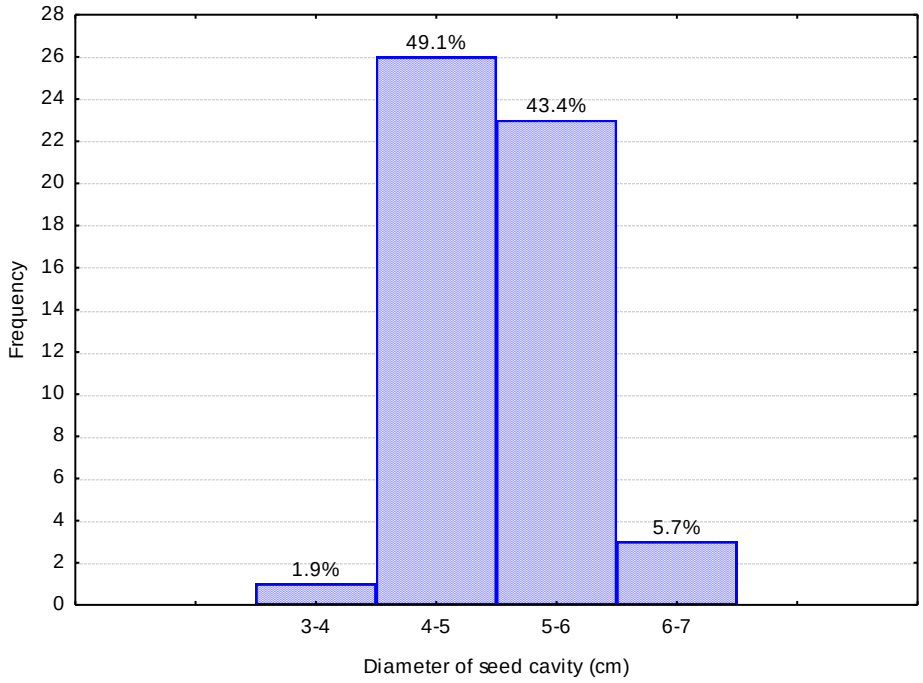

Fig. 4.16: Seed cavity diameters (cm) of fruits of avocado

Assessment of seed cavity space of the fruit gave variable results. There were some free spaces in the seed cavity of majority of the fruits studied. The results showed that 66% of the fruits had space on the seed base only, 32.1% had spaces on both the seed apex and seed base while 1.9% had space on the seed apex only. The size of the cavity space varied from fruit to fruit. Cotyledon of seeds were attached to the seeds in 30.2% of the seeds and not attached to the seeds in 69.8% of the avocados.

The weights of avocado seeds studied were between 25 g and 125 g. Seeds weighing the least (25-50 g) were 37.7% and those with the highest weight (100-125 g) were only 5.7%. The results showed that more than half (56.6%) of the seeds weighed between 50 g and 100 g.

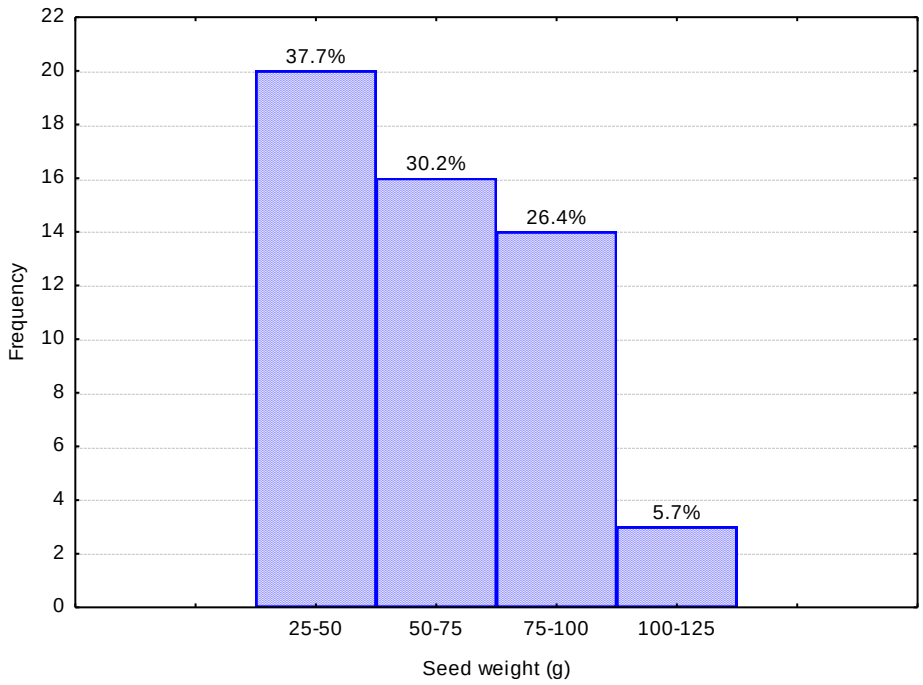

Fig. 4.17: Frequency distribution of weight (g) of avocado seed

Cluster analysis

A cluster analysis or taxonomy analysis defines a natural population of the same species into distinctively related phylogenetic main groups and subgroups. The protocol uses a set of morphological characters that have rather constant parameters to group the population. For this study, the characters used for the analysis were the characteristics of the tree, leaves, fruit and seed (Appendix 3). The relationships between all avocado plants based on 35

morphological characters are represented in a dendrogram in Fig 4.18. Each accession was represented by a number (Appendix 4). The dendrogram identified three major groups.

The results showed three major distinct groups. The first distinct group (A) was defined with samples one and eight at either ends, with four subgroups. The four subgroups had samples 1-30, 42-50, 40-33; however, sample eight stood alone. The second distinct group (B) had samples three and six at either ends. This also had four subgroups made up of samples 3-17, 15-21, 37-19 and 23-6. The third distinct group (C) was between samples 11 and 39. This group had three subgroups made up of samples 35-51, 12-41 and 48-39.

The samples with similar morphological characters were grouped together. The results showed that the samples 1, 2, 43 and 30 (Appendix 4) though collected from different districts shared a lot of morphological characters compared to samples 1 and 3 though both were collected from the same town did not have much in common. The samples in the second distinct group were closely linked. This implies that those samples share a lot of morphological features together compared with the samples in the third distinct group which appear to have much diversity within the group. Some of the samples in the third group (example sample 48) stood independent almost to the end of the link before joining the group. The Samples 16 and 39 were joined to the tree at the last cluster; this showed that they share very few morphological characters with the other samples. This showed that there was some degree of morphological diversity between the avocado accessions found in the study area.

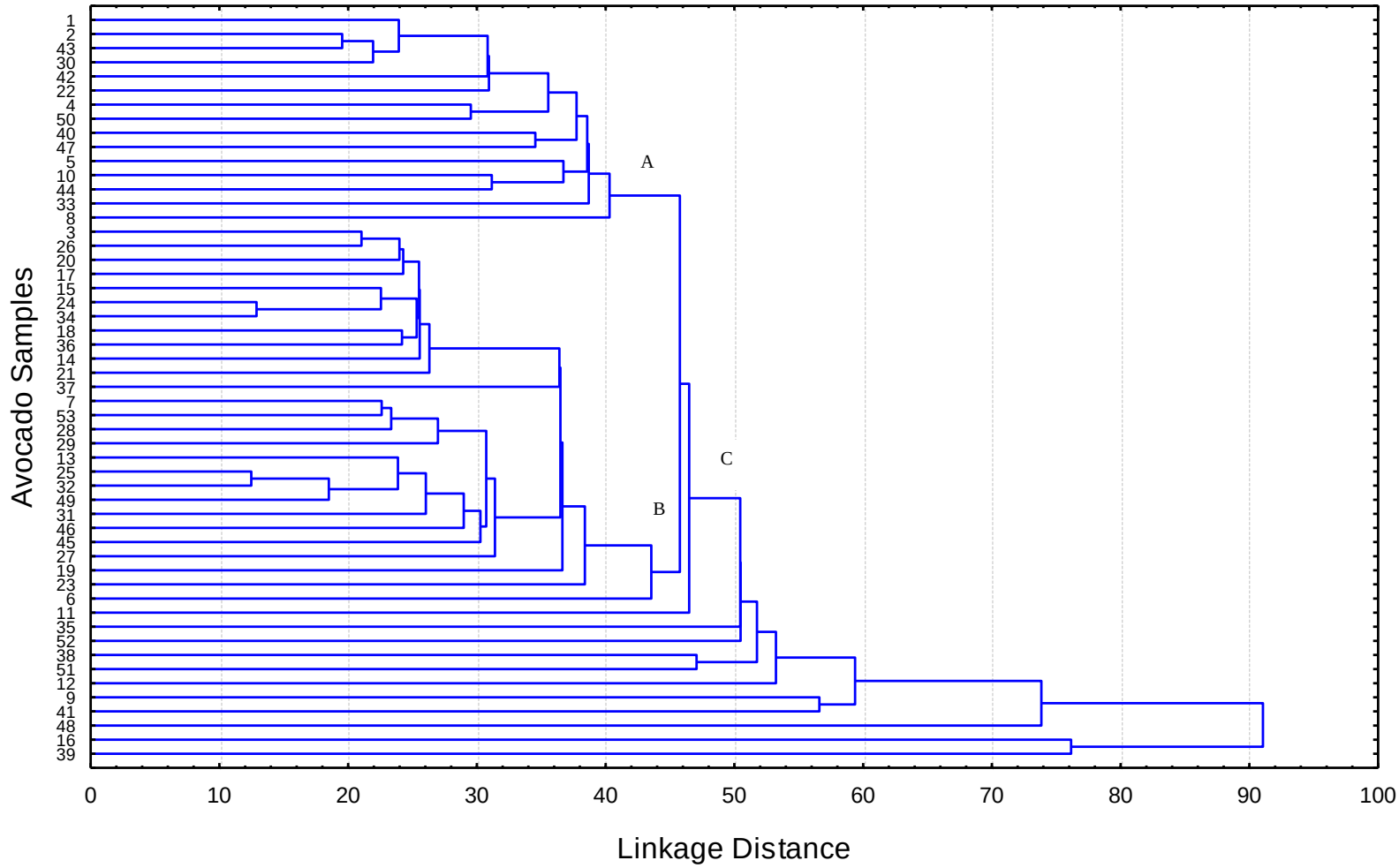

Fig. 4.18: Single-joining tree of avocado individual plants, using Euclidean distances from morphological parameters

DNA analysis and microsatellite typing

The genetic diversity of 71 avocado accessions, including 13 from the Cocoa Research Institute of Ghana (CRIG), were investigated across 10 microsatellites loci.

