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# MOLECULAR APPROACHES FOR THE CHARACTERISATION OF GHANAIAN AVOCADO PEAR (*PERSEA AMERICANA* MILL.) GERMPLASM.

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## ABSTRACT

In forested regions of southern Ghana many edible forms of avocado pear exist, but offer different fruit and fruiting quality characteristics. This is the result of unregulated seed-based propagation, use of volunteer seedlings as planting materials by farmers and germplasm introductions from a variety of sources, including the USA by the United States Agency for International Development (USAID). It is believed that West Indian, Mexican and Guatemalan races of avocado all co-exist in Ghana.

To date, no studies have been undertaken to characterise those races or their hybrids in Ghana. Some of the key determining morphological characteristics for those ecological races are outlined here.

Research currently being carried out at the University of Nottingham, UK is investigating the use of molecular markers (AFLPs) to characterise and delineate the avocado pear germplasm currently grown in Ghana.

**Key Words:** volunteer seedlings, ecological races, molecular methods, AFLPs, avocado pear.

## AVOCADO IN GHANA

Ghana is located on West Africa's Gulf of Guinea and the southern regions of the country experience a bimodal rainfall regime typified by a tropical rainforest belt, turning to coastal savannah vegetation on the Accra Plains. The northern part of the country has a single peak rainfall thus

generating a Guinea savannah eco-zone. Avocado pear is now widespread in the forest regions of Ghana (Figure 1) but there is no formal record or time frame of its introduction into the country. The first recorded planting of avocado pear was at Aburi (near Accra) in 1870, with peasant avocado pear production being recognised by 1907 (Anon, 1961). Large-scale commercial cultivation is, however, still the exception and there are no certified avocado nurseries to supply the growers with grafted material. Avocado pear growers rely on seed-propagated plants and volunteer seedlings as planting materials, the latter often infected with pests, such as the giant looper, and diseases, such as *Phytophthora* root-rot. Although avocado pear is predominantly found in the forested regions in southern Ghana (Figure 1), where the climate, soils and vegetation are optimal for cultivation, it is regarded as an important crop economically throughout the country.

## AVOCADO RACES

The complex flowering of avocado, combined with the fact that there is little or no information on the origin of germplasm, has resulted in a large pool of genetically divergent material giving variations in fruit shape, size, flesh quality and peel characteristics. The peel characteristics of the fruit can be used as a basis for morphological characterisation of the three known races (Figure 2). Avocado races may be characterised by peel that is (i) medium or thin in thickness, smooth and either leathery or brittle as found in the West Indian race (Figure 2a), (ii) thin, smooth and either leathery or brittle as found in the Mexican race (Figure 2b) and (iii) thick, pebbled and either leathery or brittle as found in the Guatemalan race (Figure 2c). Mature unripe fruits that are green will ripen yet maintain their green peel colour, however, those that are predominantly green but with some anthocyanin pigmentation ripen fully to a deep purple or dark green /purple. The shape of the fruit ranges from ellipsoid through pyriform and obovate to spheriod. The flesh of the fruit (meat) ranges from watery, through fibrous (both undesirable), and dry (desirable) to buttery (most desirable).

## MOLECULAR CHARACTERISATION OF AVOCADO

Amplified fragment length polymorphism (AFLP), a polymerase chain reaction (PCR) based-fingerprinting technology (Vos *et al.*, 1995) is being developed to characterise avocado pear germplasm. This technology is seen as suitable due to:

- The availability of several restriction enzymes and corresponding primer combinations providing flexibility to facilitate the direct manipulation of AFLP fragments for defined applications, such as genetic mapping.
- It provides appropriate performance in terms of reproducibility, resolution and time efficiency.
- It is sensitive enough for the detection of polymorphisms at the total-genomic level.
- AFLP technology does not require sequence information or probe collections prior to the gene rations of AFLP fingerprints.

Air-dried avocado leaves will be used for the characterisation, and the procedure involves:

- Isolation and purification of genomic avocado DNA. Air-dried avocado leaves from selected fruit bearing trees in Ghana are being used for DNA isolation, following modifications to the proto col of Dellaporte *et al.* (1983). A protocol for air-drying avocado leaves is being developed to assess any effects on the DNA isolation and purity (Figure 3).
- Digestion with Eco RI and MseI restriction enzymes.

