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Morphological and Genetic Diversity of *Persea americana* Mill. (Avocado) in two regions of Ghana

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Introduction

Persia americana Mill (Avocado) is a tree crop which originated from the tropics of the western hemisphere and has three general ecological races: Mexican, Guatemalan and West Indian adaptable to a wide range of climatic conditions (Bergh, 1969; Rhodes *et al.*, 1971). Avocado trees grows well in areas with over 150 mm of annual rainfall and between 55 m and 550 m altitude. The tree reaches a height of 9-18 m and trunk diameter between 30 and 60 cm. 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 fruit is pear-shaped and continues to enlarge in size while on the tree; it only ripen completely after it is harvested (RADA, 2013). The edible part of the fruit is a thick layer of greenish-yellow pulp. The oily, greenish-yellow flesh is of the consistency of firm butter and contains good proportions of both oil and proteins (Knight, 2002). The avocado plant has spread very fast to many parts of the world due to its nutritional value, preference for the fruit as food and the use of several of its parts for medicinal purposes (Campbell & Malo, 1976; Morton, 1987; Verheij & Coronel, 1991; FAOSTAT Database, 2001). In Ghana, it is widely grown in the closed forest regions (Irvine, 1961) and has the potential to contribute immensely to the economy if cultivated in large commercial farms as in the case of countries like Mexico, the Dominican Republic, the U.S.A and Brazil (Morton, 1987; FAOSTAT Database, 2001).

Archaeological evidence indicates that utilization and selection of the avocado 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; 1969). Over the past few decades, there have been several attempts to examine the classification of avocado. For example a detailed numerical taxonomic study of avocado cultivars based on morphological traits tended to cluster into three groups, more or less representing the three races (Rhodes *et al.*, 1971). However, avocado is among the least studied fruit crops in Ghana. We therefore studied its distribution, uses, morphological and genetic diversity in the Ashanti and Central regions of Ghana.

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Material and Methods

Ethnobotanical survey

An ethnobotanical survey was carried out in the Ashanti and Central regions of Ghana to determine the socio-economic uses and importance of the avocado plant to the inhabitants of these regions. A total of 14 districts, comprising 12 from the Ashanti region and two from the Central region were selected based on systematic sampling for the surveyed. Five hundred and eighteen respondents were randomly selected from 94 towns in the 14 districts and interviewed with a structured questionnaire.

Morphological studies

Morphological studies of avocado were conducted in eight districts (7 from Ashanti and 1 from Central region) selected randomly from the 14 used for the ethnobotanical studies. Fifty-three randomly selected avocado trees were studied. For each selected tree, data on tree, leaf, fruit, and seed characteristics were taken based on the guide of the International Plant Genetic Resources Institute, 1995.

Genetic data

Genetic materials were sampled from healthy leaves of 71 avocado plants in the Ashanti and Central regions, and 13 avocado plants in the vicinity of the Cocoa Research Institute of Ghana (CRIG) in the Eastern region. The samples from CRIG were standardized varieties from Israel and so were used as control for the genetic work.

Genomic DNA Extraction

The genomic DNA was extracted following a modified cetyltrimethyl ammonium bromide (CTAB) protocol (Aldrich & Cullis, 1993). DNA in each sample was quantified by comparing band intensities to 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. Samples with DNA concentration above 25 ng/µl were further diluted to bring their concentration to 25 ng/µl for polymerase chain reaction (PCR).

Simple Sequence Repeat Markers

Ten pairs of microsatellite primers designed by Sharon *et al.* (1997) were used for the PCR.

PCR amplification and electrophoresis

Amplification was done 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™ PCR PreMix (USA Bioneer Inc., Alameda, USA) The PCR reaction was carried out on a thermal cycler (AB 2720, Applied Biosystems, Singapore) and consisted of the following profile: 3 min denaturation 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°C for 10 min, followed by maintenance of the reaction mixture at 4°C. Samples were stored at -20°C.

Electrophoresis

Electrophoresis was done using 3 µl 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 at 95°C for 5 minutes and then immediately chilled on ice. Each was loaded into a 49-well plate (4 mm thick) for electrophoresis 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, Belgium) and Bio-Rad Power Pac 300 (Bio-Rad, Belgium) and 1 X TBE as running buffer. The products were visualised by silver staining using the method described by Bassam *et al.* (1991).

Data analysis

For each gel, the distance travelled by each marker size of the DNA ladder was measured using a ruler and plotted in a line graph. The equation of the relationship between them was then used to estimate the size of the unknown SSR bands of the PCR products.