Analysis of the 10 SSR loci endemic in populations

The 10 microsatellites loci had varying degrees of polymorphism, generating 115 alleles across the population sampled (Table 4.4). The number of alleles varied from five in AVAG06 to 22 in AVAG21; with an average of 11.5 alleles per locus. All SSR loci used were polymorphic (Plate 4.4). The average gene diversity was 0.7529 (0.5636 to 0.8907, Tables 4.5 and 4.6). The number, size and frequencies of alleles, observed (H_{obs}) heterozygosities, number of homozygotes, heterozygotes and null alleles across all loci are summarised in Table 4.5.

Allele size ranged from 71 bp (AVAG06) to 225 bp (AVAG21), with frequencies varying between 0.72% and 64.29%. The allelic frequencies at the different SSR loci varied significantly; ranging from 0.72 - 35.51% at AVAG01 locus, to 0.79 - 64.29 at the AVAG13 locus. Across all the accessions screened, 336 (47.3%) loci were homozygous while 312 (43.9%) loci were heterozygous. The number of null amplifications ranged from zero (AVAG22 and AVAG25) to 14 (AVMIX04), averaging 6.2 across all loci. The highest H_{obs} (0.6761) was recorded at the AVAG21 locus, while the lowest (0.3333) occurred at the locus AVAG10. The overall mean H_{obs} of all populations, calculated across all loci was (0.4765).

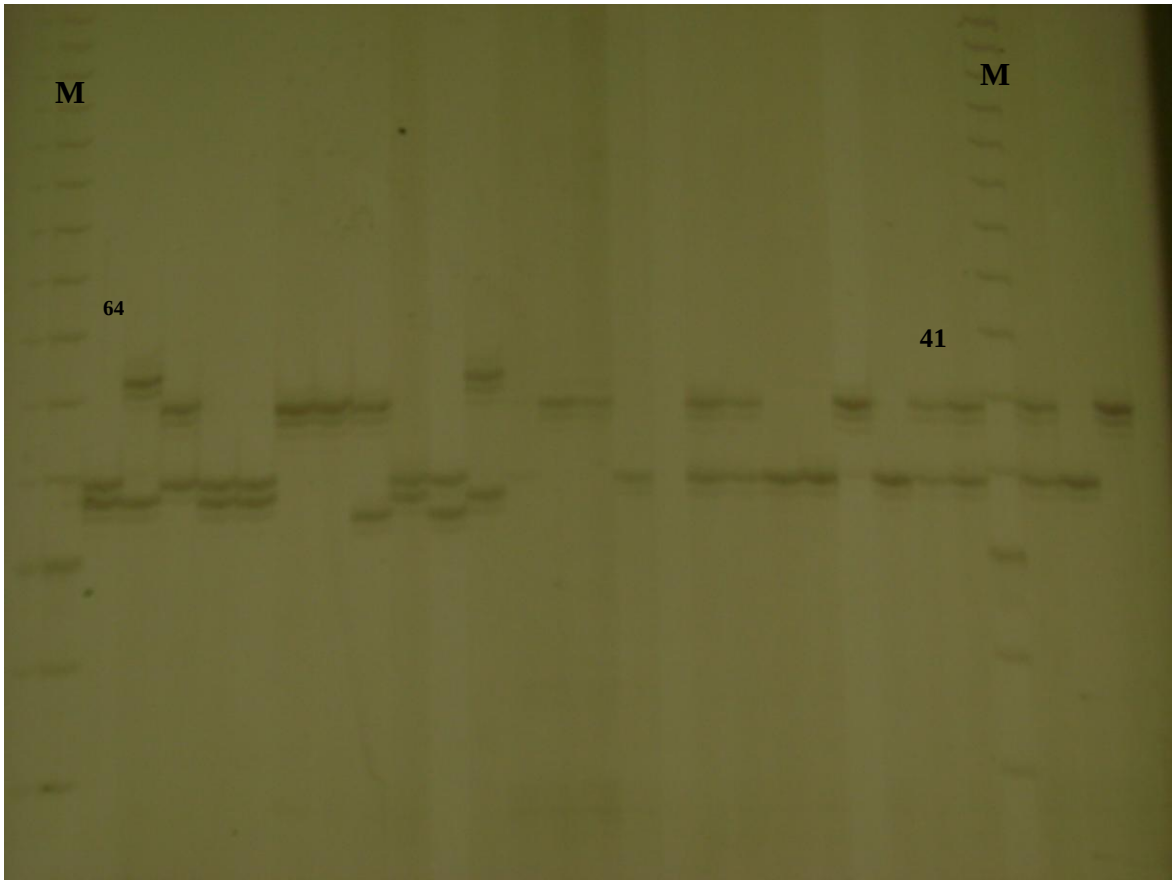

Plate 4.4: Genotyping of avocado accessions using SSR primer AVAG22 on PAGE; M = 10 bp DNA ladder

AVAG25 yielded the lowest number of homozygotes (21) for all SSRs typed. AVAG21 had the highest number of (48) heterozygotes; while AVAG10 produced the highest number of homozygotes (42) and the least heterozygotes (21). The average for a total of 62, was 6.2 - null amplifications over all loci tested; with 14 resulting from the unsuccessful amplification with AVMIX04, and two from AVAG01.

Forty-six percent (46%) of the SSR allele sizes were different from sizes previously reported; however, allele sizes in this study were estimated from gels rather than from automated sequencing equipment. The estimated major allele frequency, number of genotypes, gene diversity, heterozygosity and PIC values of the 10 SSR loci examined are presented in Table 4.6. A total of 180 genotypes and 115 alleles were detected; with a mean of 11.5 per locus. Unbiased gene diversity (H_{nb}) ranged from 0.6267 to 0.8907; with observed heterozygosity (H_{obs}) ranging from 0.3333to 0.6761.

The polymorphism information content (PIC) value ranged from 0.5441 to 0.8818 with a mean of 0.7212. AVAG21 had the highest PIC of 0.8818, followed by AVMIX04 with 0.873, the lowest PIC value of 0.5441 was recorded by AVAG13. The proportion of loci with PIC value from 0.5 and above was 100%. 70% of the accessions had PIC values of 0.6 and beyond. The mean PIC value for SSR loci was 0.7212. The Stepwise Mutation Model Index (SMMIndex) were all highly significant, with mean f value of 0.3739, ranging between 0.2324 (AVAG25) and 0.5959 (AVAG10).

Results of the Hardy-Weinberg equilibrium analysis are summarised in Table 4.7. The chi squares ranged between 81.8460 in AVAG 06 to 468.6027 in

AVAG 21. All the SSRs had chi square corresponding probabilities of 0.000. The chi-squares of all the 10 SSRs used were highly significant.

Table 4.4: Allele size (bp) and percentage frequencies (in brackets) of loci for the 71 avocado samples

Allele No	AVAC01	AVAG03	AVMIX04	AVGA05	AVAG06	AVAG10	AVGA13	AVGA21	AVAG22	AVAG25
1	102 (0.72)	98 (0.79)	107 (0.88)	93 (4.62)	71 (18.25)	122 (4.76)	96 (0.79)	157 (0.70)	104 (1.41)	80 (0.79)
2	110 (2.90)	99 (3.97)	162 (6.14)	95 (3.85)	73 (52.38)	128 (3.17)	97 (10.32)	163 (0.70)	107 (4.23)	100 (0.79)
3	111 (7.25)	103 (1.59)	164. (14.91)	97 (34.62)	75 (3.17)	161 (2.38)	99 (1.59)	168 (1.41)	108 (23.94)	102 (05.56)
4	113 (35.51)	107 (41.27)	166 (5.26)	99 (36.15)	77 (25.40)	162 (1.59)	101 (7.94)	169 (2.11)	110 (21.82)	103 (23.02)
5	115 (28.26)	108 (43.65)	168 (1.75)	101 (2.31)	79 (0.79)	173 (0.79)	103 (64.29)	173 (0.70)	116 (2.11)	114 (0.79)
6	121 (3.62)	110 (0.79)	170 (1.75)	107 (0.77)		175 (25.40)	112 (1.59)	175 (1.41)	118 (5.63)	116 (7.14)
7	123 (15.94)	113 (1.59)	172 (2.63)	117 (0.77)		176 (30.16)	120 (3.14)	177 (9.86)	120 (33.10)	118 (1.59)
8	125 (2.90)	114 (3.97)	173 (14.04)	121 (16.92)		178 (4.76)	122 (4.76)	179 (18.31)	121 (1.41)	120 (15.87)
9	127 (1.45)	116 (0.79)	174 (17.54)			181 (0.79)	126 (4.76)	181 (8.45)	123 (3.52)	122 (7.14)
10	129 (1.45)	122 (1.59)	177 (8.77)			190 (3.97)	132 (0.79)	182 (1.41)	124 (2.82)	131 (3.97)
11			178 (10.53)			194 (15.08)		191 (4.93)		134 (1.59)
12			180 (12.28)			195 (5.56)		195 (2.11)		138 (18.25)
13			186 (3.51)			197 (1.59)		203 (1.41)		139 (11.11)
14								207 (4.23)		144 (2.38)
15								209 (2.11)		
16								211 (5.63)		
17								213 (7.04)		
18								214 (0.70)		
19								216 (4.93)		
20								218 (20.42)		
21								220 (0.70)		
22								225 (0.70)		

