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Defining Genetic Diversity in the Chocolate Tree, *Theobroma cacao* L. Grown in West and Central Africa

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1. Introduction

The cacao tree, *Theobroma cacao* L., a diploid fruit tree species (2n = 20) is the source of dried cocoa beans used as the main raw material in the manufacture of chocolate, confectioneries and some cosmetics product. Although native to the humid tropical regions of the northern parts of South America and the northern parts of Central America (Bartley 2005; Cheesman 1944; Cuatrecasas 1964, Motamayor 2008), the largest cultivation of cacao, an under-storey forest tree species takes place in West and Central Africa. Originally designated a member of the Sterculiaceae family (Purseglove, 1974), *Theobroma cacao* was recently re-classified into the Malvaceae plant family (Alverson et al., 1999). Since its first introduction in the early 19th century by the Portuguese and the Spaniards, the West and Central African region has become the largest producer accounting for some 70% of the world's cocoa output (Figure 1) of more than 3.632 million metric tons (ICCO, 2010). The main producing countries are Cote d'Ivoire (43 % of global production), Ghana (14 %), Nigeria (6 %) and Cameroon (5 %) followed by Togo, Gabon, Sao Tome, Equatorial Guniea, Sierra Leone, Congo and Liberia. The major market for cocoa beans include The Netherlands, United States of America, United Kingdom, France, Germany, Spain, Italy, Japan, China and India.

With the aim of securing the future of the world's cocoa economy, and avoid the situation in the 1930s when, due to limited genetic variability in cacao collections, the swollen shoot virus disease almost destroyed the industry, the genetic structure of cacao collections in West Africa was determined using microsatellite markers.

1.1 Economic importance

1.1.1 In consuming countries

The importance of cocoa beans in the running of the multi-billion dollar annual earning chocolate and confectionery industries cannot be over-emphasised. The world grinding of cocoa beans in 2010/2011 season alone was estimated at 3.698 million metric tonnes (ICCO 2010). The world's exports amount to some US$5–6 billion/year and use of cocoa and cocoa butter in chocolate manufacturing, cosmetics, and other cocoa products drive approximately

US$70 billion market and provides over 60,000 jobs in the US alone (Guiltinan 2007). In an annual list of the top 100 global confectionery companies based on net sales in 2010 alone (Table 1), the top ten chocolate confectionery companies accounted for at least US$ 67.59 billion (Candy International, 2011). According to the Association of chocolate, biscuit and confectionery industries of Europe (CAOBISCO) based in Brussels, some 1800 companies with 245,000 direct employees are involved in use of cocoa beans in manufacturing of their products. These industries account for more than 47.8 billion Euros annual turnover, a production of 14.1 million tonnes of products and some 4.1 billion Euros of exports, that is, 10% of the total value of food exports from the European Union. The European chocolate and confectioneries industry which utilizes 50% of the world production of cocoa beans also consume some 30% of the European production of sugar, 35% skimmed milk powder at full EU price as well as a large share of the glucose, butter, wheat, eggs and dried fruit produced in the European Union.

1.1.2 In West and Central African producing countries

Cocoa production is predominantly a smallholders' enterprise in Africa with several hundred-thousand families depending on this cash crop for their livelihood and significant foreign exchange earnings for producing countries (Rice and Greenberg, 2000, Motamayor et al., 2008). Revenue derived from sale and export of crops such as cocoa provides crucial support to livelihoods of farmers in developing countries in Africa and can be a sustainable means of helping millions of households live above poverty and hunger. In West and Central African countries, domestic economies revolve around subsistence agriculture, especially from the sale of products from cash crops such as cocoa. Even in countries such as Nigeria where most of budgetary revenues come from sale of crude oil, revenue from export of cocoa beans makes significant contribution to the nations' gross domestic product (GDP). The economic growth of many of the Least Developed Countries is closely
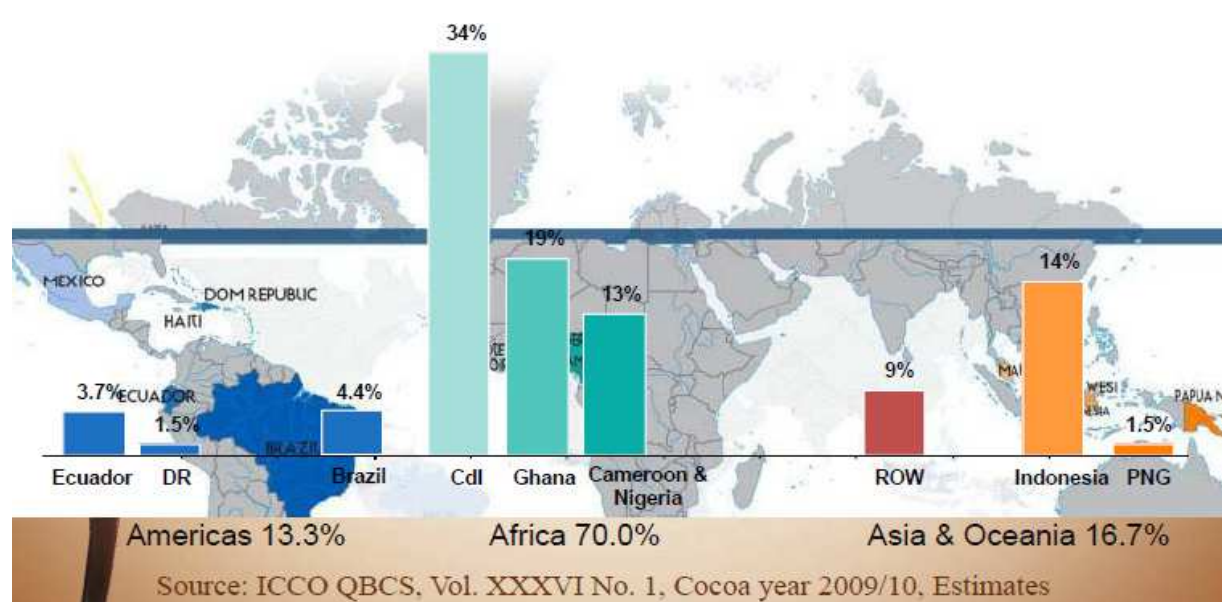

Fig. 1. World's cocoa production estimates (in percentages) according to region and countries provided by International Cocoa Organization (ICCO) for 2009/2010 crop year.

	Company	Country	Net Sales 2010 (US$ millions)
1	Kraft Foods Inc	USA	16,825
2	Mars Inc	USA	15,000
3	Nestlé SA	Switzerland	11,265
4	Ferrero Group	Italy	8,763
5	Hershey Foods Corp	USA	5,703
6	Chocoladefabriken Lindt & Sprüngli AG	Switzerland	2,602
7	Yildiz Holding	Turkey	2,180
8	August Storck KG	Germany	2,000
9	Arcor Group	Argentina	1,650
10	Meiji Holdings	Japan	1,599

Table 1. List of the top ten global confectionery companies that manufacture some form of chocolate by net confectionery sales value in 2010 (Reference: *Candy Industry*, January 2011)

linked with cocoa production, as well as other primary commodities. Many producer countries depend on cocoa exports for a large part of their foreign exchange earnings and government revenue. In Cote d'Ivoire, the largest world producer with more than 1.3 million metric tonnes (mT), cocoa contributes more than 20% of government revenue. When international cocoa prices are low, governments have difficulties meeting debt service obligations and are unable to make much needed investments in basic health, education and infrastructure. In Cote d'Ivoire, more than three of some six million people are engaged in the cocoa sector are small scale farmers. Cocoa alone makes 35 *per cent* of total export estimated at US$10.25 billion in 2010 and 15 % of the 28.2 % agriculture's contribution to the GDP estimated at US$22.82 billion in 2010 (http://www.indexmundi.com/cote_d_ivoire.html). Together with coffee, cocoa is referred to as the 'Green Gold" because of its immense contribution to the economy. It provides job for 60 % of working population and accounts for some 46 % of total export, more than a third of the nation's GDP (http://www.new-ag.info/en/country/profile.php?a=891). In Ghana, the second largest producer with more than 700,000 mT, cocoa is the primary cash crop providing about one-third of all export revenue. With higher commodity prices, gold and cocoa were the two top export revenue earning sectors for Ghana where GDP was estimated at US$38.24 billion in 2010. Cocoa remains the mainstay of Ghana's economy accounting for 40 % of agricultural exports and 12 % of country's GDP (http://www.theodora.com/wfbcurrent/ghana-economy.html). In 2007 for instance, cocoa contributed 35 % of Ghana's GDP and 60 % of employment in agriculture (Centre for the Studies of African Economies (CSAE), 2009). In spite of commencement of oil production in Ghana, agriculture, especially the cocoa sector would remain the key to rural transformation of the economy. In Nigeria, cocoa provides means of livelihood to more than five million people. Although heavily dependent on oil, agriculture contributes significantly to the economy with about 70 % of the population engaged in agriculture. The cocoa sector accounts for some 27 % of the 41.48 % of GDP attributed to agriculture. Cocoa is the single largest non-oil export earning commodity for Nigeria. In comparison with other agricultural commodities, cocoa makes the largest non-oil contribution to the nation's economic development and accounted for 65% of total agricultural export in 2004 (Aikpokpodion, 2007). In Cameroon, it is estimated that some four million people depend on cocoa and coffee for their livelihood. Most of the

cocoa is produced primarily in central southern Cameroon by millions of small scale farmers. In Cameroon, cocoa cultivation is currently one of the major sources of revenues of rural households (1 to 2 millions of people) of the forest agro-ecological zones in the country (South and South-Western parts). Cocoa is grown in more than 200 000 farms and the total cocoa growing surface is estimated to be 400 000 hectares (Efombagn et. al., 2006). In other countries such as Togo, Sierra Leone, Liberia, Equatorial Guinea, Sao Tome and Principe, Gabon and Democratic Republic of Congo, cocoa production makes significant agricultural contribution to the GDP.

2. Cacao introduction history and genetic materials

Traditionally, cacao types cultivated are subdivided into three major '*genetic*' groups: Forastero, Criollo (domesticated by the Amerindians in Central America), and Trinitario (hybrids between Forastero and Criollo, originating from Trinidad). While the Forastero trees are vigorous and more resistant to diseases, the Criollo trees are poor yielding and highly susceptible, although Criollo trees produce high premium quality beans with aromatic flavor. The Brazilian cacao of the Amelonado type (Lower Amazon Forastero) was first introduced by the Portuguese into Principe around 1822, and reached Sao Tomé in the 1850s (Bartley 2005). According to Nosti, quoted by Toxopeus (1964), it was from this collection that the Spaniards brought cacao into the Island of Fernando Po (now Bioko), Equatorial Guinea in 1854. This collection in Fernando Po became the major source of cacao introduced into mainland West Africa at several times by many persons including traders and migrant workers, agencies, missionaries among others. Available records showed that cacao was introduced from Fernando Po by workers and traders like Squiss Ibaningo into Nigeria in 1874, Tetteh Quarshie into Ghana in 1878, and Cote d'Ivoire in 1879 (Edwin and Masters 2005; N'Goran et al. 1992; Opeke 1969). Missionaries like the Basel missionaries, Royal Botanical Garden curators, colonial administrations played significant roles in the introduction of cacao types from different origins into the mainland West and Central Africa. These earlier introductions from Fernando Po formed the initial basis of cacao grown in West Africa, and was referred to as the "West African Amelonado". During the late nineteenth century, the Colonial administration also introduced some red-podded cacao materials from British West Indies into botanical gardens established in Aburi (Ghana) and Lagos (Nigeria) (Toxopeus 1964). By 1910, Ghana, followed by Nigeria, had become one of the largest producing countries, thus making the West Africa sub-region an important growing area critical to the sustainability of the world's cocoa economy, a status it still maintains today.

