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Population structure of *Garcinia kola* Heckel in Central region of Cameroon

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Declaration

I hereby declare that I have done this thesis entitled "Population structure of *Garcinia kola* Heckel in Central region of Cameroon" independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague 23.04.2021

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Irikidzai Prosper Chinheya

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Abstract

Garcinia kola is a multipurpose fruit tree species indigenous to West African communities, where it is of significant ethnomedicinal, cultural and economic importance. Faced with the threat of declining population numbers, the species was selected for conservation and participatory domestication programmes however, a lack of adequate information on genetic diversity is widely reported as a limiting factor in both processes. The aim of this study was to assess the genetic diversity of *G. kola* populations in the Central region of Cameroon using Amplified Fragment Polymorphism (AFLP) markers. Genomic DNA was extracted and then digested with *Mse*I and *Eco*RI endonucleases. From an initial 24 primers, four high-performing primer combinations were selected to assess genetic diversity within and among eight provenances of *G. kola*. A total of 1176 fragments were amplified with 98.6 % polymorphism at the species level and a mean number of 261.9 fragments per individual. The computed values for Nei's gene diversity within populations (Hj), Total gene diversity (Ht), and the Wright's fixation index (F_{ST}) were 0.1894, 0.1922 and 0.0145 respectively. The obtained results revealed a higher genetic diversity within the assessed populations than among them. Bayesian analysis of sampling groups revealed the existence of two differentiable but admixtured genetic clusters, implying a weak population structuring. Attempts to assess for correspondence between clustering and geographic distances revealed no clear patterns. Most of the outcomes of this study were comparable to those of Benin *G. kola* populations assessed using RAPD markers. The study revealed that AFLP markers are a useful tool for assessing the genetic diversity of *G. kola*. Results suggest possible human-mediated gene flow events, potentially attributed to the selection of kernels for trade or natural selection through the adaptation of the species to local environmental. This study may open the door for advancing participatory tree domestication (PTD) and conservations programmes within the study area. However, it is recommended that initiatives be undertaken to safeguard the existing genetic diversity such as the use of gene banks, sustainable utilisation of genetic diversity in PTD or the protection of important individuals within their stands.

Key words: AFLP marker, Agroforestry, AFTPs, Bitter kola, Genetic diversity, Provenance, Tree domestication.

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List of the abbreviations used in the thesis

A	=	Adenine
AFLP	=	Amplified Fragment Length Polymorphism
AFTPs	=	Agroforestry Tree Products
C	=	Cytosine
CIFOR	=	Centre for International Forestry Research
CZU	=	Czech University of Life Sciences in Prague
DNA	=	Deoxyribonucleic acid
*Eco*RI	=	"*Eco*RI" endonuclease enzyme
EDTA	=	Ethylenediaminetetraacetic acid
FTA	=	Faculty of Tropical AgriSciences
F_{ST}	=	Fixation index
G	=	Guanine
GPS	=	Global Positioning System
HPCE	=	High Performance Capillary Electrophoresis
IAA	=	Isoamyl alcohol
ICRAF	=	World Agroforestry Centre
ISSR	=	Inter-Simple Sequence Repeats
ISNAR	=	The International Service for National Agricultural Research
IUCN	=	International Union for the Conservation of Nature
JGP	=	JSTOR Global Plants Database
M	=	Molar
masl	=	metres above sea level
MCMC	=	Markov Chain Monte Carlo
*MSe*I	=	"*MSe*I" endonuclease enzyme

NaCl	=	Sodium Chloride
NTFPs	=	Nontimber Forest Products
PCA	=	Principal Component Analysis
PCR	=	Polymerase Chain Reaction
PTD	=	Participatory Tree Domestication
PVP	=	Polyvinylpyrrolidone
RAPD	=	Random Amplified Polymorphic Deoxy-ribonucleic acid
RFLP	=	Restriction Fragment Length Polymorphism
R-L	=	Restriction-Ligation
RPM	=	Revolutions Per Minute
SAFORGEN	=	Sub-Saharan Forest Genetic Resources Programme
SNP	=	Single-Nucleotide Polymorphism
T	=	Thymine
Tris-HCl	=	2-Amino-2-(hydroxymethyl)-1,3-propanediol-hydrochloride
U	=	Enzyme catalytic activity (µmol/min)
UPGMA	=	Unweighted Pair Group Method with Arithmetic mean
WFO	=	World Flora Online.