Table 4.5: Characteristics of the SSR loci among avocado populations in Ashanti and Central Regions of Ghana

SSR (Locus)	No. of Alleles	Range of Allele size (bp)	Range of Allele Frequencies (%)	$^{1}H_{obs}$	No. of Homo-zygotes	No. of Hetero-zygotes	No. of Null Ampli-fications
AVAC01	10	102-129	0.72 - 35.51	0.4058	40	29	2
AVAG03	10	98-122	0.79 - 43.65	0.4603	34	29	8
AVMIX04	13	107-186	0.88 - 17.54	0.5614	25	32	14
AVAG05	8	94-122	0.77 - 36.15	0.3538	40	24	6
AVAG06	5	71-80	0.79 - 52.38	0.4603	34	30	8
AVAG10	13	122-197	0.79 - 30.16	0.3333	42	21	8
AVAG13	10	96-132	0.79 - 64.29	0.3968	38	25	8
AVAG21	22	157-225	0.70 - 20.42	0.6761	23	48	0
AVAG22	10	104-124	1.41 - 33.10	0.4507	39	32	0
AVAG25	14	80-144	0.79 - 23.02	0.6667	21	42	8
Mean	11.5			0.4765	33.6	31.2	6.2

Table 4. 6: The major allele frequency, genotype number, gene diversity, heterozygosity and polymorphic information content (PIC) of SSR markers used for genetic diversity analysis.

Marker	Major Allele Frequency	Genotype Number	Number observed	No. of Alleles	Availability	Gene Diversity	Heterozygosity	PIC in this study	SMM Index	F
AVAC 01	0.3551	14	69	10	0.9718	0.7599	0.4058	0.7252	0.0000	0.4717
AVAG 03	0.4365	13	63	10	0.8873	0.6350	0.4603	0.5672	0.0000	0.2825
AVMIX 04	0.1754	23	57	13	0.8028	0.8843	0.5614	0.8731	0.0000	0.3728
AVAG 05	0.3615	11	65	8	0.9155	0.7166	0.3538	0.6685	0.0000	0.5119
AVAG 06	0.5238	8	63	5	0.8873	0.6267	0.4603	0.5680	0.0000	0.2729
AVAG 10	0.3016	20	63	13	0.8873	0.8104	0.3333	0.7874	0.0000	0.5939
AVAG 13	0.6429	12	63	10	0.8873	0.5636	0.3968	0.5441	0.0000	0.3032
AVAG 21	0.2042	35	71	22	1.0000	0.8907	0.6761	0.8818	0.0000	0.2476
AVAG 22	0.3310	18	71	10	1.0000	0.7822	0.4507	0.7513	0.0000	0.4296
AVAG 25	0.2302	26	63	14	0.8873	0.8600	0.6667	0.8452	0.0000	0.2324
Mean	0.3555	18	64.8	11.5	0.9127	0.7529	0.4765	0.7212	0.0000	0.3739

Table 4.7: Hardy-Weinberg equilibrium analysis

Marker	Chi-Square Value	Chi-Square d.f.	Chi-Square P-value	LRT value	LRT d.f.	LRT P-value	Exact P-value
AVAC 01	191.0008	45	0.0000	125.8237	45	0.0000	0.0000
AVAG 03	166.9236	45	0.0000	45.7613	45	0.4404	0.0003
AVMIX 04	196.8749	78	0.0000	152.7488	78	0.0000	0.0000
AVAG 05	88.7412	28	0.0000	90.0691	28	0.0000	0.0000
AVAG 06	81.8460	10	0.0000	34.3900	10	0.0002	0.0000
AVAG 10	339.2714	78	0.0000	154.2056	78	0.0000	0.0000
AVAG 13	262.7625	45	0.0000	75.8073	45	0.0028	0.0000
AVAG 21	468.6027	231	0.0000	184.6553	231	0.9889	0.0000
AVAG 22	198.3280	55	0.0000	112.7868	55	0.0000	0.0000
AVAG 25	156.8511	91	0.0000	113.5689	91	0.0548	0.0000

Phylogenetic analyses

This section presents phylogenetic tree of genetic similarity based on 10 SSRs selected for genotyping the accessions identified from the study area and others from the CRIG avocado collection.

The Dendrogram of Fig. 4.21 illustrates association between the samples studied based on the cluster analysis of their genetic similarities. The set of markers used uniquely classified 71 individual plants in this study; it also illustrated the considerable genetic diversity that was present.

The resultant dendrogram defined seven distinct groups. The most genetically distinct genotype was As 37; this did not cluster with any other accession. The largest group consisted of 27 genotypes, with As 23 at one end and As 5 at the other end. This largest group was further defined into three subgroups. The three subgroups comprised As 23 and As 32, As 28 – As 18, and As 4 – As 4. The second distinct group had Cr 49 and As 11 at the ends. The third distinct group formed between As 37 and As 24. As 37 was distinctly genotyped from the others. The fourth group had Fuchia and Borego at the ends, while the fifth had Loreta WB4-2-15 and Semil 43 WA 2-13-4 at the ends. The sixth group had K'dua and Nabal at each end while the seventh group had Cr 48 and As 8 at its ends.

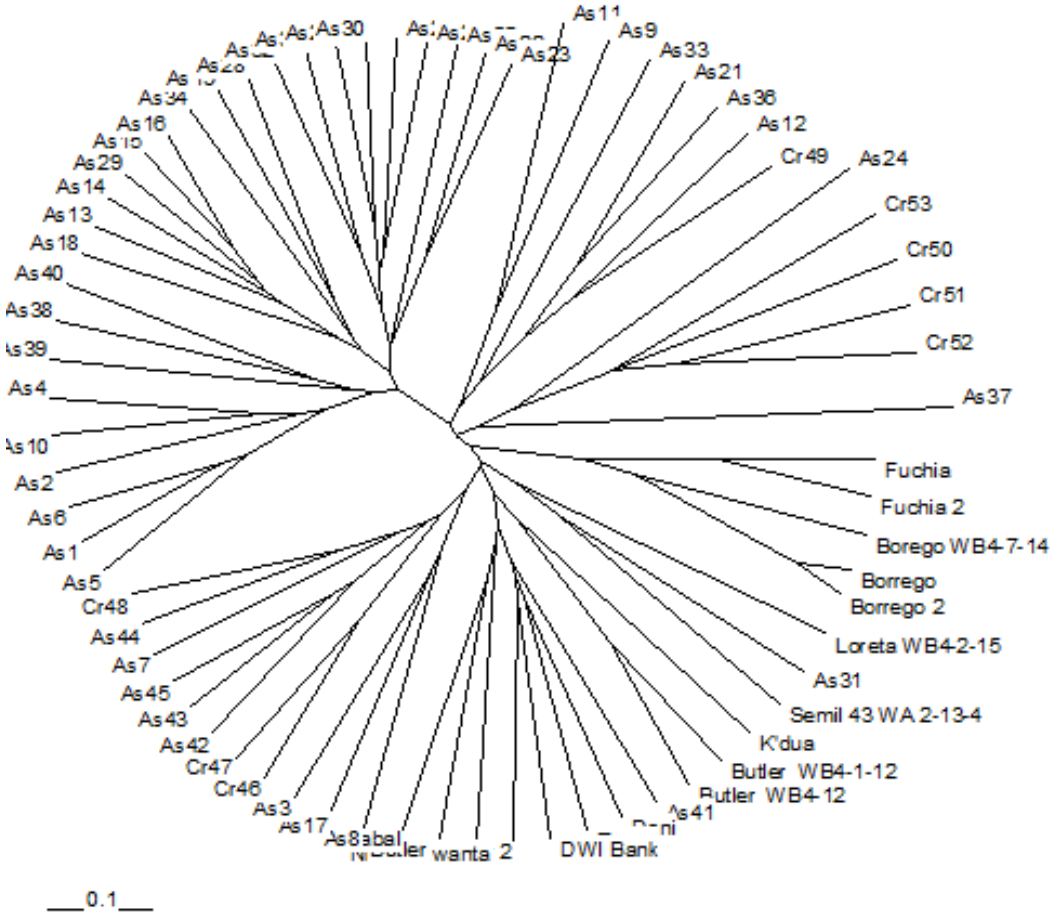

Fig. 4.19: Unweighted Paired Group Method of avocado genotypes, using 10 microsatellite markers

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

The results from the different profiles of studies of the avocado plant, and from its economic uses in the Ashanti and Central Regions of Ghana, provide the following significant conclusions. These are based mainly on ethnobotanical information on avocado, morphological characters and also the genetic diversity studied.