The introduction of cacao germplasm into island and mainland of Africa took place in response to two main waves of idea which naturally divided cacao germplasm introduction into: 1. Exploratory Colonial Period (1822 – 1909), and, 2. Expansionary Experimental Pre- and Post-Independence Period (1910 – 2010). During the first era which spanned early 19th to the end of the 19th century, cocoa seeds and plants were transported in barrels and shipments across the sea from the northern parts of Southern America and Central America to Africa. This was in response to the then imbibed and appreciated Aztec and Mayan culture of drinking '*chocolatl*', 'The Food of the Gods". The favourable similar tropical humid climatic conditions and abundant rainforest vegetation provided impetus for this transatlantic exploratory introduction which fortunately gave good results. The cultivation

of cocoa was therefore successfully established along the rainforest belt of West and Central African countries with significant economic revolution for both producing countries and chocolate lovers especially in the North. A timeline of earlier germplasm introduction efforts during the first era is presented in Table 2 below. The first materials introduced were of Amazonian origin, unlike the Criollo varieties introduced to Asiatic and Oceanic regions. The germplasm established on the island of Principe in 1822 was the main basis of the cocoa industry on the island. Original planting was said to have consisted of 30 plants which most likely were taken from a single fruit (pod). Progenies of these trees provided seeds for planting other areas of the island (Bartley 2005). Timeline of earliest cacao introduction into island and mainland West and Central Africa in the 19th and early 20th century

Date	Germplasm material & description	Origin	Remarks
Island of Sao Tome & Principe			
1822	Amazonian Bahia 'Comum' or 'Amelonado'	Brazil: States of Bahia, Espirito or Rio de Janeiro	Self-compatible and homozygous variety. Only 30 plants presumably from a single fruit (pod) was established on the island of Principe.
1840	-	-	First export of 'sizeable amount' of cocoa beans from the island of Principe.
1850	Amazonian Bahia 'Comum' or 'Amelonado' later called Sao Tome 'Creoulo'	Principe	Cocoa cultivation began in Sao Tome with seeds taken from the 'Amelonado' plantings in Principe.
1880	Non-Amazonian 'other' varieties: 1. Red fruits with smooth surfaces ('Criollo') known as Venezuela 'Caracas' 2. Narrow elongate fruits with pronounced ridges 3. 'Guayaquil' variety type 4. Very large red fruits, slightly ridged and smooth surface 5. Green fruits 6. Hybrids formed from the different types 7. 'Laranja' mutant	Ecuador, Trinidad and Venezuela	Period of active expansion of cultivation, Hybrids evolved from among the different varieties and between the non-Amazonian varieties and original 'Comum' types.

Equatorial Guinea (Fernando Po)

1854	Sao Tome 'Creoulo' and later, the 1880's Non-Amazonian 'other' varieties.	Sao Tome	Establishment of cocoa in Fernando Po (now Bioko) with seeds from Sao Tome

Cameroon

1876	1. Unknown materials likely from Trinidad.	Royal Botanic Gardens, England	13 plants shipment by a British missionary on Cameroon Mountains.
	2. Sao Tome 'varieties'	Sao Tome	Introduction by Preuss, Curator of Victoria Botanic Garden. Some of these materials became known as 'Victoria-Kakao' variety of Sao Tome
1895?	332 plants including 'Forastero'	Trinidad	Gosselin (1895), Preuss (1901)
1900	Several varieties including 'Forastero', 'Criollo', 'Puerto-Cabello', 'Venezuela', 'Maracaibo', 'Guayaquil', 'La Guira', 'Soconusco', 'Suriname' and 'Nueva Grenada' from Colombia	South and Central America (Trinidad)	Introduction by Preuss. At the beginning of the 20th century, Cameroon had established the most diverse collection of cacao varieties (Bartley, 2005)

Ghana

1857	Seeds from Suriname	Surinam	Unsuccessful attempt by the Basel Missionaries.
1861	'Amelonado'	Sao Tome & Principe	Partial success by the Basel Missionaries.
1878? 1887	'Amelonado'	Fernando Po/Sao Tome	Tetteh Quarshie brought seeds from Fernando Po. Governor Griffiths also made introduction in 1887.
1900 - 1901	1. 'Cundeamour', 'Pentagonum' from Nicaragua	Trinidad	During this period, introduction was to obtain varieties higher in quality than the widely grown 'Amelonado' from Fernando Po.
	2. 'Red Forastero', 'Criollo', 'White variety' of 'Caracas' type from Jamaica	Royal Botanic Garden, England	
1903	'Ocumare', 'Trinidad	Trinidad	Introduced plants were

	Criollo', Nicaraguan Criollo', 'Red Criollo' and 'Yellow Criollo'		established in Aburi Botanic Garden. These materials were largely self-incompatible. These are the likely ancestors of red fruited trees in Ghana.
Nigeria	*Regional differences in genetic composition of populations likely due to different routes of introduction*		
	A. *The Niger Delta Protectorate covering Midwestern to Calabar Region*		
1874	'Amelonado', Sao Tome 'Creuolo'	Fernado Po	Chief Squiss Ibaningo, a migrant worker introduced cocoa into Bonny, now in River State. This variety constituted virtually all the planting material cultivated at the middle of the 20th century.
1899	Unknown varieties. Forastero?	Royal Botanic Garden, England	Up to two shipments of cocoa plants
1900	'Pentagonum' variety	Trinidad?	Variety found at Old Calabar Station
1905	Non-Amelonado 'types'	Trinidad? SaoTome?	These were brought into Old calabar Station
1909	60 fruits of 'Forastero' type	Trinidad	Barrel shipment from Trinidad
	B. *The Lagos Colony*		
1880?	Sao Tome 'Comum' (Amelonado)	Fernando Po	First planting of cocoa in Lagos of some 1,500 plants. The plantation near Agege was owned by JPL Davies (Webster 1964)
1877-1888	Unknown.	Fernando Po Trinidad	Most likely non-Amelonado plants sent to Ceylon
Cote D'Ívoire			
1880	Amelonado	Fernando Po	First cocoa introduction
Sierra Leone			
Pre-1900	Sao Tome 'Comum' (Amelonado); Cameroon variety, Victoria-Kakao	SaoTome, Fernando Po and Cameroon	Several Sierra Leoneans were influential farmers in Fernando Po at the end of the 19th century.
1902	- 60 plants of 'Ceylon Red',	Trinidad	

	Nicaraguan Criollo', 'Forastero' and *T. pentagonum*		
	- 1000 seeds of unknown variety	Fernando Po	
Liberia 1861?	Sao Tome 'Comum' (Amelonado)	Sao Tome	Many Liberians were contract workers in Sao Tome.

Table 2.

(Bartley 2005). By 1840, some quantities of cocoa was exported from the island. The cultivation of cocoa spread to the main island of Sao Tome in the 1850s. The variety which became known as Sao Tome "Creoulo", was self-compatible and homozygous and mostly related to the 'Comum'variety in Bahia, Brazil. This variety was taken both directly and indirectly through Fernando Po to other inland West and central African countries and became the basis of cocoa grown there. However, some other varieties were also introduced into Sao Tome from Ecuador, Trinidad and Venezuela in 1880. Consequently, the bulk of cocoa grown on farmers' plantation must have consisted of a mixture of these earlier varieties, but due to differential expression of self-incompatibility systems, the self-compatible 'West African Amelonado' types must have dominated in the complex mixture of cacao of diverse origin at the beginning of the 20th century.

During the second era which began at about the end of the 19th century and beginning of the 20th century, economic considerations for higher income and premium due to greater yields and higher bean and chocolate quality were the main reasons for germplasm introduction. Previously selected individuals (clones) rather than 'types' showing potentials for high yields, resistance or tolerance to pests, diseases and abiotic stress such as drought were introduced and engaged in cultivar development processes on experimental stations. During this last decade, however, the "People, Planet and Profit'' concept of *Sustainability* has become a significant factor in cacao germplasm introduction. This has bearing with the concept of "Preventive Breeding" where clones showing resistance to regionally important diseases of cocoa growing regions could be introduced through international intermediate quarantine centers. This was to ensure that in the unlikely case of disease spread, for example, witches broom from South America to Africa, there is present in the African germplasm collections, sources of resistance to cope with the new disease in order not to paralyze the local cocoa economy as is the case during any outbreak.

Since the first successful introduction of '*Amelonado*' cacao, Lower Amazon Forastero type into West Africa in the late 19th century, there has been series of additional germplasm introductions as reviewed by (Bartley, 2005; Aikpokpodion, 2009). In Nigeria for instance, since formal selection and germplasm conservation programs around 1931 at the Nigerian Department of Agriculture in Moor Plantation, Ibadan there has been. Further germplasm introduction of Trinitario and Criollo selections from Trinidad and Ceylon (now Sri Lanka) (Jacobs et al., 1971). The British West African Colonial Administration established the West African Cocoa Research Institute (WACRI) in 1938 with headquarters in Tafo, Ghana and a mandate covering Gold Coast (Ghana), SierraLeone, Nigeria and Liberia. Several materials

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belonging to Upper Amazon Forastero and Trinitario populations were introduced from Trinidad by WACRI in 1944 (Toxopeus, 1964). Efforts to increase genetic variability in the base population in response to outbreaks of disease epidemic had provided impetus for germplasm introduction into Africa. For instance, the outbreak of cocoa swollen shoot disease in the 1930s in Ghana, Togo, and Nigeria almost destroyed the cocoa industry due to insufficient genetic variability in the base population. Consequently, new introductions were made in 1944 from Upper Amazon Forastero materials collected by F. J. Pound into the West African Cocoa Research Institute headquarters in Tafo, Ghana and Ibadan in Nigeria (Aikpokpodion et al., 2009). Due to the precocity of these materials, they were widely distributed for replanting of cut out plantations and by late 1950s, some 11 selected Upper Amazon types have been used to produce second and third generations of Amazon known as "F₃ Amazon" or "Mixed Amazon" distributed to farmers (Knight and Rogers 1955). By 1961, some 60,000 ha in Ghana and an estimated 21 million seedlings had been distributed by the government of the Western Region to plant some 9,500 ha in Nigeria (Aikpokpodion 2009). Several hybrid varieties involving crosses with local Amelonado, Trinitario, and some Criollo materials were also developed from these materials in Ghana (Lockwood and Gyamfi 1979), Nigeria (Atanda and Jacobs 1974), and Cote d'Ivoire (Besse 1975; N'Goran et al. 1992).

3. Concept and relevance of genetic diversity

Although all members of a species have certain traits in common, individual members may vary significantly. While some of these may be environmental, a significant proportion is genetic. Genetic diversity is of fundamental importance in the continuity of a species as it provides the necessary adaptation to the prevailing biotic and abiotic environmental conditions, and enables change in the genetic composition to cope with changes in the environment. The first requisite study in the survival of a species is knowledge about the level of genetic diversity (Van Delden, 1992). This refers to the determination of the number of polymorphic loci, the number of alleles, genetic architecture and spatial distribution of genetic variants. Genetic diversity in a population is preserved when the population is in Hardy Weinberg equilibrium. This means that genotypes are present in expected proportions based on the allele frequencies in the population. However, one or two of the conditions for this may not hold. For instance, introduction of genetic variants through mutation or gene flow from a genetically different population will increase the genetic diversity. Assortative mating will mainly affect genotypic proportions. Directional selection against recessives will eliminate alternative alleles and make the population monomorphic at a particular locus. As a principle, balancing selection in favour of heterozygotes will preserve genetic diversity, while inbreeding and genetic drift, on the other hand, lead to loss of genetic diversity.