1.   Introduction

Garcinia kola is a multipurpose tree species native to Western and Central Africa. *G. kola* occurs naturally in tropical lowland forests and is sometimes cultivated in home gardens as well as agroforestry systems (Maňourová et al. 2019). *G. kola* is utilised by farmers for several beneficial functions and products. The trees provide a range of agroforestry services such as shading, fencing poles and wind-breaking. All of *G. kola*'s vegetative organs are reported to be utilised as remedies for a diverse range of illnesses, thus it is commonly referred to as a "wonder plant". *G. kola* seeds, usually valued as the most important product of the tree, are mostly chewed to alleviate gastric problems and they have been demonstrated to either cure or alleviate a wide range of other medical conditions such as headaches, liver disorders and bronchitis (Iwu et al. 2002; Ebomoyi & Ekojie 2012). In Cameroon, the annual trade in *G. kola* seeds is reported at 50 t, equivalent to approximately 660,000 USD (Awono et al. 2016), whereas the annual family income based on the kernels sell varies from 300 to 1,300 USD (Fondoun & Manga 2000; Onyekwelu et al. 2015). Thus, *G. kola* has the potential to improve rural livelihoods through income generation and better healthcare.

Despite offering several benefits, *G. kola* is classified as "vulnerable" by the International Union for the Conservation of Nature (IUCN) and is reported to currently have declining population numbers (Cheek 2004; Agyili et al. 2007). This situation is largely attributed to *G. kola*'s popularity, poor natural regeneration, overexploitation and destructive harvesting methods such as bark stripping and digging out the roots (Agyili et al. 2007; Jusu & Sanchez 2014). Partly due to *G. kola*'s popularity and vulnerability, it is also listed among the species prioritised for domestication and conservation by the World Agroforestry Centre (ICRAF) and the Sub-Saharan Forest Genetic Resources Programme (SAFORGEN) respectively (Sacandé et al. 2004; Franzel & Kindt 2012). *G. kola* is currently in the early stages of the domestication process (Clement et al. 2010; Maňourová et al. 2019).

In simple terms, tropical tree domestication in agroforestry is a process of integrating selected lines of a neglected tree species into farming systems, to improve the services or the Agroforestry Tree Products (AFTPs) they offer to farmers. However,

Lengkeek et al. (2006) suggested that current tree domestication practices might be reducing the genetic base of tree resources on farms, thus compromising the productivity, sustainability and conservation ideals of agroforestry ecosystems. Maňourová et al. (2019) highlighted the lack of basic knowledge about *G. kola*, including genetic data and further suggested the development of molecular markers for analysis of different populations is a prerequisite to advance the ongoing *G. kola* domestication efforts. In practice, agroforestry plantings are often weakened by the use of genetically poor germplasm (Simons 1996). Genetic diversity should therefore be considered when selecting *G. kola* lines for domestication and conservation processes. Diversity is needed to enhance the capacity of planting material to adapt to changing user requirements and environmental conditions, while sophisticated molecular techniques are required to assess overall genetic diversity (Leakey 2014). Therefore, the thesis aimed to assess the genetic diversity of *G. kola* populations in the Central region of Cameroon using AFLP markers.

2.   Literature Review

2.1.   Agroforestry

Agroforestry is an ancient agricultural practice that involves the cultivation of trees in combination with crops, livestock, or pastures. There are several widely accepted definitions of the term "agroforestry". In simple terms, the World Agroforestry Centre (ICRAF) defines agroforestry as "the interaction of agriculture and trees, including the agricultural use of trees". Lundgren and Raintree (1983) described agroforestry as "a collective name for land-use systems and technologies where woody perennials (trees, shrubs, palms, bamboo, etc.) are deliberately used in the same land management units as agricultural crops and/or animals, in some forms of spatial arrangement or temporal sequence". In agroforestry, the most typical component, the woody perennial, may not always be cultivated mixed with or adjacent to the other components, but rather it may be separated from the other components in time and space. Agroforestry farming units are often referred to as agroforestry systems.