Ethnobotanical survey

Age distribution of respondents

The results of the age distribution showed that the elderly (ages 51 years and above) in the population were involved in avocado cultivation as compared to the younger generation. Several factors account for this scenario. In Ghana where the retirement age is 60 years; some pensioners move to resettle in their villages, to engage in farming. Some people actually start farming in their native villages when they are nearing their retiring age. Many go into cocoa farming or buy cocoa farms. Cocoa being a shade loving tree, many of these farmers plant the avocado plant to shade their cocoa plants; especially, in the nurseries. Further investigations revealed that many of the elderly group were old cocoa farmers, who used the avocado plant as shade plant for their cocoa seedlings. They also planted avocado on their cocoa farms to provide food for them and their workers during the harvesting season.

The practice of using avocado trees as shade trees has changed over the years; however, juvenile cocoa farms of late no longer use avocado plants as shade tree. This is because the farmers now believe that the avocado plants tend to introduce mistletoe onto their cocoa farms. This information on mistletoe comes from the agricultural extension officers, who work with the farmers. Some of the farmers were advised by the officers to cut down the trees, to enhance the growth of the cocoa trees and prevent the spread of mistletoes. They were also advised to use other alternate methods to nurse the cocoa seeds and avoid the use of avocado. As a result, there has been a drastic reduction in the number of avocado trees in these juvenile cocoa farms. The CRIG has now revised the above recommendations and are encouraging farmers to inter-crop avocado with cocoa. Mistletoes will grow on any free-standing tree as it is usually spread by birds.

The Youth (10–30 age group) who go into farming are also very often more interested in fast growing crops, such as tomatoes, carrot, cabbage, green pepper and cassava. These crops are ready for harvest within a year but an avocado plant has a juvenile period of 7 to 8 years (Arumuganathan and Earle, 1991). Avocado has a slower growth rate than many food crops which have similar economic benefits to the people. As a result, its cultivation is not appealing to young farmers who are interested in a quick harvest to meet the demands of the market. Additionally, the other crops tend to have higher market value than the avocado fruit. Younger people tend to need money within the shortest possible time to support their young families or start a productive life than the old who think of long term issues and their effects after their death. This

compels younger farmers to plant vegetable crops and other crops which mature within a short period than avocado. This explains the lack of interest in avocado cultivation.

Agro-ecological distribution of the avocado plant in Ghana

All the 14 districts used for the study were found within the forest zone of the study area. The plant had a wider distribution in the forest zone of the study area than the coastal savannah zone. Irvine (1961) reported that avocado is widely cultivated in the close forest regions in Ghana.

Ecologically, many parts of the Ashanti Region are in the transitional zone, comprising the moist semi-deciduous forest zone of Ghana. In contrast, the greater part of the Central Region lies in the Coastal Savannah; specifically, in the Southern Marginal Forest zone of Ghana (Hall and Swaine, 1976; 1981). The soil in the Ashanti Region is more fertile than the coastal savannah soil in the Central Region. The environmental advantages of the forest area offer greater opportunity for the growth, diversity and abundance of the avocado plant. The rather higher altitude of the Ashanti Region and the forest areas of the Central Region provide more favourable rainfall and other climatic and edaphic factors. These factors are optimum, for avocado growth. Soils are generally more fertile in the forest zone than in the coastal savannah zone (Hall and Swaine, 1976, 1981); and inevitably, soils in the coastal savannah are more saline. High soil salinity is not conducive for avocado growth. With the more favourable soils in the Ashanti Region, more people are encouraged to engage in farming in the Ashanti Region than in the

Central Region. Traditionally, people in the Central Region, especially, those close to the coast, are normally fisher folks. Thus, they would rather go fishing than do land based farming.

Cultivation levels of avocado

The results showed that as many as 85.6% of the respondents had between one and 20 avocado trees either on their farms or on their home compounds (Appendix 3a). This was so because, interviews conducted during the survey showed that commercial avocado cultivation in the area was not profitable. According to the respondents, they were advised by agricultural extension officers not to plant avocados in their cocoa farms in order to allow more light onto the farms. Although cocoa is a shade loving tree, it will need some minimum amount of sunlight to do well. Some farmers who had a few avocado plants on their farms were also destroying them because they claimed the avocado introduce and spread mistletoes on their farms. The mistletoes grow on the cocoa plants and reduce their yield. The destruction of avocados on farms threatens the conservation of the avocado. There is therefore the need for the establishment of well planned avocado plantations.

Agricultural extension officers are probably, not doing enough to ensure the conservation of the avocado plant. Poor understanding of the nutritional, medicinal and other economic values of the avocado plant may also account for the low interest in the cultivation of avocado. As a result, newly established cocoa farms do have only limited stock of avocado plants. Another reason for the

limited numbers of avocado stands is that most people prefer to cultivate avocado just for their own subsistence or as a hobby. Also, because of the long juvenile period of the plant coupled with the rather high financial capital to establish and sustain the farm, do not encourage most farmers venture into commercial avocado farming. Some farmers just do not have the financial input to go into commercial avocado farming. Other farmers also lack the technical know-how of commercial avocado farming. The Ashanti Region has quite conducive environment and market to sustain commercial avocado farms.

Traditional uses of avocado

The study showed that avocado has several important uses. In fact, almost every part of the avocado plant has some use. However, different parts have different uses in different localities.

Quite evidently, the fruit has the most important use in the study area; as 91.3% of people eat the fruit as food. Another important use of the fruit is that, it provides income. Unfortunately, the people appear to be earning too little from avocado probably because they do not take avocado cultivation as a business. Farmers earned between $60 (GH¢ 60) and $100 (GH¢ 100) from the tree per annum in engaging in avocados cultivation. On the other hand, the market women who convey the fruit to the cities and to other regions where the plant is not grown earn more. In the cities, one avocado fruit is sold for $0.20 (GHp 20) or more, while a basket full of avocado fruits (between 30 and 40 fruits) sells at $ 2.0 (GH¢ 2.0) in the rural communities. Cash earnings from avocado can be

maximised if farmers take avocado cultivation as a business. An avocado tree yields between 300 and 400 fruits yearly, on the average (Ghosh, 2000). It can therefore be inferred that, 1 ha of avocado plantation with about 490 plants could yield a minimum about 147,000 fruits and a maximum of about 196,000 fruits per year. The sale of one avocado fruit at the lowest price of $ 0.01 (GHp 1) each will earn a farmer between $ 1,470 (GH¢ 1,470) and $ 1,960 (GH¢ 1,960) per ha each harvesting year. Selling avocado fruits at a more realistic but yet a low price of $0.05 (GHp 5) each will earn farmers higher revenues of between $ 7, 350 (GH¢ 7,350) and $ 9,800 (GH¢ 9,800). Taking establishment cost out, a farmer will still have enough revenue from avocado plantations. It promises to be a profitable venture if markets are found. If farmers are given the relevant education, they can earn far more money from avocado plantations than the fast growing crops that they cultivate.

The leaves of avocado also have several uses. They are used as medicine and food for nursing mothers. However, the most important use of the leaves was its use as medicine. Over 50% of the people use the leaves alone for the treatment of various ailments and conditions including stimulation of breast milk for nursing mothers, treatment for malaria, heart disease, jaundice, diabetes, typhoid fever, stomach aches and anaemia. The use of other parts of avocado as medicine was also quite evident from the study. The other parts use for medicinal purposes are the seed, the bark and the root. The most important use of the seed however was as a propagule. An important use of the tree that cannot be overlooked was the use of the stem and branches as fuelwood. In the rural areas where electricity

and gas are rare, wood is an important source of energy. Avocado trees and branches are important fuelwood source**.** The wood is also used for making furniture and mortar in some communities.

Morphological studies of *Persea americana* Mill.

The heights of the avocado plant studied indicated that most of the plants were fully matured. All the plants studied had heights above 5 m. For instance the tallest plant studied was above 16 m. Observation however indicates that avocado plants between the heights of 3 m and 4.5 m are relatively easier to manage and more productive (Partida Jr., 1996). This implies that, it will be very difficult for the farmers to manage their tall plants. Harvesting from such plants is very difficult and expensive. Shorter avocado trees with good canopy development produce more fruits than taller ones. Also it has been reported that in California, avocado fruit yield reduced from about 2,177 kg/ha to 725 kg/ha over a three year period when the canopies were crowded such that there was not enough sunlight through (Partida Jr., 1996). When such trees were pruned to open up the canopy to allow more light through, and the tree heights limited to about 3.6 m, yield increased again. As well, it was observed that not only did the fruit yield increase but cost of harvesting was also significantly reduced on pruned trees. It is thus possible that, Ghanaian avocado farmers might have better yield and income from their avocado farms and plantations when the trees are pruned down to between 3 m and 4.5 m in height. The average canopy spread of avocado plants studied was 8.4 m, and the crowns ranged between 4.9 m and 13.17 m. Such large crowns may

be too much for proper management. Management of such trees will be relatively easier when the crowns are pruned. The trunk circumference of avocados ranged between 46.30 cm and 283.10 cm; equivalent to diameters between 14.74 cm and 90.11 cm. Such plants are considered very large fruit plants. However the sizes of the avocados are evidence of good and healthy growth.

The most common branching distribution of the plants studies was irregular. However, Paz-Vega (1997) noted that horizontal branching could enhance flowering. By tipping the branches, more side shoots were formed. This created complex branching systems in avocado. This method could thus be effectively used to control the size and shape of avocado plants, as well as enhance yield. Removal of excess branches by girdling could reduce vegetative growth; and thus increase flowering and fruiting. The study results showed that, nearly half of the avocado plants had intensive branching.