4. Utility of genetic markers in defining genetic diversity in cacao

Genetic markers are inherited variations that can be used to understand genetic events. These include any gene or other DNA variations that are useful for explaining observed genetic event in a population of interest. The use of genetic markers has been useful in the study of population genetics and evolution by providing methods for detecting genetic

differences among individuals. The majority of genetic markers are variations in DNA at sites that may or may not be part of a functional gene. However, their transmission to offspring follows Mendelian rules for inheritance. There are three main properties of a genetic marker: It must be locus-specific, polymorphic in the studied population and easily genotyped. The quality of a genetic marker is measured by its heterozygosity in the population of interest, and for a molecular marker, its polymorphism information content (PIC) as described by Botstein *et al*. (1980). Genetic markers that have been used in population genetics are grouped into three main classes: phenotypic or morphological markers, biochemical markers or isozymes and molecular markers utilizing variation at the nuclear DNA level.

4.1 Morphological or phenotypic markers

These are phenotypes for which variation observed in the population of interest can be explained by Mendel's law of inheritance e.g. colour variation, growth habit and fruit shape. Traditionally, morphological markers have been used to characterize varieties based on the assessment of a range of phenotypic characteristics. Several studies have been carried out using morpho-agronomic characteristics of the pods, seeds and flowers to elucidate population structure and genetic diversity of cacao populations (Aikpokpodion, 2010; Bekele and Bekele, 1996; Engels, 1992). Although morphological genetic markers proved useful in several cases, they are subject to several limitations. These include subjectivity in the analysis of character, environmental influences, limited diversity (morphological variants) among cultivars, and restriction of characterization of some useful characters to a particular stage of development, such as flowering or fruit ripening, and limitation to only one locus.

4.2 Allozymes

Allozymes are allelic variants of enzymes encoded by structural genes. Enzymes are proteins consisting of amino acids, some of which are electrically charged. Due to change in the net electric charge resulting from mutation, allelic variation can be detected by gel-electrophoresis and subsequent specific enzymatic staining. Usually, two or more loci can be distinguished per enzyme, and they are termed isoloci. Therefore, allozyme variation is also referred to as isozyme variation. The study of genetic variation in plant populations was greatly facilitated by the development of protein-based markers (i.e. allozymes). The primary contribution of allozymes to plant population biology has come from their utilization as neutral (or nearly neutral) genetic markers. Allozymes have been employed to characterize patterns of genetic variation within and among populations, and to examine the processes of dispersal and the patterns of mating that influence levels of genetic differentiation. In cacao, several workers such as Ronning & Schnell (1994) and Warren (1994) used isozyme systems to explore genetic diversity among cacao populations.

4.3 Molecular markers

Use of molecular markers has allowed the complete sampling of the genome, and helped to overcome the limitations of morphological markers and the isozyme markers. DNA markers have been successfully applied in cultivar identification, controlling seed purity of hybrids

and checking the genetic relatedness between cultivars. Some of the techniques developed for DNA manipulation in order to detect variations are:

4.3.1 Random Amplified Polymorphis DNA (RAPD)

In cacao, the first study of the use of molecular markers in cacao was reported by Wilde *et al.* (1992). These authors used Random Amplified Polymorhism DNA (RAPD) to study relationships among cocoa groups. Their work was soon followed by those of several authors who also used RAPD to determine genetic relationships among cacao populations (Russel *et al.*, 1993; Laurent *et al.*, 1994; Lerceteau *et al.*, 1997; Whitkus *et al.*, 1998). N'Goran *et al.* (1994) analyzed the genetic diversity of 106 genotypes in Cote d'Ivoire belonging to the various morphogeographic groups within Criollo, using 49 repeatable polymorphic RAPD products. They showed a clear structure among Forastero and Criollo groups with clear differentiation between Upper and Lower Amazon Forastero. Lerceteau *et al.* (1997) analysed the genetic diversity of Ecuadorian Nacional clones, Forastero, Trinitario and Criollo cacao clones using forty-three genomic probes. They found that within-group genetic diversity was almost identical between Forastero, Trinitario and Criollo. Their results showed that the populations of Amazon Forasteros and Criollo studied were highly diverse and that the Criollo and Trinitario populations showed some overlap.

4.3.2 Restriction Fragment Length Polymorphisms (RFLP)

RFLPs are generally found to be moderately polymorphic and can be applied in comparisons ranging from the individual level to closely related species. Because of their high genomic abundance and random distribution throughout the genome, RFLPs have been used by several workers to determine the genetic diversity of cacao populations (N'Goran *et al.*, 2000; Lerceteau *et al.*, 1997; Motamayor and Lanaud, 2002; Motamayor *et al.*, 2002).

4.3.3 Microsatellites

Microsatellites, also known as Simple Sequence Repeats (SSRs) are molecular marker loci consisting of tandemly repeated DNA of short oligonucleotide sequences of two to six bases in length. They form a class of genetic markers that show variation in the number of repeats of a simple DNA sequence. They are extremely common in eukaryotic genome (Tautz and Rentz, 1984) and are highly polymorphic in length. The development and application of SSRs facilitate the acquisition of a large quantity of genetic information relevant to genotype identification, which provides opportunities to characterize germplasm collections (Mitchell *et al.*, 1997). Such information generated are used by plant breeders to better understand their germplasm, guide breeding plans and better exploit genetic variation available (Lu *et al.*, 2005). Microsatellites are recommended as an international standard for defining genetic identity and has been widely used in the study of genetic diversity of cacao genetic resources (Aikpokpodion *et al.*, 2009, 2010; Saunders *et al.*, 2000; Zhang *et al.*, 2006).

5. Utility of phylogenetic tree in revealing genetic diversity

In the assessment of genetic diversity it is important to define relationships existing within or among sets of germplasm collection of a species and their evolutionary history within or

with related species. Phylogenetic trees serve as extremely powerful tools for organizing and illustrating these relationships. Phylogenetic trees have been successfully used in guiding conservation and biodiversity efforts (Sul et al., 2009) and establish relationship of cacao with its wild relatives (Figuera et al., 1994). A phylogenetic tree is a diagrammatic branching "tree" illustrating evolutionary relationships among entities within a species or various biological species based on similarities and differences in their physical and/or genetic characteristics. Organisms with similar morphologies or DNA sequences are likely to be more closely related than organisms with different structures or sequences. Entities that are joined together in the tree are implied to have evolved from a common ancestor. Each branch point represents the divergence of two species while sister taxa are groups that share an immediate common ancestor. Phylogenetic trees can either be rooted or unrooted. A rooted phylogenetic tree clearly shows relationship of each entity with the (usually imputed) most recent common ancestor of all the entities. Rooted trees are often constructed with the use of a definitive related 'outgroup' taxa. An 'outgroup' is a species or group of species that is closely related to the 'ingroup', the various species being studied. Unrooted trees, on the other hand, depict the relatedness of the entities without making assumptions about their ancestry. The principles of maximum parsimony and maximum likelihood are often used to analyze phylogenetic relationships with computer programs. The principle of maximum parsimony assumes that the tree that requires the fewest evolutionary events (appearances of shared derived characters) is the most likely. The principle of maximum likelihood states that, given certain rules about how DNA changes over time, a tree can be found that reflects the most likely sequence of evolutionary events.

6. Genetic diversity analysis results from West and Central Africa

Prior to recent studies in Cameroon, Cote d'Ivoire, Ghana and Nigeria, there was no useful information on the extent of genetic diversity in the cocoa cultivated in West and Central African countries. These studies were conducted within the framework of the Sustainable Tree Crops Program, a public-private partnership platform endowed by the United States Agency for International Development, US Department of Agriculture and chocolate industry partners such as the Mars Incorporated. These studies assessed genetic diversity in the introduced primary cacao clones and germplasm accessions used to develop improved hybrids distributed to farmers and cacao accessions on farmers' fields across the sub-region. Microsatellite markers were used to assess genetic diversity in these accessions. In addition to the microsatellite studies, some studies were also carried out to determine variation in agro-morphological and phenotypic characteristics of cacao germplasm in farmers and genebank collections.

6.1 Cameroon (Efombagn et al., 2006; Efombagn et al., 2008; Efombagn et al. 2009)

In their study of some 194 cocoa accessions collected in farms in Southern Cameroon during field surveys (Plate 1 d) and 71 Trinitario and Upper Amazon clones available in genebank collections on-station were assessed using 13 SSR markers. The gene diversity, genetic differentiation and genetic similarities were analyzed for the different populations. In total, 282 alleles were detected within all the populations studied (Plate 1c). The farm accessions were strongly differentiated based on their geographical origin, with accessions coming from the East province clustering together with local Trinitario accessions from the

genebank while accessions from the Centre-South provinces clustered with Amazon and hybrid accessions (Plate 1b), suggesting greater uptake of seed garden materials in farms in these provinces. The genetic diversity parameters indicate that the farmers' planting material was not highly diverse, but genetically close to parental genotypes available in genebanks (Plate 1a and 1c). However, some promising Upper Amazon clones (T-clones) that have also been used as parents of released hybrid varieties were genetically distant from the accessions. Their result suggested that the progenies of the Upper Amazon parents have so far been poorly used in the cocoa farms surveyed. A large genetic diversity was observed in the farm (H_{nb} = 0.34 – 0.72) and genebank (H_{nb} = 0.64 – 0.66) materials (Plate 1c). The large variability observed in farmer plantations was attributed to the large variation of first cocoa introductions (Bartley 2005), and the introduction of UA germplasm in the 1950's with its subsequent use in the cocoa breeding program. They also observed a higher private allelic richness in farm genotypes (A_P= 2.03) than those of SNK (Selection of Nkoemvone), ICS (Imperial College Selection), T (Trinidad) and UPA (Upper Amazon) clones. This indicated that farm accessions also harbor some genes that are not present in current national field genebanks. Evidence were found of admixture in farmers' fields which must have been due to hybridization (in seed gardens) and to substantial natural recombination in farmers' fields. Also, since farmers tend to use seeds issued from open pollination in their plantations for new plantings (replacement of dead cacao trees, extension or the creation of new cacao farms), these must have resulted in the presence of admixture as observed in these materials. Surprisingly however, was the observation that there was no relationships between the 'Criollo' reference materials with cacao accessions collected from farmers plantations (Plate 1a).

In a study to determine morphological diversity existing in cacao farms in relation to genetic diversity in gene bank accessions a total of 300 farm accessions (FA) were selected in the two major cocoa producing areas (Southern and Western) of Cameroon. Seventeen quantitative and qualitative descriptors used in this study were related to leaf (flush colour), flower (ligule colour), pod (weight, length, width, apex form, shape, rugosity, colour, husk hardness, basal constriction and pod index) and seed (number, length, width, dry weight and colour) characters. For the qualitative characters evaluated, considerable morphological variation was observed using the Shannon Weaver diversity index (SWDI) within FA and gene bank accessions. Among the FA, a differentiation between southern and western regions was only possible when using quantitative pod traits. Mean quantitative traits values of FA were not too different than those of most gene bank AGs, except for a few traits of agronomical interest (seed weight and pod index). No significant variation was observed for seed traits in all FA groups (southern/western). The morphological structure (quantitative traits) showed spatial differentiation between western and southern FA and a closer relationship between gene bank and some farm accessions.