The Food and Agriculture Organisation (FAO) (2020) describes agroforestry systems as multifunctional systems with the potential to provide a diverse range of economic, sociocultural, and ecological benefits. Therefore, there are economic and ecological interactions among the agroforestry system components. Silvoarable, silvopastoral and agrosilvopastoral systems are widely recognised as the major agroforestry systems (FAO 2020). Silvoarable (agrosilvicultural) and silvopastoral systems involve the cultivation of trees in combination with conventional crops and pastures respectively, whereas an agrosilvopastoral system involves maintenance of trees, crops and pastures in one system. However, these three major agroforestry systems can be further subdivided into more characteristic agroforestry systems such as improved fallows, homegardens, and taungyas. Other agroforestry systems are defined based on specific tree species which form the integral part of the system. Cocoa agroforests, for example, are plantations of multiple species dominated by trees of *Theobroma cacao*.

Cocoa agroforests are widespread in Asia, Latin America and Africa (FAO 2020), and therefore vary in structure and composition according to the regions. However, they are similar in that the cocoa trees usually occupy the lower strata of the agroforest and

therefore benefits from the provision of shade and microclimate by other tree species which dominate the higher canopies. Despite having provided farmers in Cameroon with food, medicinal plants and income for over 70 years, cocoa agroforests only became widely adopted in the mid-1980s (Malleson 2001; FAO 2013). Inspired by a mid-1980s drop in cocoa price, Cameroonian farmers began to widely supplement their income by incorporating fruit trees into their cocoa plantations. To date, *Garcinia kola* is regarded as one of the major shading tree species in cocoa agroforests in Cameroon (FAO 2013). This practice, besides providing shade cover, further reduces land degradation and contributes to regular and stable rural family income generation through product diversification (FAO 2013).

When designed and implemented correctly, agroforestry systems provide several benefits to farmers and the environment (FAO 2013). Agroforestry is a simple and inexpensive farming practice that is available to almost all farmers and it has the potential to address numerous agriculture-related issues such as poor productivity, sustainability, and biodiversity and environmental conservation. There is a growing body of scientific literature on the multiple beneficial roles of agroforestry (Schroeder 1994, Current et al. 1995; Franzel et al. 2001). However, as Leakey (2010) put it summarily, agroforestry is unique in that it is the only agricultural system which combines the following three attributes altogether:

(i) making use of underutilised and marketable indigenous tree products for income generation and the enhancement of local livelihoods

(ii) producing complex, mature, and functioning agroecosystems similar to natural woodlands and forests

(iii) producing linkages with culture through food and other products of traditional importance to local people.

Agroforestry has been demonstrated, among several other benefits, to sequester carbon from the atmosphere (Shi et al. 2018), innovate diversified farm enterprises (Orwa et al. 2009) and to make agricultural landscapes more resilient (Vågen & Winowiecki 2013). Carsan et al. (2014) highlighted how agroforestry can sustain agricultural intensification through the regulation of ecosystem functions such as nutrient recycling, water use, species diversity and agrochemical contamination. Simons and Leakey (2004)

even suggested that the most viable remedy to the current levels of global deforestation lies in incorporating trees onto agricultural land, i.e., agroforestry.

However, despite the enormous well-documented potential benefits, it is quite clear that agroforestry is not a solution to all food security and environmental degradation problems. Sometimes there are factors, such as local environmental laws, discouraging farmers from adopting the practice (FAO 2013). For example, to curb the prevalent unsustainable timber harvesting practices destructive to rainforests, some South American governments introduced laws which inadvertently restricted the harvesting and transport of timber produced on farms through sustainable practices such as agroforestry (Detlefsen & Scheelje 2012). In other regions, farmers are generally disinterested in agroforestry involving tree species that they perceive would lower the yield of their primary crop, hinder the smooth movement of farm machinery, promote pest infestation and those that would compete with the primary crop for water and other resources (FAO 2013). It is therefore important that the choice of promoted tree species, besides easily adapting to local weather conditions, is technically, socially and economically favourable to the farmers. For these and other reasons, numerous research institutions work in collaboration with farmers to assess the viability of various agroforestry practices.

Multipurpose trees are the fundamental concept of agroforestry (Lojka & Preininger 2006). By definition multipurpose trees are; "trees and shrubs which are deliberately kept and managed for more than one preferred use, product, and/or service; the retention or cultivation of these trees is usually economically but also sometimes ecologically motivated, in a multiple-output land-use system" (Lojka & Preininger 2006). Farmers benefit from multipurpose trees through the various products or services offered by the trees. Common products include fuelwood, pulp for paper, resin or latex, leaves, fruits, roots, fodder, medicine, and construction materials. Services derived from multipurpose trees commonly include shelter, control of soil erosion, improvement of soil fertility, maintenance or improvement of soil structure and the conservation of biodiversity (Roshetko & Verbist 2000). Products that are obtained from agroforests are termed Agroforestry Tree Products (AFTPs) (Simons & Leakey 2004). Diversity within multipurpose tree species populations has led to the evolution of ideotypes, which are better adapted to local conditions. Farmers typically take advantage of these ideotypes for the improved AFTPs and services they offer.