Avocado leaves have variable lengths. They may have lengths up to 22 cm (Irvine, 1961) or 40 cm (Morton 1987). The leaves of the avocado plants sampled in this study had leaf blade length between 12.92 cm and 28.40 cm long. Large leaf is distinctive of both West Indian and Guatemalan races, and their hybrids, but the West Indian species are said to have the largest leaf size among the species (Bergh and Lahav, 1996). Hence, the plants studied might very well relate to these two species. Almost 50% of the trees had leaves with acute apex. This is a feature of a normal avocado leaf. However, improved cultivars have some leaf shape variations. The observation therefore means that new improved cultivars

have not been introduced to the area. The accessions spreading are the ones which were introduced over a century ago.

The lengths of the fruit studies ranged between 7 cm and 19 cm. About half of the fruits were between 10 cm and 13 cm long. The fruits of Guatemalan species range from small to large while that of West Indian species are between medium and very large (Bergh and Lahav, 1996). The same authors mentioned that Guatemalan fruits are mostly round while West Indian fruits are variable in shape. The results in Plates 4.1 and 4.2 show some morphological variations of the fruit. There are a few rounded fruits and a lot of variable shaped fruits. From the morphological characteristics studied, most of the avocados studied exhibited characteristics of the West Indian accessions than the Guatemalan.

A typical avocado fruit weighs about 200 g to 300 g fresh weight (Paz-Vega, 1997). In the study, more than 50% of the avocado fruits sampled weighed between 220 g and 370 g. These weights are impressive in a developing country like Ghana where no Plant Growth Regulators (PGR) (Lovatt, 2005) are used to enhance growth and development of crops. This implies that, there are potentially a lot of avocado accessions in Ghana that could do without the use of PGRs. Majority of the fruits had thin skin thickness with a few thick skin thickness of 5 mm. Thin and medium fruit skin thickness is a characteristic feature of West Indian species and their varieties.

The results showed five different seed shapes (Plate 4.3). Majority of the fruits had large seeds while a few had small seeds. Literature shows that large seeds are characteristic features of Mexican and West Indian races while small

seeds are typical characteristic features of the Guatemalan race (Bergh and Lahav, 1996).

A dendrogram (Fig 4.18) was defined based on 35 morphological characters described in the descriptors for avocado used for the study. Three major clusters were obtained using the characters measured. The results showed that samples from the various districts were not exclusively different. The same set of accessions had been shared between the districts. This implies that, the same set of materials circulates in the area and that one should not expect much morphological variations from the study area. This might so because there were a lot of migratory farmers in the study area. It came to light that fruits from the study area were marketed in the Greater Accra, part of the Western, Central and some parts of the Volta Regions. The seeds of these fruits are used as propagules in these places.

Genetic diversity

The conservation of crops in the world crucially depends on the knowledge of their genetic diversity, which provides much useful insights than their morphological characters. Molecular markers, such as SSRs, reveal diversity at the DNA level; and thus provide a fundamental tool for germplasm conservation and genotyping.

SSR polymorphism and genetic diversity

A high proportion (40%) of the alleles scored fall outside the range of sizes previously scored by Schnell *et al*. (2003) and varied by one base pair more than the ones confirmed by Schnell *et al.* (2003) (Table 4.4). However, the projected size given by Schnell *et al.* (2003) involved sequencing data using capillary electrophoretic methods; whereas the data set produced in this study was by gel electrophoresis. Accurate scoring of bands depends on the discriminative ability of gel – separation technology, effective use of molecular-weight marker ladders and visual checking of scoring (Smith *et al.*, 1997). Artificial stutter bands can also cause incorrect genetic scoring of bands (Smith *et al.*, 1997); although these stutter bands can aid automated genotyping (Perlin *et al.*, 1995). In this study, the most intensely amplified band was scored as an allele for that locus. The differences in the scores could have derived from the method of determination of molecular sizes. The eye level reading of the bands from a ruler could have some margin of error; this would affect the bands scored, in one way or the other. This would also influence the sizes defined, in one way or the other.

Microsatellites are said to be highly polymorphic and useful as genetic markers that have been used in defining genetic similarities in crops such as wheat (Roder *et al***.,** 1995), maize (Smith *et al.*, 1997), Sorghum (Taramino *et al.*, 1997: Uptmoor *et al.*, 2003; Menz *et al.*, 2004). A high level of polymorphism was obtained in most of the loci studied, since eight of the 10 loci revealed 10 or more alleles in the accessions analysed. AVAG 05 and AVAG 06 had 8 and 5 alleles, respectively. AVAG 21 and AVAG 25 were the most polymorphic loci;

containing 22 and 14 alleles, respectively. The features of the locus of AVAG 21 were not much different from that of an earlier work by Schnell *et al.* (2003). In this, it was reported to be one of the most polymorphic loci, when they genotyped 258 accessions from the National Germplasm Repository (NGR), Miami, and California South Coast Field Station (SCFS), Irvine, California.

The average of 11.5 allele per locus obtained in this study was similar to the 10.4 alleles per locus reported by Ashworth and Clegg (2003). using 25 SSR loci and 180 genotypes. This is significantly higher than the 37 genotypes obtained in another study (Alcaraz and Hormaza, 2007). Schnell *et al.* (2003) identified 256 alleles from the 14 SSRs loci used, ranging from eight to 30 per locus; they obtained an average of 18.8 from the 14 SSRs used. The difference observed between their work and the present might be due to differences in the number of the accessions used.

The observed heterozygosity (H_{obs}) value calculated from the study ranged from 0.333 in AVAG 10, to 0.6761 in AVAG 21. The average H_{obs} was 0.4765; this is lower than the 0.64 obtained by Schnell *et al.* (2003). This indicates a narrower genetic base for the populations analysed. Unique alleles were identified within some samples (Table 4.4). However, their frequencies were too low to provide for any meaningful inferences.

Polymorphic information content (PIC), a measure of the discrimination ability of a locus that has been found to be comparable between SSRs and RFLPs (Smith *et al.*, 1997), of AFLPs (Menz *et al.*, 2004) or even higher PIC value for SSRs (Pejie *et al.*, 1998). The mean PIC (0.72) and the mean number of alleles for

this study were 0.72 and 11.5, respectively. Four SSRs with allele number from 13 to 22 also had high PIC values of (0.79 to 0.88).

Similarity relationships among accessions

A phylogenetic tree of the accessions (Fig. 4.19) was drawn. A number of clusters were obtained using differences in allele sizes of the SSRs associated with some avocado traits (Fig 4.19). Almost all the exotic hybrids from CRIG were found to be closer in clustering with others from the same parental line. The parental lines of avocado are the Mexican, West Indian, and Guatemalan races or hybrids of any two of the three races. The introduced hybrids from the CRIG avocado farm used in this work were predominantly of West Indian origin with one (Nabal) from a Guatemalan parent and a few inter hybrid varieties.

Some of the accessions from the study area clustered close to some of the varieties from CRIG (Fig 4.19). K'dua, a local accession from the CRIG avocado farm, was found to be genetically similar to Butler WB4-1-12, Butler WB4-12, DWI Bank and Doni; all of these are of West Indian parents, and were developed in the US. These West Indian hybrids were all recently introduced into the country by Takrama (2005). Nabal, a Guatemalan hybrid, was also highly related to the Nkrankwanta variety; these were planted in the CRIG avocado farm, just as As 8 and As 17 from the study area. These samples were collected from different districts in the Ashanti region. It indicates that there are some plants that are West Indian and Guatemalan hybrids in the study area. This confirms the observation of Taah *et al.* (2003) that there are some West Indian and Guatemalan hybrids in the

country. Loreta WB4-2-15 was also found to be similar to As 31 while As 41 also shared similar alleles with Doni, Tower and DWI Bank. These developments suggest that there are a number of unknown accessions of avocados in the study area which should be further studied and analysed.

The phylogenetic tree suggests a wide genetic variation among the accessions genotyped. The phylogenetic tree, Fig. 4.19, showed high varying levels of genetic differences between their groups. The differences in the dendrogram (Fig. 4.18 and Fig. 4.19) show that some morphological variations observed in the avocado accessions might not always have a genetic basis. The variation might have been influenced by some environmental factors.

Conclusions

It may be concluded from the ethnobotanical studies and survey that adults, mostly above the age of 30 years, are those mostly involved in avocado cultivation. Even within this age group, those older than 40 years are more frequently involved than the younger folks. The study also showed that the avocado plant is not cultivated on large scales in the study areas. This scenario is not quite different for the other parts of the country where avocado can be found.

The usefulness of the avocado plant in the socio-economic lives of the people living in the study area cannot be over emphasised. The fruit is an important food staple, and food supplement for many of the people. The majority of the respondents eat the fruit as food. Its medicinal value is overwhelming. More than half of the respondents use the leaves alone for various medical

conditions. Further studies would substantiate its efficacy for treating all ailments reported by the local people.

All the avocado trees studied had height above the 3 m and 4.5 m range recommended for high fruit productivity (Partida Jr., 1996). This means that the trees studied may not reach their optimum productivity as expected and harvesting may be expensive for the farmers. The leaves of the avocados studied exhibited characteristics that relate to the West Indian and Guatemalan races. The results of the field work showed that the samples from the various districts were not exclusively different. A probable reason may be that the seeds of the same accessions might have been used as propagules by migratory farmers. The accessions in the area might be the same as the ones introduced over a century ago.

The genetic diversity analysis shows that the SSRs used were highly polymorphic in structure. There is a wide range of diversity between the accessions; this might have resulted from cross pollination and genetic mutations. The missionaries might have introduced mostly West Indian race into the country, but there are a few Guatemalan hybrids in the cultivations. There are hybrid varieties of both races in the study area.

Recommendations

On the basis of the findings from this study, the following recommendations may be made to improve economic gains and genetic conservation of the avocado crop.