6.2 Cote D'Ivoire (Pokou et al., 2009; Tahi et al., 2008)

Since the introduction of the 'Amelonado' type in 1880 that was widely cultivated in cocoa growing regions (Plate 2a), the first step at genetic improvement of the locally available germplasm took place with mass selection of 'better' types in local farms between 1947 and 1958. Mostly due to low genetic variability available in local types as is the case in other West African countries, the Upper Amazon Forastero types were introduced in 1954. This

a)

b)
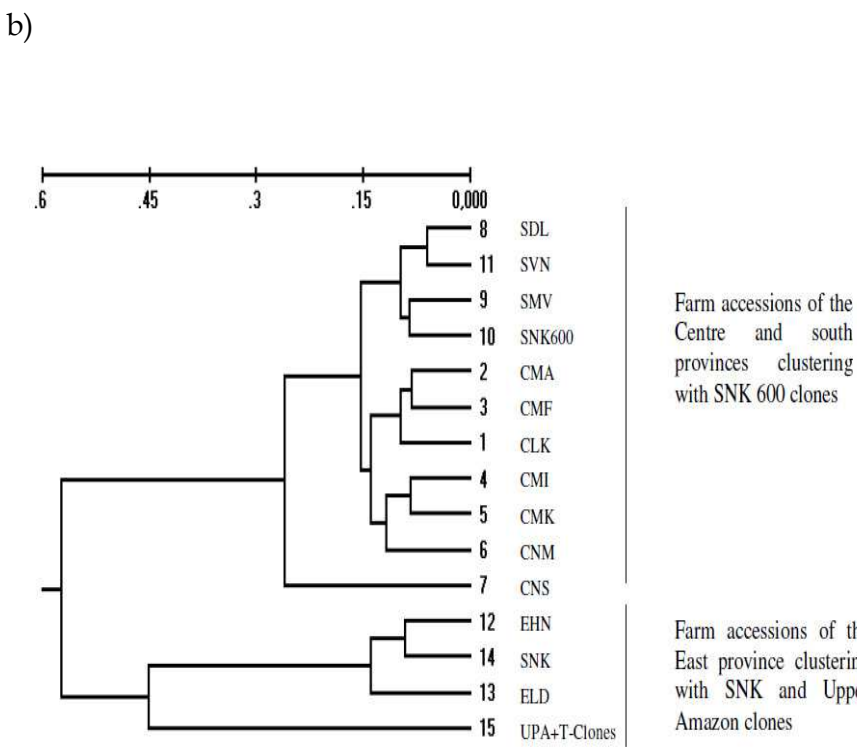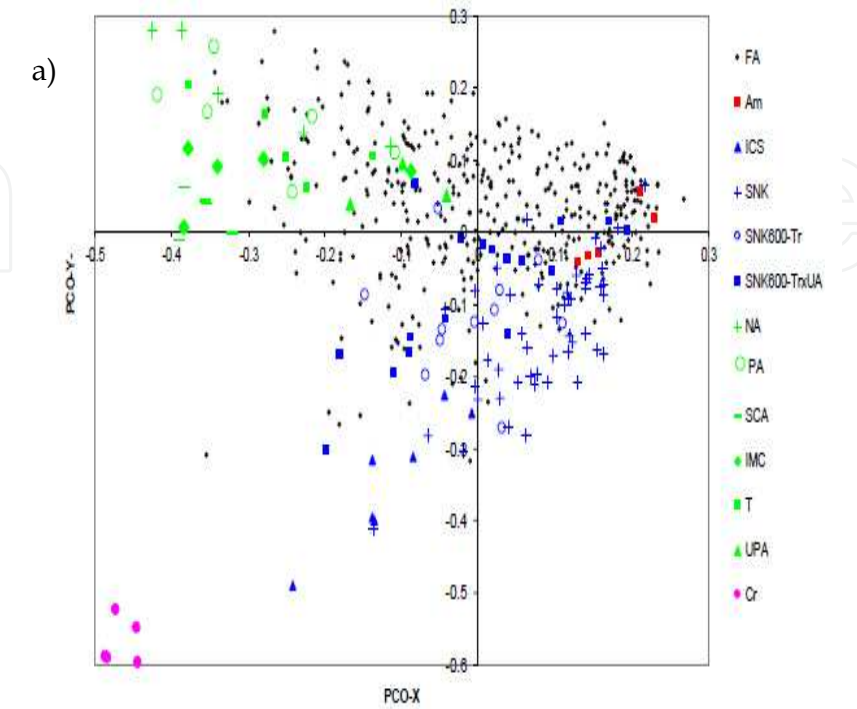
c)

FAC group I						
Population	Collection site	H$_E$	H n.b.	H$_o$	P(0.95)	Mean A/L	Effective A/L
Farm accessions							
CLK	Lékie	0.65(0.20)[1]	0.67(0.21)	0.66(0.21)	1.00	6.53	3.92
CMA	Mefou-et-Akono	0.63(0.14)	0.66(0.15)	0.51(0.15)	1.00	5.53	3.53
CMF	Mefou-et-Afamba	0.68(0.16)	0.70(0.17)	0.65(0.20)	1.00	7.61	4.76
CMI	Mbam-et-Inoubou	0.62(0.22)	0.66(0.23)	0.53(0.32)	0.92	5.15	4.53
CMK	Mbam-et-Kim	0.60(0.21)	0.61(0.22)	0.51(0.25)	1.00	9.53	3.61
CNM	Nyong-et-Mfoumou	0.69(0.12)	0.72(0.12)	0.63(0.20)	1.00	6.00	4.46
CNS	Nyong-et-So'o	0.68(0.17)	0.74(0.18)	0.72(0.16)	1.00	5.30	5.30
SDL	Dja-et-Lobo	0.47(0.20)	0.50(0.21)	0.49(0.29)	1.00	4.23	4.07
SMV	Mvila	0.63(0.17)	0.66(0.18)	0.58(0.19)	1.00	6.76	4.30
SVN	Vallée du Ntem	0.57(0.21)	0.58(0.22)	0.54(0.27)	1.00	6.46	4.23
Genebank material							
SNK600	Nkoemvone	0.64(0.15)	0.65(0.15)	0.59(0.21)	1.00	9.00	3.53
UA (UPA)	Nkoemvone	0.66 (0.17)	0.70(0.18)	0.58(0.36)	1.00	5.23	3.43

FAC group II						
Population	Collection site	H$_E$	H n.b.	H$_o$	P(0.95)	Mean A/L	Effective A/L
Farmers accessions							
EHN	Haut-Nyong	0.34(0.24)	0.35(0.24)	0.16(0.16)	0.92	4.07	2.84
ELD	Lom-et-Djerem	0.61(0.21)	0.62(0.21)	0.55(0.18)	1.00	7.85	3.92
Gene bank material							
SNK	Nkoemvone	0.61(0.14)	0.61(0.14)	0.47(0.22)	1.00	7.38	3.38

H$_E$: Expected Heterozygosity   H n.b.: non biased heterozygosity   H$_o$:observed heterozygosity
P(0.95): proportion of polymorphic loci when the most frequent allele do not exceed 95%
Mean A/L: Mean number of alleles per locus effective A/L: Effective number of alleles per locus
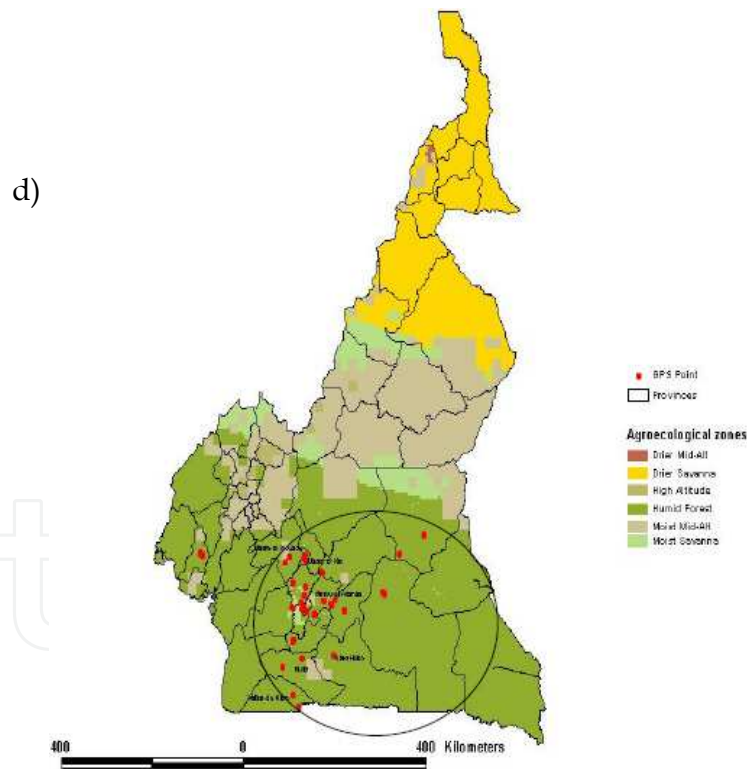[1]: standard deviation in parentheses

d)

Plate 1. a. A scatter plot showing genetic structure of planting materials in farmers and genebank materials in Cameroon; b. Spatial genetic differentiation between farmers accessions in central and south provinces from accessions in eastern province of Cameroon; c. genetic diversity indices of farmers and genebank accessions of cocoa in Cameroon; d. locations of cacao accessions collected within cocoa producing area of Cameroon used for the study (Source: Efombagn et al., 2006; Efombagn et al., 2009).