- Ligation with Eco RI and MseI adaptors at the restricted ends.
- Preselective amplification with Eco RI and MseI primers
- Selective amplification
- Generation of DNA fingerprints and cluster analysis (ABI Prism Genotyper) to group avocado pear present in Ghana into races.

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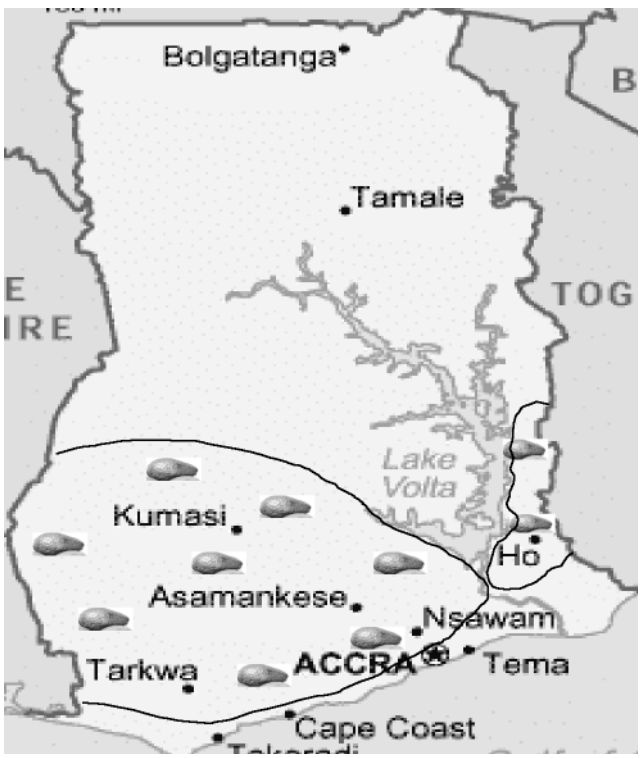


**Figure 1.** Avocado growing areas in Ghana.

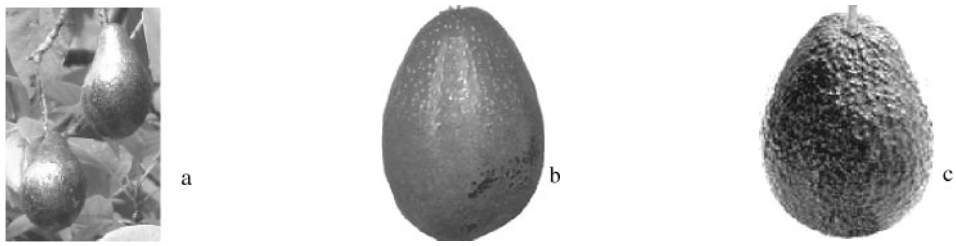


**Figure 2.** Fruits of three races of avocado. (a) West Indian (b) Mexican (c) Guatemalan source: http://www.ucavo.ucr.edu/ (Accessed 13/05/03)

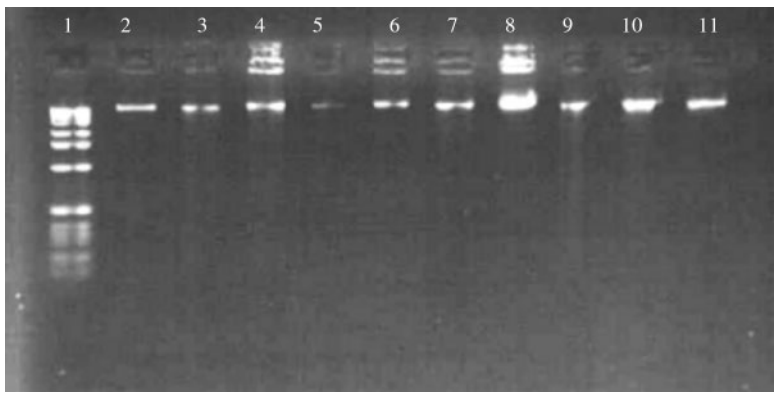


**Figure 3.** Gel electrophoresis of avocado DNA extracted from leaves of seedlings (cultivar Hass) raised in a controlled environment room.
Lane 1 represents 1 kb ladder. With the exception of lanes 10 and 11, which represent freeze-dried avocado leaves from seedling number 4, all other lanes are in pairs and represent fresh and dried leaves respectively from the following seedlings: lanes 2 & 3 from seedling number 1; lanes 4 & 5 from seedling number 2; lanes 6 & 7 from seedling number 5; and lanes 8 & 9 from seedling number 18.