- Further molecular genetic work must be carried out to determine the avocado accessions found in the country
- The agricultural research institutions should embark on mass germplasm collection, to enhance preservations of the varieties in the country to avoid extinction.
- To improve the economic values of avocado in the country, farmers should be educated to allow the fruits to mature fully before harvest; so as to attract higher prices on the market.
- The height of avocado trees should be scaled down to within 3-4.5 meters. The canopy should be managed to allow adequate light.
- Use grafting to raise seedlings that will mature early (short gestation period) and of reduced plant architecture.

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APPENDICES

Appendix 1

Ethnobotanical study research questionnaire

UNIVERSITY OF CAPE COAST
DEPARTMENT OF MOLECULAR BIOLOGY AND BIOTECHNOLOGY
Genetic Diversity of Avocado (*Persea americana* Mill)

The research will help identify the specific accessions of avocado of economic importance in the Ashanti Region. It will also identify the natural habitat of the various varieties where they could be cultivated for the highest yield. The study will also relate to the economic importance of the plant to the indigenes. It will also cover an assessment of the potential of the crop for revenue generation by the indigenes.

NB: Information provided will be treated confidential.

1. Personal Information

1.1 Date:……
1.2 Respondent Number:…….…….
1.3 Collection Number:……
1.4 Name: ……
1.5 Age:……
1.6 Gender: male / Female
1.7 Locality: Region…..... District……Town/ Village…...…….
1.8 Occupation: Farmer [ ] Trader [ ] Herbalist [ ] others (specific)……

2. Tree Plant (avocado)

2.1 Do you have avocado (Pea) tree on your farm or home? Yes [ ] / No [ ]
2.2 How many trees do you have in your farm or home? ...……
2.3 What variety of Avocado tree do you have? …..……..
2.4 When does it start fruiting? ……..
2.5 How many times does it fruit in a year? ……..
2.6 Give a brief description of the fruits? (i.e. the nature of the fruit; Shape, color, etc) : .....……
……
…….....
.....
.....
.....
.....
.....
.....
.....

2.7 What is the local name of the variety of avocado fruit you have? ……
2.8 What are the uses of the avocado tree you have (fill in the table below).

Part of plant	**Uses / Importance**
Fruit	
Leaf	
Seed	
Stem bark	
Root	
Wood	

2.9 What is the scale of production of the Avocado fruit?
Commercial [ ] Domestic [ ] Fun [ ]
2.10 Where do you obtain planting material?…….....
2.11 What varieties of Avocado do you prefer?……..
Why?……
2.12 How much income did you get from the Avocado last year? ……
and this year? …….

Appendix 2

Some results from the ethnobotanical studies

Appendix 2a

Number of plants cultivated by respondents in the study districts

Districts	How many trees do have in your farm or home									Total
	Non	1-5 trees	up to 10 trees	up to 15 trees	up to 20 trees	up to 25 trees	up to 30 trees	above 30 trees but less or equal to 50 trees	300 trees	
Sekyere West Disrtict	0	10	16	6	4	3	4	6	1	50
Afigya Sekyere Disrtict	0	11	16	6	5	0	3	9	0	50
Sekyere East District	0	10	12	2	11	2	7	6	0	50
Adansi South District	0	9	18	3	2	0	1	2	0	35
Obuasi Municipal Assembly	0	0	4	3	0	0	0	0	0	7
Adansi North District	0	16	7	4	2	0	1	3	0	33
Offinso District	0	20	7	9	3	2	1	2	0	44
Ahafo Ano North District	0	14	10	4	3	1	0	2	0	34
Atwima Mpoa District Assemble	1	19	12	1	2	0	0	1	0	36
Asante Akim South District	0	24	16	4	3	0	3	0	0	50
Asante Akim North District	0	6	11	4	2	1	0	3	0	27
Ejisu Juaben District	0	3	12	4	8	0	3	1	0	31
Twifo Hemang Lower Denkyire District	0	25	12	3	1	0	0	2	0	43
Upper Denkyira District	1	8	5	3	3	0	3	5	0	28
Total	2	175	158	56	49	9	26	42	1	518

Appendix 2b

Local uses of avocado

District	Indicate the folk uses of the fruit					Total
	no idea	Food	Generates income	Stew	source of food and income	
Sekyere West Disrtict	0	38	1	1	10	50
Afigya Sekyere Disrtict	0	42	0	0	8	50
Sekyere East District	0	37	0	1	12	50
Adansi South District	1	30	0	0	4	35
Obuasi Municipal Assembly	0	6	0	0	1	7
Adansi North District	0	32	0	0	1	33
Offinso District	0	43	0	0	1	44
Ahafo Ano North District	0	34	0	0	0	34
Atwima Mpoa District Assemble	0	36	0	0	0	36
Asante Akim South District	0	50	0	0	0	50
Asante Akim North District	1	26	0	0	0	27
Ejisu Juaben District	0	29	0	0	2	31
Twifo Hemang Lower Denkyire District	0	43	0	0	0	43
Upper Denkyira District	0	27	0	1	0	28
Total	2	473	1	3	39	518

Appendix 2c

Uses of the avocado leaves

District	Indicate the folk uses of the leaf												Total
	no idea	Soup for nursing mother	diabetes	treats fever, malaria	blood tonic	treating heart problems	soup, blood tonic	typhoid fever	feed for animals	treat boils	stomach problems	jaundice	
Sekyere West Disrtict	14	23	1	5	1	5	1	0	0	0	0	0	50
Afigya Sekyere Disrtict	17	21	1	6	0	3	0	1	1	0	0	0	50
Sekyere East District	22	9	0	13	0	0	0	1	4	1	0	0	50
Adansi South District	23	5	0	5	0	1	0	0	0	0	1	0	35
Obuasi Municipal Assembly	6	0	0	1	0	0	0	0	0	0	0	0	7
Adansi North District	14	4	0	11	1	0	3	0	0	0	0	0	33
Offinso District	13	19	0	7	2	2	0	0	0	0	1	0	44
Ahafo Ano North District	11	14	1	3	2	0	0	1	1	0	1	0	34
Atwima Mpoa District Assemble	17	16	0	2	0	0	0	0	1	0	0	0	36
Asante Akim South District	19	2	1	22	1	3	0	0	1	0	0	1	50
Asante Akim North District	15	5	0	5	0	0	0	0	0	0	0	2	27
Ejisu Juaben District	23	2	0	1	0	3	1	0	0	0	0	1	31
Twifo Hemang Lower Denkyire District	38	0	0	5	0	0	0	0	0	0	0	0	43
Upper Denkyira District	17	2	0	5	1	2	0	0	0	0	0	1	28
Total	249	122	4	91	8	19	5	3	8	1	3	5	518

Appendix 2d

Uses of the seed

District	Indicate the folk uses of the seed							Total
	no idear	planting material	vegetable	feed for animals	heart problems	stomach problems	diabetes	
Sekyere West Disrtict	6	42	1	1	0	0	0	50
Afigya Sekyere Disrtict	12	34	0	1	1	1	1	50
Sekyere East District	25	23	0	2	0	0	0	50
Adansi South District	20	14	0	1	0	0	0	35
Obuasi Municipal Assembly	1	6	0	0	0	0	0	7
Adansi North District	21	9	1	1	1	0	0	33
Offinso District	26	17	1	0	0	0	0	44
Ahafo Ano North District	29	3	0	1	1	0	0	34
Atwima Mpoa District Assemble	35	0	0	1	0	0	0	36
Asante Akim South District	48	2	0	0	0	0	0	50
Asante Akim North District	26	0	0	0	0	1	0	27
Ejisu Juaben District	30	1	0	0	0	0	0	31
Twifo Hemang Lower Denkyire District	43	0	0	0	0	0	0	43
Upper Denkyira District	26	0	0	0	2	0	0	28
Total	348	151	3	8	5	2	1	518

Appendix 2e

Uses of the bark

District	Indicate the folk uses of the stem bark										Total
	no idea	malaria treatment	fire wood	treatment of typhoid fever	stomach problems	tooth arch	soup	hernia	sickle cell treatment	blood tonic	
Sekyere West Disrtict	49	1	0	0	0	0	0	0	0	0	50
Afigya Sekyere Disrtict	36	3	3	3	4	1	0	0	0	0	50
Sekyere East District	44	4	0	1	0	0	1	0	0	0	50
Adansi South District	30	1	0	1	1	0	0	2	0	0	35
Obuasi Municipal Assembly	7	0	0	0	0	0	0	0	0	0	7
Adansi North District	27	3	0	0	1	0	1	0	1	0	33
Offinso District	29	11	0	0	2	0	0	0	0	2	44
Ahafo Ano North District	31	0	0	0	2	0	0	0	0	1	34
Atwima Mpoa District Assemble	30	3	0	0	3	0	0	0	0	0	36
Asante Akim South District	46	4	0	0	0	0	0	0	0	0	50
Asante Akim North District	26	0	0	0	1	0	0	0	0	0	27
Ejisu Juaben District	30	1	0	0	0	0	0	0	0	0	31
Twifo Hemang Lower Denkyire District	43	0	0	0	0	0	0	0	0	0	43
Upper Denkyira District	27	0	0	0	0	0	0	0	0	1	28
Total	455	31	3	5	14	1	2	2	1	4	518