In agroforestry, significant resources are channelled towards the identification of the species, varieties, ideotypes or cultivars of multipurpose trees that are most likely to accomplish certain functions (Lojka & Preininger 2006). As such, in the 1990's ICRAF began a global initiative, partnering local stakeholders to identify species that had the potential to provide multiple benefits to farmers, but more specifically the best performing ideotypes of those species (Wiesberg et al. 2016).

2.2. Tree domestication

Smallholder farmers in tropical rural areas rely on wild tree species as a source of nutrition and other services such as shading, fodder and medicine. Due to deforestation, overexploitation and forest fragmentation, some of the species have not only begun to decline in population numbers but have also weakened in diversity and the quality of the Agroforestry Tree Products (AFTPs) they produce (Weber et al. 2001). Naturally, this situation prompted farmers to select from the wild species of their interest and begin their cultivation in the fields, thus also contributing to the conservation of the vulnerable species. In the other scenario, farmers have traditionally been inspired to maintain in their homegardens highly performing ideotypes. Both schemes have led to the traditional or cultural plant/tree domestication by smallholder farmers in tropical rural areas. Tropics are hotspots of biodiversity, upon which resource-poor families rely for the trade of various AFTPs to sustain their livelihood (Schreckenberg et al. 2006). This is why the tree domestication programme was initiated by ICRAF in the mid-1990s, also inspired by a pursuit for sustainable methods for poverty reduction in developing countries (Simons & Leakey 2004). Initially focusing on a few indigenous species in isolated regions of the world, ICRAF later standardised the tree domestication strategy and expanded it globally. To date, researchers have become involved either by leading the process or helping through conducting joint research in a process now referred to as "participatory tree domestication (PTD) programme".

Alternatively defined, tree domestication is a process encompassing the socio-economic and biophysical processes involved in the identification, characterization, selection, multiplication and cultivation of high-value tree species in managed ecosystems. Domestication of a plant population is therefore a "co-evolutionary process

by which human selection of the phenotypes of promoted, managed or cultivated individual plants results in changes in the descendent population's phenotypes and genotypes that make them more useful to humans and better adapted to human management of the landscape" (Clement 1999). Roshetko and Verbist (2000) described tree domestication as "the naturalisation of a species to progress its cultivation and use by humanity". Tree domestication is therefore a form of *in-situ* conservation which serves to enhance the AFTPs and services of agroforestry systems (Jamnadass et al. 2019).

The term "tree domestication" is often incorrectly applied. There is a distinct difference between tree domestication and classical tree improvement (Simons & Leakey 2004). The major difference lies within the selection process and choice of species for improvement (Franzel et al. 1996). In conventional plantation forestry the focus is on a single end-product which already has abundant scientific background data and is traded mostly by companies and governments, whereas in tree domestication a single tree species with limited background research data is utilised for numerous products and services, by a more diverse group of clients (Franzel et al. 2008). Furthermore, tree domestication involves perennial woody species and is a far more recent phenomenon relative to classical annual crop domestication (Simons & Leakey 2004).

Several studies have demonstrated that women and children, vulnerable segment of most rural communities in developing countries, are the major beneficiaries of most of these indigenous fruits (Ndoye et al. 1997; Wynberg et al. 2003; Schreckenberg 2004; Schreckenberg et al. 2006). Indigenous fruit trees also present opportunities for low-income countries to meet their development goals, such as poverty reduction, environmental sustainability, improvement of health and advancing education levels (Schreckenberg et al. 2006). Furthermore, the tree domestication programme is beneficial in promoting food and nutritional security, it diversifies farmers income opportunities, and it builds on traditional and cultural uses of AFTPs of domestic and local commercial importance (Simons & Leakey 2004). Through the promotion of local level processing and entrepreneurship, tree domestication creates employment and promotes off-farm economic development (Simons & Leakey 2004). All these benefits can inspire a self-help approach to development and present poor people an opportunity to empower themselves. Simons and Leakey (2004) further described the programme as one that has immediate impact and bypasses several potential delays which are characteristic of

traditional international aid flow patterns, thereby going straight into implementation at a village level. Therefore, simply put, the tree domestication program aims to improve the living standards of resource-poor farmers, conserve biodiversity and reduce environmental degradation in the tropics (Weber et al. 2001; Simons & Leakey 2004).