Results and Discussion

Ethnobotanical survey

Ethnobotanical studies showed that most of the people involved in avocado farming were above 40 years old (Table 1). This situation most likely contributes to the cultivation of avocados in small scale since farming is largely done manually and the aged may not have enough energy to farm in large scale. However, avocado has the potential to contribute more to the economy if cultivated in large-scale commercial farms (Morton, 1987; FAOSTAT Database, 2001). The indigenes have several uses for avocado (Table 2). They eat the fresh fruits as food or sell them for income, the leaves, seeds, roots and bark are used for treating several medical conditions and the dead wood of avocado is used as fuelwood. The bark is also used as a dye for cloth.

Table 1. Age group and corresponding number of respondents engaged in avocado farming in the Ashanti and Central regions of Ghana

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

GT= Grand total

Besides the fact that more respondents (>82%) who engage in avocado farming were above 40 years, a significant number (36.5%) of them were also above 61 years old.

Growth of avocado was better in the Ashanti region than the Central region. It thrived best in old cocoa farms and was cultivated on small scale. The plant was used for various medicinal and economic purposes.

Indigenous use of avocado

Table 2. Avocado tree parts and their uses in the Ashanti and Central regions of Ghana

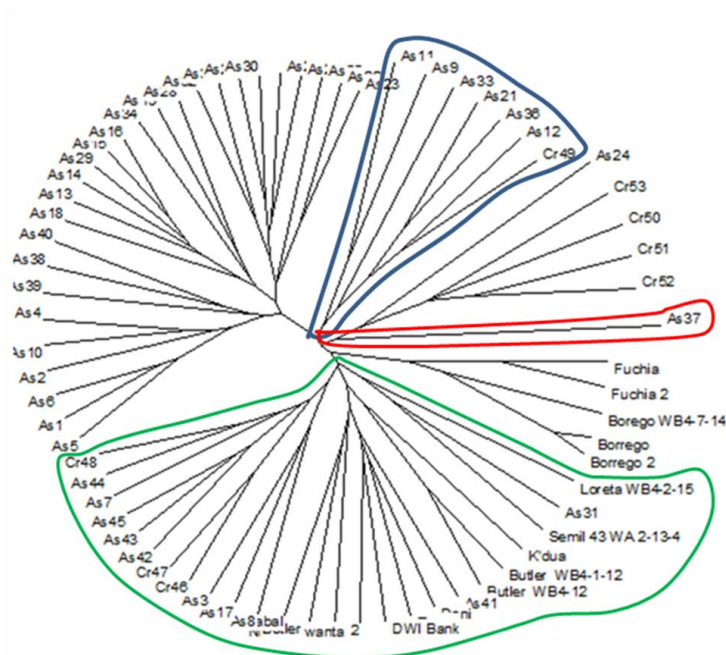
Avocado tree part	Most important use	%
Fruits	Food	91
Leaves	Medicinal	27
Seeds	Planting material	29
Roots	Medicinal	2
Bark	Medicinal	12
Wood	Fuel	91.5

Morphological analysis

Morphologically, the avocados studied were mainly of Western Indian origin. However, accessions from the Ashanti region were more diverse in plant and fruit characters than those from Central region.

Phylogenetic analyses

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 microsatellites were highly informative with both genetic diversity and polymorphic informative content higher than 0.5. Using the unweighted pair group method with arithmetic averages, the genotypes were clustered into six different groups (Figure 1). The genetic diversity analysis shows that the SSRs used were highly polymorphic in structure. A total of 180 genotypes and 115 alleles were detected. There is a wide range of diversity between the accessions; which might have resulted from cross pollination and genetic mutations.



The clusters were obtained from the phylogenetic tree of the accessions using differences in allele sizes of the SSRs associated with some avocado traits. They were found to cluster with those of same parental lines. 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. This 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 Ghana. The phylogenetic tree suggests a wide genetic variation among the accessions genotyped.

Figure 1: Unweighted Paired Group Method of avocado genotypes using 10 microsatellite markers

Conclusion

Our study has revealed that most of the people involved in avocado farming are above 40 years old and could be the reason for small scale avocado farming in the two regions we studied. There is therefore the need to encourage more younger people to engage in avocado farming which could subsequently lead to large scale avocado farming. The avocado plant is very useful to the people living in the Ashanti and Central regions of Ghana as they use it for several purposes. Our study has highlighted the distribution, uses, morphological and genetic diversity of avocado in the Ashanti and Central regions of Ghana and has opened the way for more research on this important crop in Ghana. The wide genetic diversity among the accessions indicates a wide genetic base for improvement of avocado through breeding and selection in Ghana.

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