Appendix 2f

Uses of the roots

	Indicate the folk uses of the root							Total
District	no idea	for treating cough	treating typhiod fever	medicine for expectant mothers	impotency	enhances walking in children	stomach problems	
Sekyere West Disrtict	49	1	0	0	0	0	0	50
Afigya Sekyere Disrtict	47	0	2	1	0	0	0	50
Sekyere East District	48	0	0	0	1	1	0	50
Adansi South District	34	0	0	0	0	0	1	35
Obuasi Municipal Assembly	7	0	0	0	0	0	0	7
Adansi North District	32	1	0	0	0	0	0	33
Offinso District	43	1	0	0	0	0	0	44
Ahafo Ano North District	33	1	0	0	0	0	0	34
Atwima Mpoa District Assemble	36	0	0	0	0	0	0	36
Asante Akim South District	50	0	0	0	0	0	0	50
Asante Akim North District	27	0	0	0	0	0	0	27
Ejisu Juaben District	31	0	0	0	0	0	0	31
Twifo Hemang Lower Denkyire District	43	0	0	0	0	0	0	43
Upper Denkyira District	28	0	0	0	0	0	0	28
Total	508	4	2	1	1	1	1	518

Appendix 2g

Uses of the wood

District	Indicate the folk uses of the wood			Total
	no idea	Fire wood	Furniture	
Sekyere West Disrtict	0	50	0	50
Afigya Sekyere Disrtict	3	47	0	50
Sekyere East District	2	48	0	50
Adansi South District	10	25	0	35
Obuasi Municipal Assembly	3	4	0	7
Adansi North District	2	30	1	33
Offinso District	10	34	0	44
Ahafo Ano North District	1	33	0	34
Atwima Mpoa District Assemble	3	33	0	36
Asante Akim South District	4	46	0	50
Asante Akim North District	0	27	0	27
Ejisu Juaben District	1	30	0	31
Twifo Hemang Lower Denkyire District	0	43	0	43
Upper Denkyira District	4	24	0	28
Total	43	474	1	518

Appendix 3

Field guide to morphological studies

UNIVERSITY OF CAPE COAST
DEPARTMENT OF MOLECULAR BIOLOGY AND BIOTECHNOLOGY
Genetic Diversity of Avocado (*Persea americana* Mill)

The research will help identify the specific cultivars of Avocado of economic importance in the Ashanti and Central Regions of Ghana. It will also identify some morphological characters of taxonomic importance for further work on the Avocados in the Regions. It will help throw more light on the cultivars that can do well in these areas to facilitate commercial production of the plant in the country. The natural habitat of the various varieties and where they could be cultivated for the highest yield will also be identified. The study will also relate to the economic importance of the plant to the indigenes.

NB: Information provided will be treated confidential.

1. Personal Information
1.1 Date:……
1.2 Respondent Number:…….
1.3 Collection Number:……
1.4 Locality: Region…..…....District…..……Town/ Village…...……
1.5 Collecting source: Wild habitat Farm land Backyard

2. Morphological Characters
2.1 Tree characteristics
2.1.1 Tree vigour: 3 Weak 5 Intermediate 7 Strong
2.1.2 Tree spread (m^2)
2.1.3 Tree height (m) : 1) 1-4m 2) 5-8m 3) 9-12m 4) 13-16m 5) More than 16m
2.1.4 Tree shape
2.1.5 Trunk surface: 3 smooth 7 Rough 9 Very rough
2.1.6 Trunk circumference (cm) 30cm above ground level
2.1.7 Branching pattern 1 Extensive 2 Intensive 3 Both pattern
2.1.8 Distribution of branches
2.1.9 Crotch angle of main branches

2.2 Leaf characteristics
2.2.1 Leaf shape
2.2.2 Leaf base shape
2.2.3 Leaf blade length (cm) average of 10 leaves
2.2.4 Colour of mature leaves

2.2.5 Crotch angle of leave petiole
2.2.6 Leaf margin
2.2.7 Number of primary veins
2.2.8 Primary leaf vein divergence to the main vein (^{o})
2.2.9 Leaf apex shape
2.2.10 Leaf texture

2. 3 Fruit characteristics

2.3.1 Fruiting habit: 1 Single isolated fruit 2 Clusters
2.3.2 Fruit shape
2.3.3 Fruit length (cm)
2.3.4 Fruit diameter (cm) average of 5 fruits
2.3.5 Fruit weight (g)
2.3.6 Fruit base shape
2.3.7 Fruit apex shape
2.3.8 Fruit apex position
2.3.9 Ridges on fruit
2.3.10 Pedicel position on fruit
2.3.11 Pedicel shape
2.3.12 Nalhead pedicel apex shape
2.3.13 Peduncle length (cm)
2.3.14 Peduncle diameter (mm)
2.3.15 Pedicel length (cm)
2.3.16 Fruit skin surface 3 Smooth 5 Intermediate 7 Rough
2.3.17 Fruit skin colour (ripe fruit)
2.3.18 Fruit skin thickness (average of 5 fruits)
2.3.19 Adherence of skin to flesh 3 Slightly 5 Intermediate 7 Strong
2.3.20 Flesh texture
2.3.21 Sweetness of flesh
2.3.22 Bitterness of flesh 3 low 5 Intermediate 7 High

2.4 Seed characteristics

2.4.1 Seed shape
2.4.2 Seed weight (g)
2.4.3 Cotyledon surface 3 Smooth 5 Intermediate 7 Rough
2.4.4 Attachment of cotyledons 0 Not attached 1 Attached
2.4.5 Length of seed cavity (cm)
2.4.6 Diameter of seed cavity (cm)
2.4.7 Length of seed (cm)
2.4.8 Diameter of seed (cm)
2.4.9 Free space of the seed cavity

Source: Descriptors for Avocado (*Persea spp.)* by International Plant Genetic Resources Institute

Appendix 4

Geographical data on plants for morphological and genetic analysis

Sample	Region	District	Town	Longitude	Latitude	Elevation (Ft)
1	Ashanti	Sekyere West	Nintin	06^0 59.576'	001^0 26.262'	1592
2	Ashanti	Sekyere West	Nintin	06^0 59.580'	001^0 26.252'	1567
3	Ashanti	Sekyere West	Nintin	06^0 59.997'	001^0 26.416'	1566
4	Ashanti	Sekyere West	Nintin	07^0 00.014'	001^0 26.538'	1584
5	Ashanti	Sekyere West	Nintin	07^0 00.240'	001^0 26.244'	1633
6	Ashanti	Sekyere West	Abonkosu	07^0 00.603'	001^0 23.816'	1548
7	Ashanti	Sekyere West	Abonkosu	07^0 00.605'	001^0 23.812'	1539
8	Ashanti	Sekyere West	Abonkosu	07^0 00.592'	001^0 23.800'	1538
9	Ashanti	Sekyere West	Nkwanta	07^0 07.514'	001^0 23.354'	1260
10	Ashanti	Sekyere West	Nkwanta	07^0 07.487'	001^0 23.918'	1244
11	Ashanti	Sekyere West	Nkwanta	07^0 07.468'	001^0 23.820'	1275
12	Ashanti	Sekyere West	Nkwanta	07^0 07.527'	001^0 23.720	1308
13	Ashanti	Asante Akim North	Hwiediem	06^0 45.477'	001^0 06.046'	1159
14	Ashanti	Asante Akim North	Hwiediem	06^0 45.488'	001^0 06.027'	1136
15	Ashanti	Asante Akim North	Hwiediem	$06^0$45.505'	001^0 06.010'	1117
16	Ashanti	Asante Akim North	Hwiediem	06^0 45.508'	001^0 06.018'	1120
17	Ashanti	Asante Akim North	Hwiediem	06^0 45.507'	001^0 06.039'	1147
18	Ashanti	Asante Akim North	Hwiediem	06^0 45.492'	001^0 06.008'	1100
19	Ashanti	Ejisu Juaben	New Koforidua	06^0 37.589'	001^0 19.245'	1055
20	Ashanti	Ejisu Juaben	New Koforidua	06^0 37.579'	001^0 19.316'	735
21	Ashanti	Ejisu Juaben	New Koforidua	06^0 37.494'	001^0 19.343'	752
22	Ashanti	Ejisu Juaben	New Koforidua	06^0 37.495'	001^0 19.348'	742
23	Ashanti	Ejisu Juaben	New Koforidua	06^0 37.499'	001^0 19.345'	731
24	Ashanti	Ejisu Juaben	New Koforidua	06^0 37.435'	001^0 19.320'	719
25	Ashanti	Ejisu Juaben	New Koforidua	06^0 37.412'	001^0 19.363'	746
26	Ashanti	Ejisu Juaben	New Koforidua	06^0 37.411'	001^0 19.363'	746
27	Ashanti	Ejisu Juaben	Duampompo	06^0 38.407'	001^0 20.743'	727
28	Ashanti	Ejisu Juaben	Duampompo	06^0 38.405'	001^0 20.748'	718
29	Ashanti	Ejisu Juaben	Juaben	06^0 48.536'	001^0 25.822'	1012
30	Ashanti	Sekyere East	Apemso-Asokore	06^0 50.437'	001^0 21.859'	1110
31	Ashanti	Sekyere East	Apemso-Asokore	06^0 50.434'	001^0 .853'2	1127
32	Ashanti	Sekyere East	Apemso-Asokore	06^0 50.429'	001^0 21.891'	1119