2.2.1.   Participatory Tree Domestication

Tree domestication is most effectively achieved by combination of traditional and scientific knowledge (Fondoun & Manga 2000). Therefore, participatory tree domestication involves researchers collaborating with farmers by offering their advice and in some cases conducting dual on-farm research (Leakey & Akinnifesi 2008). Thus, an effective domestication strategy requires close cooperation between farmers and researchers from the beginning (Weber et al. 2001). Due to this cooperation between farmers and researchers, participatory approaches to tree domestication are preferred for the advantage of building on already established traditions and culture, while speeding the species adoption by farmers to enhance livelihood and environmental benefits (Leakey et al. 2003; Simons & Leakey 2004). Fondoun & Manga (2000) described the *G. kola* cultivation in Cameroon as a form of *in-situ* conservation which involved the following four major strategies;

1) Transplanting of regenerants in tree-based cropping systems such as homegardens and cocoa fields
2) Selective land clearing and protection of regrowth in cocoa plantations
3) Nursery development and creation of artificial plantations in mixed cropping
4) Community effort to protect illegal exploitation of the plants in the wild particularly by 'outsiders'.

The participatory tree domestication procedure standardised by ICRAF is a multi-step process, which involves several distinct stages and multiple stakeholders. These stakeholders are; the rural households, research scientists, development practitioners and policy makers (Akinnifesi et al. 2008). Farmers are vital in that they are the principal beneficiaries of tree domestication; they are best able to recognise their needs in a research programme and they possess valuable ethnobotanical knowledge which is

instrumental in furthering the research programme. The domestication strategies employed for individual species vary depending on the purpose of their use, biology, target environment as well as on the domesticator/researcher or farmer. The ICRAF-standardised procedure consists of these steps; (Franzel et al. 1996; Akinnifesi et al. 2008);

1) Prioritisation of species
2) Selection of elite ideotypes
3) Development and applying efficient vegetative propagation and nursery management techniques for producing quality propagules of on-farm dissemination
4) Integration of improved germplasm into farming systems
5) Post-harvest handling, processing and marketing research of fresh and processed products from domesticated species.

Numerous species in all ecoregions have the potential to be domesticated for the production, sale of marketable AFTPs and to be utilised for their various services (Simons & Leakey 2004). For example, the rural communities in Burkina Faso, Mali, Niger and Senegal were reported to value more than 115 indigenous tree species for their products and services (Faye et al. 2011). However, only a few selected species can be accommodated for participatory domestication at a time. Hence "prioritisation", the first stage of domestication, was designed to identify and select high-value tree species that would potentially have the greatest impact on the local communities (Franzel et al. 2008). Another standardised sequence of steps is used to come up with the list of species during the process of prioritisation;

1) Team building among stakeholders to agree on approaches and refine the method to local conditions
2) Identifying clients and assessment of users' needs (farmers, marketers, etc.)
3) Inventory of all species used by clients, including potentially useful ones
4) Identifying the most important products in the target region, considering only those with the greatest importance
5) Selection of a few species with the highest impact
6) Estimating the production value of key species to set priorities

7) Synthesizing previous results, reviewing the process and selecting the final choice of species (Franzel et al. 1996).

Prioritisation is important because it takes into consideration the ease of researchability of a species, expected adoption rates and the extent to which the species is likely to benefit the vulnerable members of the community such as women in rural areas (Franzel et al. 1996; Akinnifesi et al. 2008).

When prioritisation and identification of species are complete, cultivar development of the selected species follows. The identification of species involves the selection of ideal ideotypes of the species from the wild, their testing followed by introduction into breeding programmes (Leakey & Akinnifesi 2008). To select the best ideotype, mature trees which have already expressed their genetic potential at a particular site over many years of growth, are selected, propagated vegetatively and the propagules are planted either in clonal performance trials or directly into compatibility trials. Despite the potential use of sexual reproduction through seed germplasm being an alternative for plant propagation, clonal breeding strategies such as vegetative propagation have widely been adopted for species that produce fruits, nuts and medicinal products (Leakey & Assah 2013). This is largely due to their speed and efficiency in fixing the genetic traits into the developed final cultivar.