culminated in the development and distribution of selected hybrids between from the 1960s. However, much impact was made with the distribution on the hybrids developed and distributed in 1975 (Besse, 1975). In a survey conducted recently, it was shown that 71 % of materials grown on farms were locally selected 'Amelonado' type, 23 % selected improved hybrid types, while 6 % of the farms were grown with a mixture of local and improved types (Plate 2c).
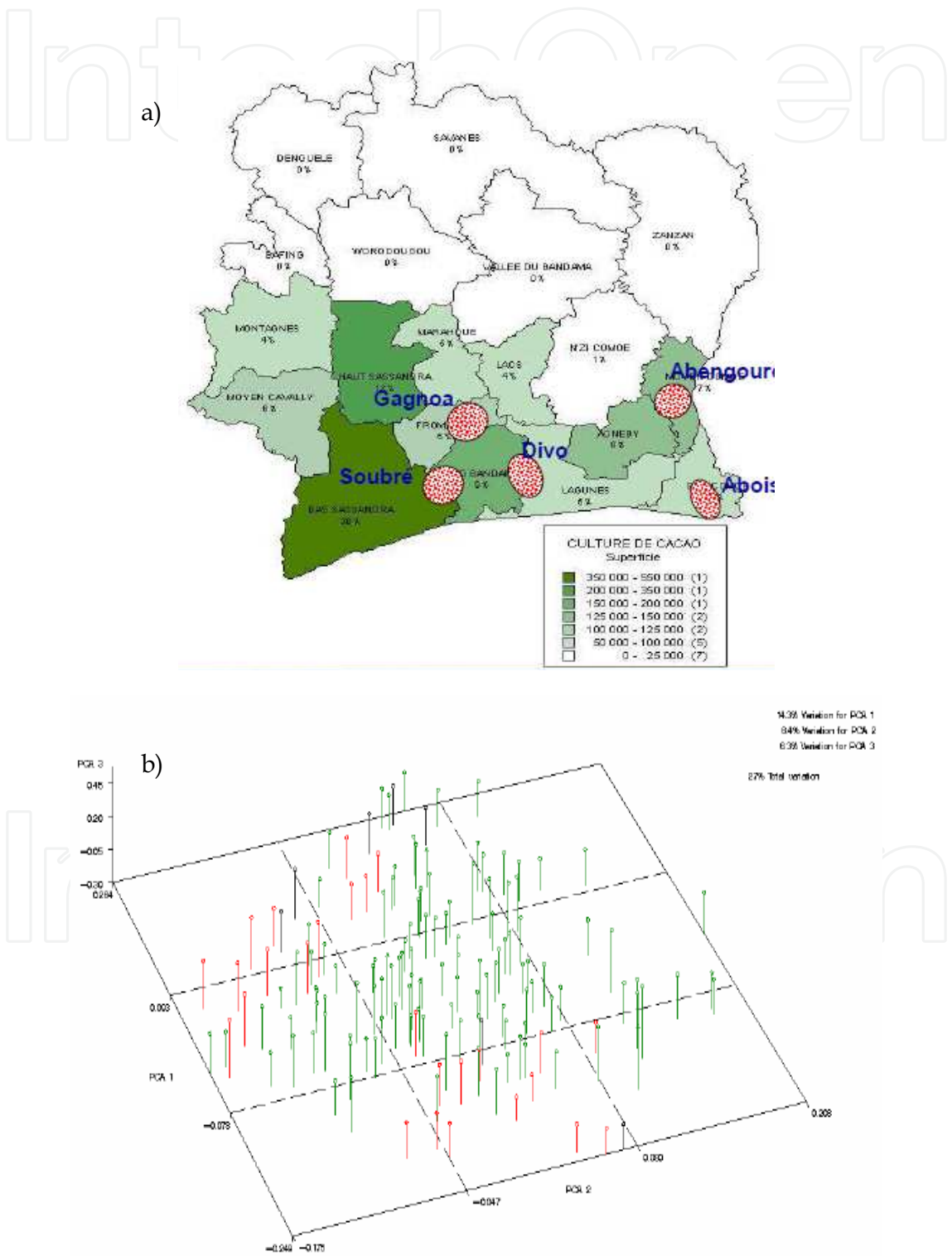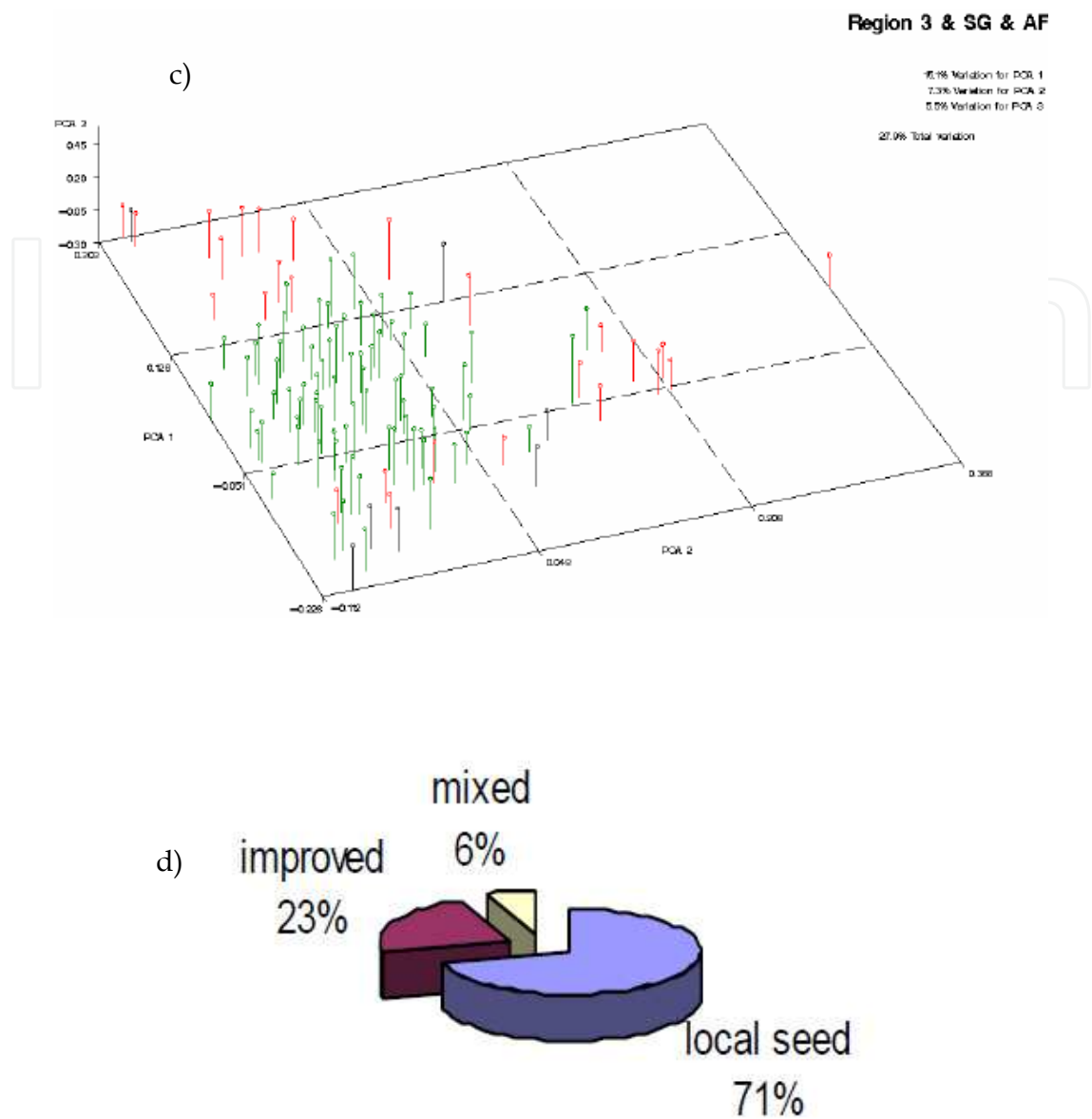
Plate 2. a. Cocoa producing regions in the humid forest of Cote d'Ivoire; b. genetic diversity in cacao accessions in farmers fields in Abengourou (green vertical lines) in relation to seed garden materials (red vertical lines); c. genetic diversity in cacao accessions in farmers fields in Divo (green vertical lines) in relation to seed garden materials (red vertical lines); d. distribution of different cacao types present in farmers fields in Cote d'Ivoire.

In a study conducted between 2003 and 2005 (Pokou et al., 2009), 12 microsatellites (simple sequence repeats marker) were used to asses genetic diversity of cacao types. Results showed considerable diversity in farmers accessions reflecting largely hybridization between local Amelonado types and Upper Amazon types distributed in the 1970s (Plates 2b & 2c). However, a significant proportion of diversity in seed garden materials was yet to diffuse to the farms indicating that farmers still largely used their own 'selected' materials

against those developed in research institutions. A further analysis was carried of the reciprocal recurrent selection programme set up in 1990. This involved two main genetic groups: Upper Amazon Forastero (UA) and a mixture of Lower Amazon Forastero (LA) and Trinitario (T). Based on data obtained from 12 microsatellite primers, the genetic diversity and genetic distances of the parental populations used in the first and second selection cycles are presented. The results revealed that the diversity of populations UA0 and UA1 on the one hand and (LA+T)0 and (LA+T)1 on the other is similar. The genetic distances were small between the parental populations used for the first and second cycles. Genetic diversity was greater in the UA group than in the LA+T group. The number of rare and of private alleles was reduced for both genetic groups, as well as the number of the frequent alleles in the LA+T group.

6.3 Ghana (Opoku et al., 2007)

In order to assess the genetic diversity of cacao types grown, some 377 accessions including farmers' accessions, breeders' collection and parental clones were collected from all cocoa growing regions of Ghana (Plate 3a), and analyzed using 17 microsatellite markers. Genetic diversity indices indicated that average gene diversity was high in all populations, with mean observed heterozygosity of 0.738 (Plate 3b). Although the highest was recorded in accessions from breeders' and parental collections, genetic diversity in the farmers' collection was comparatively high. Included in the study were a few extant trees among one of the earliest Tetteh Quarshie's introduction in the late 19th century.
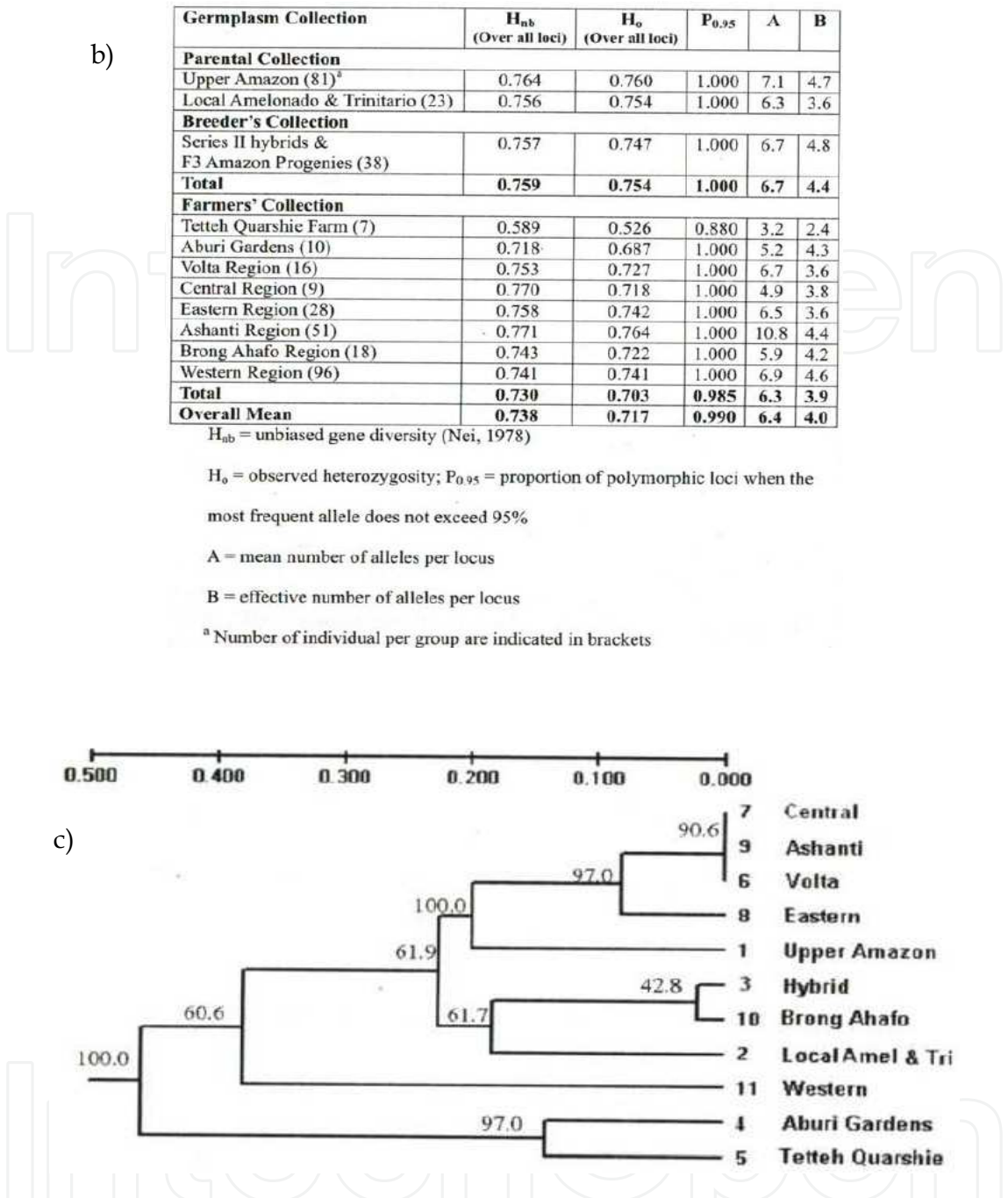
b)