Appendix 4 continued

Sample	Region	District	Town	Longitude	Latitude	Elevation (Ft)
33	Ashanti	Sekyere East	Apemso-Asokore	06^0 50.406'	001^0 21.961'	1103
34	Ashanti	Sekyere East	Apemso-Asokore	06^0 50.427'	001^0 22.007'	1109
35	Ashanti	Afigya Sekyere	Bipoah	06^0 57.867'	001^0 29.738'	955
36	Ashanti	Afigya Sekyere	Bipoah	06^0 57.972'	001^0 29.749'	931
37	Ashanti	Afigya Sekyere	Bipoah	06^0 58.036'	001^0 29.627'	929
38	Ashanti	Adansi South	Amponyase Junction	06^0 03.106'	001^0 45.784'	563
39	Ashanti	Adansi South	Finaso Nkwanta	06^0 05.581'	001^0 46.686'	462
40	Ashanti	Obuasi	Obuasi	06^0 12.254'	001^0 40.331'	716
41	Ashanti	Obuasi	Obuasi	06^0 12.265'	001^0 40.332'	705
42	Ashanti	Obuasi	Obuasi	06^0 12.250	001^0 40.267'	759
43	Ashanti	Obuasi	Obuasi	06^0 12.327	001^0 39.843'	772
44	Ashanti	Obuasi	Obuasi	06^0 12.331	001^0 39.843'	799
45	Ashanti	Obuasi	Obuasi	06^0 12.357	001^0 39.832'	768
46	Central	Upper Denkyira	Denyase	05^0 50.850	001^0 47.683'	624
47	Central	Upper Denkyira	Denyase	05^0 50.491'	001^0 47.828'	600
48	Central	Upper Denkyira	Denyase	05^0 50.468'	001^0 47.819'	565
49	Central	Upper Denkyira	Denyase	05^0 50.481'	001^0 45.850'	596
50	Central	Upper Denkyira	Denyase	05^0 50.496'	001^0 47.851'	567
51	Central	Upper Denkyira	Denyase	05^0 50.802'	001^0 47.510'	603
52	Central	Upper Denkyira	Dunkwa	05^0 51.117'	001^0 48.105'	606
53	Central	Upper Denkyira	Dunkwa	05^0 56.952	001^0 48.502	447

Appendix 5

Names of plant samples used for genetic analysis

Sample Label	Name of samples	Region	District	Town
1	As 1	Ashanti	Sekyere West	Nintim
2	As 2	Ashanti	Sekyere West	Nintim
3	As 3	Ashanti	Sekyere West	Nintim
4	As 4	Ashanti	Sekyere West	Nintim
5	As 5	Ashanti	Sekyere West	Nintim
6	As 6	Ashanti	Sekyere West	Abonkosu
7	As 7	Ashanti	Sekyere West	Abonkosu
8	As 8	Ashanti	Sekyere West	Abonkosu
9	As 9	Ashanti	Sekyere West	Nkwanta
10	As 10	Ashanti	Sekyere West	Nkwanta
11	As 11	Ashanti	Sekyere West	Nkwanta
12	As 12	Ashanti	Sekyere West	Nkwanta
13	As 13	Ashanti	Asante Akim North	Hwiediem
14	As 14	Ashanti	Asante Akim North	Hwiediem
15	As 15	Ashanti	Asante Akim North	Hwiediem
16	As 16	Ashanti	Asante Akim North	Hwiediem
17	As 17	Ashanti	Asante Akim North	Hwiediem
18	As 18	Ashanti	Asante Akim North	Hwiediem
19	As 19	Ashanti	Ejisu Juaben	New Koforidua
20	As 20	Ashanti	Ejisu Juaben	New Koforidua
21	As 21	Ashanti	Ejisu Juaben	New Koforidua
22	As 22	Ashanti	Ejisu Juaben	New Koforidua
23	As 23	Ashanti	Ejisu Juaben	New Koforidua
24	As 24	Ashanti	Ejisu Juaben	New Koforidua
25	As 25	Ashanti	Ejisu Juaben	New Koforidua
26	As 26	Ashanti	Ejisu Juaben	New Koforidua
27	As 27	Ashanti	Ejisu Juaben	Duampompo
28	As 28	Ashanti	Ejisu Juaben	Duampompo
29	As 29	Ashanti	Ejisu Juaben	Juaben
30	As 30	Ashanti	Sekyere East	Apemso-Asokore
31	As 31	Ashanti	Sekyere East	Apemso-Asokore
32	As 32	Ashanti	Sekyere East	Apemso-Asokore
33	As 33	Ashanti	Sekyere East	Apemso-Asokore
34	As 34	Ashanti	Sekyere East	Apemso-Asokore
35	As 35	Ashanti	Afigya Sekyere	Bipoah
36	As 36	Ashanti	Afigya Sekyere	Bipoah

Appendix 5 continued

Sample Label	Name of samples	Region	District	Town
37	As 37	Ashanti	Afigya Sekyere	Bipoah
38	As 38	Ashanti	Adansi South	Amponyase Junction
39	As 39	Ashanti	Adansi South	Finaso Nkwanta
40	As 40	Ashanti	Obuasi	Obuasi
41	As 41	Ashanti	Obuasi	Obuasi
42	As 42	Ashanti	Obuasi	Obuasi
43	As 43	Ashanti	Obuasi	Obuasi
44	As 44	Ashanti	Obuasi	Obuasi
45	As 45	Ashanti	Obuasi	Obuasi
46	Cr 46	Central	Upper Denkyira	Denyase
47	Cr 47	Central	Upper Denkyira	Denyase
48	Cr 48	Central	Upper Denkyira	Denyase
49	Cr 49	Central	Upper Denkyira	Denyase
50	Cr 50	Central	Upper Denkyira	Denyase
51	Cr 51	Central	Upper Denkyira	Denyase
52	Cr 52	Central	Upper Denkyira	Dunkwa
53	Cr 53	Central	Upper Denkyira	Dunkwa
54	Fuchia	Eastern	CRIG	Akim Tafo
55	Doni	Eastern	CRIG	Akim Tafo
56	Nabal	Eastern	CRIG	Akim Tafo
57	Butler	Eastern	CRIG	Akim Tafo
58	Borrego	Eastern	CRIG	Akim Tafo
59	Borrego 2	Eastern	CRIG	Akim Tafo
60	Bernercker	Eastern	CRIG	Akim Tafo
61	Bernercker 2	Eastern	CRIG	Akim Tafo
62	Nkan Nkwanta	Eastern	CRIG	Akim Tafo
63	Fuchia 2	Eastern	CRIG	Akim Tafo
64	Tower	Eastern	CRIG	Akim Tafo
65	K'dua	Eastern	CRIG	Akim Tafo
66	Butler WB4-1-12	Eastern	CRIG	Akim Tafo
67	Borego WB4-7-14	Eastern	CRIG	Akim Tafo
68	Loreta WB4-2-15	Eastern	CRIG	Akim Tafo
69	Butler WB4-12	Eastern	CRIG	Akim Tafo
70	Semil 43 WA 2-13-4	Eastern	CRIG	Akim Tafo
71	DWI Bank	Eastern	CRIG	Akim Tafo

Appendix 6

Morphological data

Morphological character	Valid N	Mean	Minimum	Maximum	Std.Dev.
Tree spread (m)	53	8.4338	4.9000	13.1700	1.8511
Tree height (m)	53	3.0189	2.0000	5.0000	0.7203
Trunk surface	53	7.6038	3.0000	9.0000	1.1492
Trunk circumference (cm)	53	133.0434	46.3000	283.1000	46.8193
Branching pattern	53	2.0189	1.0000	3.0000	0.7203
Distribution of branches	53	2.0566	1.0000	5.0000	1.0454
Leaf shape	53	5.7547	1.0000	9.0000	2.7168
Leaf blade length (cm)	53	19.0277	12.9200	28.6400	3.3675
Number of primary veins	53	14.6038	12.0000	18.0000	1.4850
Leaf apex shape	53	4.1509	1.0000	7.0000	1.5114
Fruit shape	53	6.0943	1.0000	9.0000	2.0872
Fruit length (cm)	53	11.0987	7.8000	16.1000	2.1506
Fruit diameter (cm)	53	7.7464	5.5000	9.5500	0.8441
Fruit weight (g)	53	328.7415	179.0000	612.5000	102.1630
Ridges on fruit	53	1.8868	1.0000	3.0000	0.6697
Gloss of fruit skin	53	5.1509	3.0000	7.0000	1.6100
Pedicel position on fruit	53	1.4906	1.0000	2.0000	0.5047
Nailhead pedicel apex shape	53	0.3962	0.0000	1.0000	0.4938
Peduncle length (cm)	53	4.7108	1.5500	12.3000	1.8162
Peduncle diameter (mm)	53	5.9575	4.0000	10.1000	1.2678
Pedicel length (cm)	53	1.1230	0.6800	8.0000	0.9938
Fruit skin surface	53	4.5094	3.0000	7.0000	1.3534
Fruit skin colour (ripe fruit)	53	4.9245	1.0000	9.0000	2.3685
Fruit skin thickness (mm)	53	4.0566	3.0000	7.0000	1.1505
Adherence of skin to flesh	53	3.9057	3.0000	7.0000	1.4447
Colour of fruit next to skin	53	5.2642	3.0000	6.0000	0.5599
Colour of flesh next to seed	53	2.5283	2.0000	3.0000	0.5040
Seed shape	53	6.4717	3.0000	8.0000	1.4088
Seed weight (g)	53	61.5553	25.0000	122.5000	23.6801
Attachment of cotyledon	53	0.3019	0.0000	1.0000	0.4635
Length of seed cavity (cm)	53	6.0853	4.4000	9.0000	1.0708
Diameter of seed (cm)	53	4.9251	3.9700	6.4300	0.5517
Length of seed (cm)	53	5.2155	3.8700	7.7000	0.8091
Diameter of seed (cm)	53	4.5828	3.4300	5.8600	0.5235
Free space of the seed cavity	53	2.3019	1.0000	3.0000	0.5033