When carried out according to the set guidelines and up to completion, PDTPs have several potential benefits to all stakeholders involved. However, the major benefits of tree domestication are the conservation of species that are vulnerable or threatened with extinction and the improvement of the livelihood of farmers through the provision of diverse AFTPs and their marketing. Despite domesticating indigenous fruit trees being a route to harnessing the potentials of genetic diversity and indigenous knowledge in rural communities, it has its drawbacks. The major constraint is that it results in shifts and/or losses in underlying genetic diversity in cultivated tree populations (Jamnadass et al. 2000; Cornelius et al. 2006).

2.3. Genetic diversity of tree species

"Biodiversity is the variety of life at genetic, species and ecosystem levels" (FAO 2019). Diversity within a species can be either genetic or phenotypic. Phenotypic or

morphological diversity is the variation of physical characteristics from one organism to the other, whereas genetic diversity is the variation in the hereditary material, that is nucleic acids of which genomic deoxyribonucleic acid (DNA) is the major basis of genetic diversity studies. In agriculture, plant genetic diversity is key to production as it increases resilience to shocks and stresses, offers opportunities to adapt to emerging challenges such as climate change, and is an instrumental resource for sustainable ways of increasing production (FAO 2019). Plant genetic diversity in the agricultural sector is therefore indispensable for food security and has increasingly become recognised in international policy programmes through strategies such as tree domestication, an *in-situ* conservation strategy (Carsan et al. 2014; FAO 2019).

The tree domestication procedures encompass the maintenance and use of three interlinked populations (Leakey and Akinnifesi, 2008), which are; i) the gene resource population for genetic conservation; ii) the selection population, for the development of improved cultivars; and iii) the production population, utilised by farmers. The process, therefore, has an impact on both genetic and phenotypic diversity of the "selection" and "production" populations of a tree species. Generally, in both these populations, phenotypic diversity is reduced for features on which the desirable characteristics are selected, such as fruit size and taste. Due to the selection, domesticated populations typically have a lower genetic variation (Weber et al. 2001; Cornelius et al. 2006). The degree of induced change in tree populations varies along with a range from the wild (which lacks human-induced variation), through the incipiently domesticated, to semi-domesticated and domesticated.

While the selection of high-performing ideotypes is important when selecting tree populations for cultivation, genetic diversity is required to enhance the capacity of germplasm to adapt to changing user requirements and environmental conditions (Simons et al. 1994). Most farmers generally utilise germplasm from a narrow range of parental generation for plantation establishment (Lengkeek et al. 2006), which is partly attributed to the farmers' poor comprehension of germplasm quality (Roshetko & Verbist 2000). However, the selection of germplasm with low genetic diversity for use in the production population is thought to be detrimental by leading to serious inbreeding problems in subsequent generations (Leakey et al. 2003). Generally, inbreeding makes a plant population more vulnerable to a complete destruction for example by pests and diseases.

It, therefore, appears ideal that greater genetic diversity is perpetuated within plant populations through the deliberate selection of relatively large numbers of unrelated ideotypes (Simons & Leakey 2004). Inbreeding also reduces the vigour and productivity of future species generations (Weber et al. 2001). When properly maintained, genetic diversity can provide various genes and alleles combinations, that produce plant cultivars upon which agriculture relies. Genetic diversity in plants therefore plays a fundamental role in satisfying numerous basic needs of local communities in tropics (Dah-Nouvlessounon et al. 2016). Tree domestication practices can therefore only have a conservation function if the germplasm included is genetically diverse (Weber et al. 2001). Despite the importance of genetic diversity, Lengkeek et al. (2006) suggested that the intraspecific genetic diversity of the on-farm trees established during the development of tropical agroforestry is mostly uncharacterised. The characterisation of existing genetic diversity in the gene resource population is therefore a vital first step in the utilisation and preservation of genetic diversity for future uses (Weber et al. 2001).