Germplasm Collection	H$_{nb}$ (Over all loci)	H$_o$ (Over all loci)	P$_{0.95}$	A	B
Parental Collection					
Upper Amazon (81)[a]	0.764	0.760	1.000	7.1	4.7
Local Amelonado & Trinitario (23)	0.756	0.754	1.000	6.3	3.6
Breeder's Collection					
Series II hybrids & F3 Amazon Progenies (38)	0.757	0.747	1.000	6.7	4.8
Total	**0.759**	**0.754**	**1.000**	**6.7**	**4.4**
Farmers' Collection					
Tetteh Quarshie Farm (7)	0.589	0.526	0.880	3.2	2.4
Aburi Gardens (10)	0.718	0.687	1.000	5.2	4.3
Volta Region (16)	0.753	0.727	1.000	6.7	3.6
Central Region (9)	0.770	0.718	1.000	4.9	3.8
Eastern Region (28)	0.758	0.742	1.000	6.5	3.6
Ashanti Region (51)	0.771	0.764	1.000	10.8	4.4
Brong Ahafo Region (18)	0.743	0.722	1.000	5.9	4.2
Western Region (96)	0.741	0.741	1.000	6.9	4.6
Total	**0.730**	**0.703**	**0.985**	**6.3**	**3.9**
Overall Mean	**0.738**	**0.717**	**0.990**	**6.4**	**4.0**

H$_{nb}$ = unbiased gene diversity (Nei, 1978)

H$_o$ = observed heterozygosity; P$_{0.95}$ = proportion of polymorphic loci when the

most frequent allele does not exceed 95%

A = mean number of alleles per locus

B = effective number of alleles per locus

[a] Number of individual per group are indicated in brackets

c)

Plate 3. a. Cocoa regions of Ghana indicating sites of cacao accessions collection (dots); b. Table of genetic diversity indices in cacao form the different regions and germplasm collection; c. Relationships among cacao accessions in the different regions and field genebank accessions

The cocoa produced at the Tetteh Quarshie farm is of the Amelonado type, which originated from Brazil. Results of diversity analysis showed a clear separate clustering of accessions from Tetteh Quarshie farm and Aburi garden compared to rest of cocoa populations presently grown n Ghana (Plate 3c). These accessions were among the first introductions into the country and then spread across the country; however, the results indicated that presently these accessions have little or no influence on the current plantings in farmers'

fields. Subsequent hybridization with later introductions and adoption of farmers' own or newly released improved germplasm might have been responsible for observation made. The accessions from Western region clustered separately from those of breeders' collection and populations of other regions indicating that the breeders' germplasm had less impact on planting materials in the West of the country in comparison with other regions. This proved to be in agreement with the historical records that cocoa cultivation in Ghana had spread from other adjacent regions to the West. Additionally, the seed gardens from which farmers could obtain improved planting materials developed by breeders are fewer in the region and are inaccessible due to poor road network. On the other hand, farmers from other regions mostly collected seeds from the 'Seed gardens'. This explained why the farmers' collections from these regions clustered with the Breeders' collections. Another interesting observation was that the germplasm from Central, Ashanti, Volta, and Eastern regions, which constituted the earliest cocoa-growing regions of Ghana, clustered together and separately from the Parental clones and Breeders' collection, whereas the accessions from Brong Ahafo region clustered with the Breeders' collections. This showed that most of Brong Ahafo plantings were done at the time when the "Series II hybrids" had been developed and were popular in the country. Most farmers in this region might have used those varieties as planting material. However, in the case of the other regions, a substantial number of farmers had, in addition to breeders' varieties, used materials from their own farms or other neighboring sources.

6.4 Nigeria (Aikpokpodion 2007; Aikpokpodion et al., 2009; Aikpokpodion 2010; Aikpokpodion et al., 2010)

In a recent study, 12 microsatellite markers were used to determine genetic diversity in 574 accessions representing eight groups covering parental populations in West Africa, genebank, and farmers' accessions collected from cocoa growing regions of Nigeria (Plate 4a). From this study, it was shown that appreciable genetic diversity was present in on-farm and field genebank collections. A total of 144 alleles were detected in these accessions with a mean allelic richness of 4.39 alleles/ locus. The largest genetic diversity was found in the Upper Amazon parent population (H_{nb}=0.730), followed by the 1944 Posnette's Introduction (H_{nb}=0.704), and was lowest in the Local parent population (H_{nb}=0.471). Gene diversity was appreciably high in the farmers' populations (H_{nb}=0.563– 0.624); however, the effective number of alleles was lower than that found in the genebank's Posnette population. Fixation index estimates indicated deficiency of heterozygotes in the Upper Amazon and the Local parent populations (F_{is}=0.209 and 0.160, respectively), and excess of heterozygotes in the Trinitario parent population (F_{is}=−0.341). The presence of inbreeding in the Local parent populations and substructure (Wahlund effect) in the Upper Amazon were suggested for the deficiency of heterozygotes observed. In Nigeria, restricted gene flow and spatial differentiation was evident in cacao varieties grown by farmers in Nigeria (Plate 4b). Cacao trees grown on farmers' fields in southwestern and mid-western Nigeria are mainly hybrids of the Upper Amazon and the local Amelonado varieties, while the local Amelonado variety predominates in southeastern Nigeria (Plate 4c).The non-significant genetic differentiation observed between the genebank's and farmers' populations indicated significant impact of national breeding programs on varieties grown in farmers' plantations (Plate 4c). Results also showed that a small proportion of the genetic diversity available in field gene banks at the Cocoa Research Institute of Nigeria (CRIN) had been used to develop improved varieties

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in the Institute's cacao breeding programs and that 'Scavina' and 'Imperial Mixed Calabacillo' cacao varieties of the Upper Amazon Forastero have not been significantly utilized in Nigerian cacao breeding programs. Population structure analysis cacao types grown in farmers' fields showed that the Upper Amazon Forastero constitute 66 % of cacao grown, Amelonado made up 24 %, Trinitario accounted for 6 % while other types made up 4 % of cacao grown (Plate 4d). Large number of alleles were found in the farmers' populations (7.67–9.00), and compared with the number recorded for the genebank's collection, although the effective number of alleles (4.49–4.80) was lower than in Posnette's population (5.65). This situation is encouraging as it indicated that genetic diversity held in farmers' collection in commercial plantations is much greater than, and showed a major shift from, what it was since introduction in the late nineteenth century till the 1950s when the highly uniform local Amelonado cocoa was predominant on the field. We can conclude from the molecular data used in this study that there has been a significant variety replacement of the Amelonado cacao grown on fields in West Africa with Upper Amazon and Amazon × Amelonado hybrids.

In a study to determine phenotypic variation among cacao grown in Nigeria, 17 agro-morphological traits were studied in 184 accessions collected from farmers' fields (138) and field genebank collections (46). Fruit and bean traits of Upper Amazon Forasteros observed in farmers' accessions provided evidence of a shift from previously grown local 'West African Amelonado' from the Lower Amazon Forastero population. The large variation observed in this study for cacao grown by farmers indicated a high level of heterogeneity
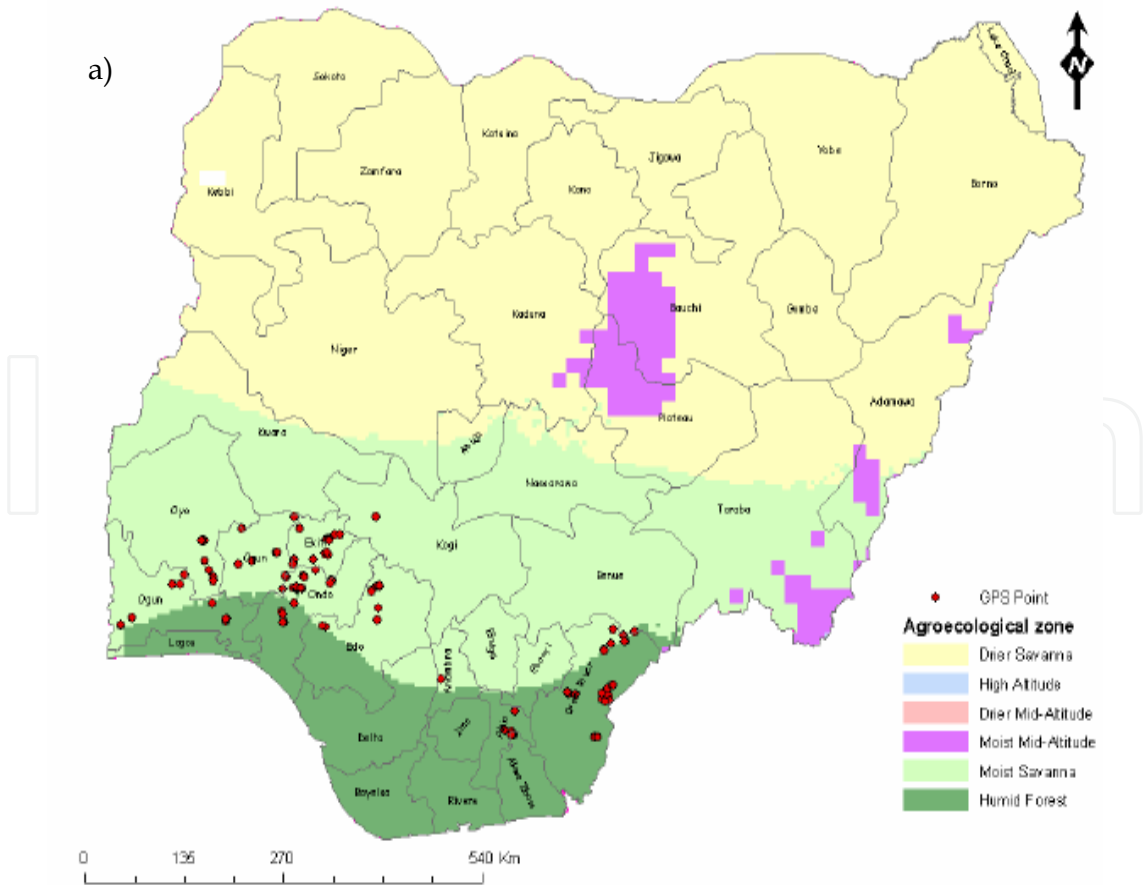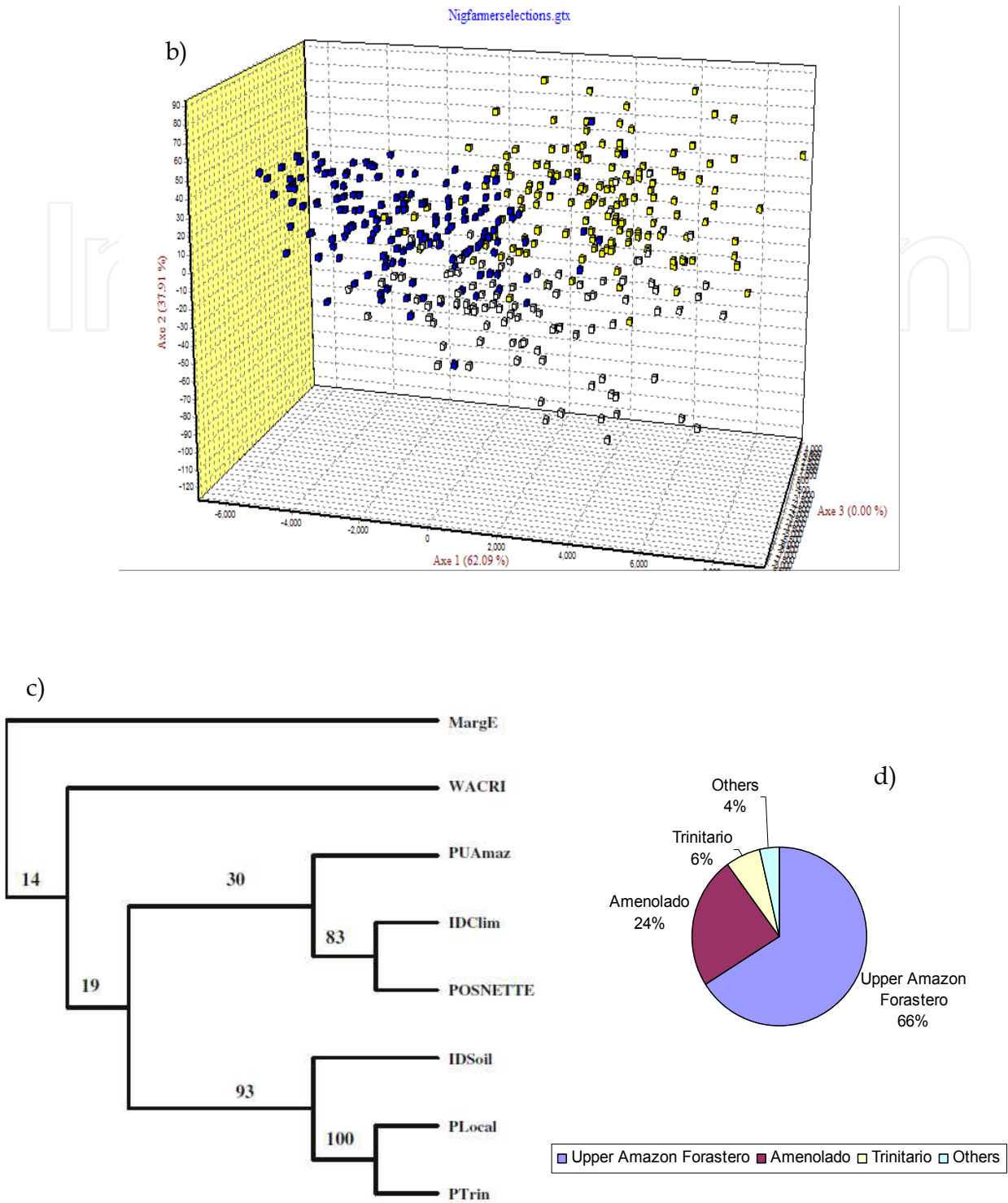
Plate 4. a. Sites of cacao germplasm collection(red dots) in cocoa producing region of Nigeria; b. Relationships showing spatial differentiation among cacao accessions collected in farmers' fields in ideal climate (yellow), ideal soil (blue) and marginal climate (white) conditions in Nigeria; c. Relationships between farmers and field genebank cacao accessions in Nigeria; d. Population structure indicating cacao types grown on farmers fields in Nigeria.

in materials maintained on-farm. The low percentage of fruit traits that are typical of 'Amelonado' and 'Trinitario' types provides some evidence of variety replacement of 'West Africa Amelonado' (WAA) cacao types in farmers' fields with Upper Amazon-derived types. This showed a radical shift from the situation preceding the 1950s, when uniform 'Amelonado' cacao types were mainly grown. This would have resulted from the use Upper Amazon-derived cacao varieties distributed to farmers through the seed gardens. Significant variation observed for bean and fruit characteristics among cacao accessions in this study also indicated the importance of on-farm collections as a valuable reservoir of genetic diversity. Some of these traits are of commercial importance and have been used as selection criteria by farmers in the choice of parent trees for raising seedlings to make new plantings and farm expansion. From this study, the complete absence or slight anthocyanin pigmentation on the ridge of the mature fruit of more than 88% of accessions indicated that most of the cacao now grown in Nigeria was apparently derived from the Amazonian Forastero origin. The preponderance of 'Cundeamour' fruit shape (76%) with slight to strong basal constriction (88%), obtuse to attenuate apex forms (95%) and intermediate to intense rugosity (86%) showed that, possibly, the Upper Amazon Forastero (UAF) 'Parinari' population, characterized by pronounced bottleneck, conspicuous apex form and the intermediate to intensely warty fruit (Bartley 2005) and to a lesser extent, the 'Nanay' population, had the most impact on cacao On the other hand, the low percentage (less than 15%) of red pigmentation in fruits, a trait associated with some 'Criollo' populations indicated that 'Criollo' and red-podded 'Trinitario' populations have, at present, only a minimal influence on field-grown cacao in Nigeria.

7. Conclusion

Cocoa beans either as export commodity or processed into cocoa products remain a significant revenue earner for the government and a veritable means for livelihood sustenance for the people of West and Central Africa. It was therefore pertinent to determine the extent of genetic diversity in this crop in order to safeguard, not only the livelihood of the people and revenue base of the government, but also the multi-billion dollars industry of processing and consuming countries. From results obtained in recent studies, the presence of appreciable genetic diversity in farmer-grown cacao indicated that there is enough resilience in the cacao types now grown in West and Central African countries to withstand any major disease outbreak, which may constitute a serious threat to the cocoa industry. This is in contrast to the situation in the 1930s and 1940s, when, due to a small range of genetic variability in materials grown in farms, the Cocoa Swollen Shoot Virus almost ruined the industry. At that time, more than 20 million trees were cut down as a control measure. The outbreak of Witches' Broom disease in the late 1980s in Brazil provides a recent example of how disease attack can affect farmers' livelihoods and the cocoa industry. The outbreak led to a drop in cocoa production from 380,000 tons per year (at that time Brazil was the world's second largest cocoa producer) to 90,000 tons in the late 1990s, when cocoa actually had to be imported. The presence of some private alleles in farmers' population also gives an opportunity to select useful recombinants on the field that have shown greater adaptation and possibly accumulated genes for resistance to prevalent diseases such as *Phytophthora* pod rot, mirid attack (*Sahlbergella singularis* and *Distantiella theobromae*), and abiotic stress (e.g., drought). Restricted gene flow and spatial differentiation as observed in Cameroon, Cote d'Ivoire, Ghana and Nigeria indicated the existence of

inefficient seed delivery systems in some cases, which are not extending the benefits of improved varieties developed by researchers to farmers. There is a need, therefore, for the establishment of a functional and efficient seed delivery system to facilitate farmers' easy access to improved planting materials.

These studies revealed that research efforts to develop improved varieties over the years have been limited to a narrow range of diversity present in germplasm collections. This, in turn, has limited the gains that would have been made from utilizing useful attributes, such as resistance genes against *Phytophthora* pod rot disease in the Scavina variety and the large bean size of the Iquitos Mixed Calabacillo variety. This information is useful for future cocoa breeding efforts and a guide for future germplasm introduction. This knowledge is also important to enable the development of appropriate breeding strategies to improve planting materials, with particular attention to the integration of available genetic diversity into future cocoa improvement programmes. However, the discontinued use of the Amelonado cocoa by farmers have significant implication for the conservation of this stock, some of which are completely homozygous at all loci used in this study. It is hereby suggested that conservation strategies should be developed to preserve the local Amelonado landraces in order to exploit their useful values in future breeding programs.

Although useful information have now been obtained on genetic diversity in the major cocoa producing (Cameroon, Cote d'Ivoire, Ghana and Nigeria) West and Central African countries, much information is still needed on the genetic diversity situation in other countries in the region and others such as Eastern and Southern African countries. For instance, information on genetic diversity in Sao Tome & Principe island and Fernando Po (Bioko) in Equatorial Guinea, the earliest places of cocoa introduction in Africa will be useful to determine the sources of many private alleles that were found in farmers accessions but absent in field genebanks. It is also very important to capture the diversity that might be present in order to exploit these for benefits of the ever increasing sophistication of the cocoa market including 'origin' and 'specialty' emphasis of the consumers. It will be very useful if funding could be made available to determine the genetic diversity of cacao types in Madagascar, Malawi, East African countries such as Tanzania and Uganda in addition to the rest West and Central African countries. This is because a thorough knowledge of genetic diversity in on-farm and field genebanks, particularly in an introduced crop species as cacao is crucial to the utilization of the genetic resources available. This will also be important for the sustainability of the global cocoa industry.

8. Acknowledgement

9. References

Aikpokpodion, P.O. (2007). Genetic diversity in Nigerian cacao, *Theobroma cacao* L. collections as revealed by phenotypic and simple sequence repeats marker. PhD Thesis submitted to the University of Ibadan, Nigeria. 147 pp

Aikpokpodion, P. O. (2010). Variation in agro-morphological characteristics of cacao, *Theobroma cacao* L. in farmers' fields in Nigeria. *New Zealand Journal of Crop and Horticultural Science* Vol. 38 (2):157-170

Aikpokpodion, P.O.; Motamayor, J.C., Adetimirin, V.O., Adu-Ampomah, Y., Ingelbrecht, I., Eskes, A.B., Schnell, R.J. & Kolesnikova-Allen, M. (2009). Genetic diversity assessment of sub-samples of cacao, *Theobroma cacao* L. collections in West Africa using simple sequence repeats marker. *Tree Genetics and Genome* 5:699-711

Aikpokpodion, P. O.; Adetimirin, V. O., Guiltinan, M. J., Eskes, A. B., Motamayor, J-C., Schnell, R. J. & Kolesnikova-Allen, M. (2010). Population structure and molecular characterization of Nigerian field genebank collections of cacao, *Theobroma cacao* L. *Silvae Genetica* 59 (6):273-285

Alverson, W.S.; Whitlock, B. A., Nyfeller, R., Bayer, C. & Baum, D.A. (1999). Phylogeny of the core Malvales: Evidence from *ndhF* sequence data. *American Journal of Botany* 86: 1474 - 1486

Atanda, O.A. & Jacobs, V.J. (1974). Assessment of WACRI Series I and II varieties of cocoa in Nigeria. 1. Establishment and yield. *Ghana J Agric Sc* 7:215–220

Bartley, B. G. D. (2005). The genetic diversity of cacao and its utilization. CABI, Wallingford, 341 pp

Bekele, F. L. & Bekele, I. (1996). A sampling of the phenetic diversity of cacao in the international cocoa gene bank of Trinidad. *Crop Science* 36: 57-64

Besse, J. (1975). La selection generative du cacaoyer en Cote d'Ivoire: bilan et orientation des recherches en 1975. In 5th International Cocoa Research Conference, Ibadan Nigeria, 1–9 September. pp. 95–103

Botstein, D.; White, R. L., Skolnick, M. & Davis, R. W. (1980). Construction of a genetic linkage map in human usung restriction fragment length polymorphisms. *American Journal of Human Genetics* 32:314-331

Cheesman, E. E. (1944). Notes on nomenclature, classification and possible relationships of cacao populations. *Tropical Agriculture* 21:144–159

CSAE (Center for the study of African Economies) (2009): Fertile Ground: BoostingCaocoa production among Ghanaian Smallholder. Briefing Paper 01, August 2009. www.csae.ox.ac.uk

Cuatrecasas, J. (1964). Cacao and its allies: A taxonomic revision of the genus *Theobroma*. *Contributions to the U.S. Natural Herbarium* 35(6): 379 – 614.

Edwin, J. & Masters W. A. (2005). Genetic improvement and cocoa yields in Ghana. Expt Agric 41:491–503

Efombagn, M. I. B; Sounigo, O., Nyassé, S., Manzanares-Dauleux, M., Cilas, C., Eskes, A.B. & Kolesnikova-Allen, M. (2006). Genetic diversity in cocoa germplasm of southern Cameroon revealed by simple sequences repeat (SSRs) markers. *African Journal of Biotechnology* 5(16):1441-1449.

Efombagn, M. I. B; Motamayor, J.C., Sounigo, O., Eskes, A.B., Nyassé, S., Cilas, C., Schnell, R., Manzanares-Dauleux, M., & Kolesnikova-Allen, M. (2008). Genetic diversity

and structure of farm and genebank accessions of cacao (*Theobroma cacao* L.) in Cameroon revealed by microsatellite markers. *Tree Genetics and Genome* 4:821-831.

Efombagn, M. I. B; Sounigo, O., Nyassé, S., Manzanares-Dauleux, M. & Eskes, A.B. (2009). Phenotypic variation of cacao (*Theobroma cacao* L.) on farms and in the genebank in Cameroon. *J. of Plant Breeding and Crop Science* 1(6):258-264.

Engels, J. M. M. (1992). The use of botanical descriptors for cocoa characterisation: CATIE experiences. *Proceedings of the International Workshop Conservation Characterization Utilization Cocoa Genetic Resources 21st Century*. Haines Clark& Co. (UK) Ed. Cocoa Research Unit, The University of the West Indies, Port-of-Spain, Trinidad. 69-76

Figuera, A.; Janick, J., Levy, M. & Goldsbrough, P.B. (1994): Reexamining the classification of *Theobroma cacao* L. using molecular markers. J Amer Soc Hort Sci 119, 1073-1082

Gosselin, M. (1895). Report for the year 1893-1894 0n the German Coloniesin Africa and the South Pacific. Miscellaneous Series, no. 352, 1895. Foreign Office, London, 94 pp

Guiltinan, M.J. (2007). Cacao. Biotechnology in agriculture and forestry, vol. 60. In: Pua EC, Davey MR (eds) Transgenic crops V. Springer, Berlin

ICCO (International Cocoa Organization) (2010). Quarterly Bulletin Cocoa Statistics. Vol. XXXVI. 2009/2010

Jacob, V.J.; Atanda, O.A. & Opeke, L.K. (1971). Cacao breeding in Nigeria. In: *CRIN Commemorative Publication – Progress in Tree Crops Research in Nigeria.*9-12

Knight, R. & Rogers, H. H., 1955. Incompatibility in *Theobroma cacao. Heredity* 9: 69 – 77

Laurent, V.; Risterucci, A.M. & Lanaud. C. (1994). Genetic diversity in cacao revealed by cDNA probes. *Theor Appl Genet* 88:193–198

Lerceteau, E.; Robert, T., Petiard, V. & Crouzilat, D. (1997). Evaluation of the extent of genetic variability among *Theobroma* accessions using RAPD and RFLP markers. *Theor Appl Genet* 95:10–19

Lockwood, G. & Gyamfi, M.M.O. (1979). The CRIG cocoa germplasm collection with notes on codes used in the breeding programme at Tafo and elsewhere. Cocoa Research Institute, Ghana Technical Bulletin No. 10 Tafo

Lu, H.; Redus, M.A., Coburn, J.R., Rutger, J.N., McCouch, S.R. & Tai, T.H. (2005). Population structure and breeding patterns of 145 U.S. Rice cultivars based on SSR marker analysis. *Crop Science* 45:66–76

Mitchell, S.E.; Kresovich, S., Jester, C. A., Hernandez, C. J. & Szewc-McFadden, A. K. (1997). Application of multiplex PCR and fluorescence-based, semi-automated allele sizing technology for genotyping plant genetic resources. *Crop Science* 37:617-624

Motamayor, J.C. & Lanaud, C. (2002). Molecular analysis of the origin and domestication of *Theobroma cacao* L. *Managing Plant Genetic Diversity* Engels, J.M.M., Ramanatha Rao, V., Brown, A.H.D. & Jackson, M.T. Eds. IPGRI. 77–87

Motamayor, J.C.; Risterucci, A.M., Lopez, P.A., Ortiz, C.F., Moreno, A. & Lanaud, C. (2002). Cacao domestication I: the origin of the cacao cultivated by the Mayas. *Heredity* 89:380–386

Motamayor, J.C.; Lachenaud, P., Wallace, J., Loor, G., Kuhn, D. N., Brown, J. S. & Schnell II, R. J. (2008). Geographic and genetic population differentiation of the Amazonian chocolate tree. *PLoS One* 3: e3311.doi:10.1007/s12042-008-9011-4

N'Goran, J.A.K.; Laurent, V., Risterucci, A.M. & Lanaud, C. (2000). Comparative genetic diversity of *Theobroma cacao* L. using RFLP and RAPD markers. *Heredity* 73:589-597

N'Goran, J.A.K.; Lachenaud, Ph. & Bastide, Ph. (1992). Cocoa germplasm conservation initiatives in Cote d'Ivoire. In: Proceedings of the International Workshop on Conservation, Characterization and Utilization of Cacao Genetic Resources in the 21st Century. Cocoa Research Unit, Port of Spain, Trinidad 13–17 September 1992, pp 221–227

N'Goran, J.A.K.; Laurent, V., Risterucci, A.M. & Lanaud, C. (2000). The genetic structure of cocoa populations (*Theobroma cacao* L.) revealed by RFLP analysis. *Euphytica* 115:83–90

Opeke, L.K. (1969). Development of cacao industry in Nigeria. In: Proceedings of the 6th Conference of the Agricultural Society of Nigeria, pp. 1–5.

Opoku, S.Y.; Bhattacharjee, R. , Kolesnikova-Allen, M., Motamayor, J.C., Schnell, R., Ingelbrecht, I., Enu-Kwesi, L. & Adu-Ampomah, Y. (2007). Genetic diversity in cocoa (*Theobroma cacao*, L.) germplasm collection from Ghana. *Journal of Crop Improvement* 20(1/2):73–87.

Pokou, N. D.; N'Goran, J. A. K., Lachenaud, P., Eskes, A. B., Montamayor, J. C., Schnell, R., Kolesnikova-Allen, M., Clément, D. & Sangaré, A. (2009). Recurrent selection of cocoa populations in Côte d'Ivoire: comparative genetic diversity between the first and second cycles. *Plant Breeding* 128: 514–520. doi: 10.1111/j.1439-0523.2008.01582.x

Preuss, P. (1901). Expedition nach Central- und Sudamerika 1899/1900. Kolonial-Wirtschaftlichen Komitees, Berlin, 374 pp

Purseglove, J. W. (1974). *Tropical crops, dicotyledons*. John Wiley & Sons, New York

Rice, R. A. & Greenberg, R. (2000). Cacao cultivation and the conservation of biological diversity. *Ambio* 29(3):167-173.

Ronning, C. M. & Schnell, R. J. (1994). Allozyme diversity in a germplasm collection of *Theobroma cacao* L. *Journal of Heredity* 85: 291-295.

Russell, J. R.; Hosein, F., Johnson, E., Waugh, R. & Powell, W. (1993). Genetic differentiation of cocoa, *Theobroma cacao* L. populations revealed by RAPD analysis. *Molecular Ecology* 2: 89-97.

Saunders, J.A.; Hemeida, A.A. & Mischke, S. (2000). USDA DNA Fingerprinting programme for identification of *Theobroma cacao* accessions. *Proceedings of the International Workshop on New Technologies and Cocoa Breeding 16-17 October*. Kinabalu, Sabah, Malaysia: INGENIC, p. 108-114.

Sul, S-J.; Matthews, S., & Williams, T.L. (2009). Using tree diversity to compare phylogenetic heuristics. BMC Bioinformatics 10(Suppl 4)S3

Tahi, G. M.; N' Goran, J.A.K., Pokou, N.D., Lachenaud, P. & Eskes, A.B. (2008). Current status of planting materials in Côte d'Ivoire. In: Proceeding of CFC/ICCO/Bioversity Project Workshop on Collaborative and Farmer Participatory Approaches in Cocoa Breeding in Africa held 18 – 22 February 2008, Abidjan, Cote d' Ivoire

Tautz, D. & Rentz, M. (1984). Simple sequences are ubiquitous components of eukaryotic genomes. *Nucleic Acid Research* 12: 4127-4138

Toxopeus, H. (1964). F_3 Amazon cacao in Nigeria. *Report of West African Cacao Res. Inst. Nigeria*, 1963/64, pp 13–22

Van Delden, W. (1992). Genetic diversity and its role in survival of species. *Biodiversity and Global Change*. O.T. Solbrig, H.M. Van Emden and P.G.W.J. Van Oordt. Eds. CAB international and IUBS. 1992. Chapter 6: 41-56

Warren, J.M. (1994). Isozyme variation in a number of populations of *Theobroma cacao* L. obtained through various sampling regimes. *Euphytica* 72: 121-126

Webster, J.B. (1964). The Bible and the Plough. *Journal of Historical Society of Nigeria* II, 4:418-434

Whitkus, R.; de la Cruz, M., Mota-Bravo, L. and Gomez-Pompa, A., 1998. Genetic diversity and relationships of cacao (*Theobroma cacao* L.) in southern Mexico. *Theoretical and Applied Genetics* 96:621–627.

Wilde, J.; Waugh, R. & Powell, W. (1992). Genetic fingerprinting of *Theobroma* clones using randomly amplified polymorphic DNA markers. *Theoretical and Appliled Genetics* 83: 871–877.

Zhang, D.; Arevalo, E., Mischke, B., Zuniga, L. & Barreto, A. (2006). Genetic diversity and population structure of cocoa, *Theobroma cacao* L. in the Hullaga and Ucayali valleys of Peru. *Annals of Botany* 98:647–655
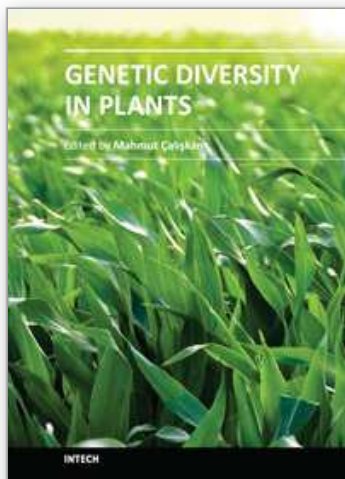
Genetic Diversity in Plants

Edited by Prof. Mahmut Caliskan

Genetic diversity is of fundamental importance in the continuity of a species as it provides the necessary adaptation to the prevailing biotic and abiotic environmental conditions, and enables change in the genetic composition to cope with changes in the environment. Genetic Diversity in Plants presents chapters revealing the magnitude of genetic variation existing in plant populations. The increasing availability of PCR-based molecular markers allows the detailed analyses and evaluation of genetic diversity in plants and also, the detection of genes influencing economically important traits. The purpose of the book is to provide a glimpse into the dynamic process of genetic variation by presenting the thoughts of scientists who are engaged in the generation of new ideas and techniques employed for the assessment of genetic diversity, often from very different perspectives. The book should prove useful to students, researchers, and experts in the area of conservation biology, genetic diversity, and molecular biology.

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