Sophisticated molecular techniques are required to assess the overall genetic diversity of ideotypes (Weber et al. 2001). Recent developments in molecular genetic techniques coupled with geographic information systems present a great opportunity to assess and manage the diversity of tropical trees. The application of landscape genetics, which takes into consideration species population genetics and aspects of landscape topography can provide insights into microevolutionary processes, such as genetic drift and selection (Manel et al. 2003; Manel & Holderegger 2013). This approach is advantageous in combining the high-resolution property of molecular techniques with spatial data and statistical methods to assess the role that landscape variables play in influencing genetic diversity and population structure (Storfer et al. 2007). This study, therefore, took into consideration the spatial distribution of *Garcinia kola* in assessing the genetic variation among the plants in the Central region of Cameroon.

3.   Genetic markers

Traditionally, before the discovery of molecular markers, genotype identification research was based on morphological markers such as height, weight, and quality parameters such as colour (Mba & Tohme 2005; Larranaga & Hormaza 2016). However, besides being a slow and expensive process, which not only limits the number of studies that can be carried out simultaneously, morphology-based studies had also low accuracy (Mba & Tohme 2005). The low accuracy is largely due to the fact that morphological traits are influenced by environmental factors (Larranaga & Hormaza 2016). Genetic markers, an alternative to morphological markers, are unique sequences of DNA which enable the identification of particular species or organisms. Genetic marker technology has developed over time since it was first used in the 1980s (Mba & Tohme 2005; Larranaga & Hormaza 2016).

Generally, depending on their ability to distinguish between heterozygous and homozygous genotypes, genetic markers can be classified into two main categories; dominant markers, such as Random Amplified Polymorphic DNA (RAPDs) and Amplified Fragment Legnth Polymorphism (AFLPs) or codominant, such as Restriction Fragment Polymorphism (RFLPs), microsatellites, or Single Nucleotide Polymorphism (SNPs). Codominant markers permit the identification of all the alleles positioned on a specific locus, whereas dominant markers reveal only a single dominant allele (Freeland et al. 2011). Another important distinguishing feature that is considered when selecting genetic markers is their ability to assay several loci at a single time (Mba & Tohme 2005). Since their discovery by Jeffreys (1979), RAPDs found wide use in the assessment of fruit tree genetic diversity. However, in recent times they have had relatively less utilisation owing to the development of more recent genetic markers (Larranaga & Hormaza 2016). Among the dominant markers, Inter-Simple Sequence Repeat (ISSR) markers arose as a potential tool in plant genomic studies especially in cultivar identification (Wang 2002). The ISSR marker provides a rapid, consistent and highly informative tool for DNA fingerprinting (Wang 2002). After the development of the Polymerase Chain Reaction (PCR) by Kary Mullis in 1983 (Mullis et al. 1986), a technique that amplifies the amount of DNA, PCR-based genetic markers were subsequently developed.

3.1. AFLP markers

AFLPs were subsequently developed in the 1990s (Vos et al. 1995) and have since been used in studies of fruit trees such as *Prunus mira* (Li et al. 2014), *Phoenix dactylifera* (Sabir et al. 2014), and the *Actinidia species complex* (Li et al. 2014). This DNA fingerprinting technique combines restrictive digestion of genomic DNA with selective amplification to create a cluster of fragments and form precise profiles for separate organisms (Partis et al. 2007). AFLPs are "DNA fragments, usually in the range of 80–500 base pairs that are obtained from endonuclease restriction, followed by ligation of oligonucleotides to the fragments and selective amplification by PCR" (Mba & Tohme 2005). AFLP assay is therefore a PCR-based technique which makes use of restriction enzymes to produce DNA fragments. Resultant oligonucleotides are ligated with adaptors to the sticky ends to produce priming sites for amplification of a subset of fragments. This produces a complex mixture of fragments. The amplified fragments are separated and visualised by chromatographic techniques or by automated capillary sequencing techniques. The AFLP assay is unique in that the resulting data is not scored as length polymorphisms, but rather as presence-absence polymorphisms (Vos et al. 1995). The AFLP assay can be separated into the following distinct stages (Mba & Tohme 2005; Arif et al. 2010; Paun & Schönswetter 2012);

1) Extraction of highly purified DNA
2) Restriction of genomic DNA (enzyme mixture, usually *Eco*RI + *Mse*I).
3) Ligation of adaptors; (Double-stranded adaptors, specific to *Eco*RI and *Mse*I sequences, are added with the help of a ligase enzyme)
4) Preselective PCR amplification of a subset of the restricted fragments
5) Selective PCR amplification, reducing further fragment number; labelled primer pair (Primer + 3 base pairs; forward labelled, reverse unlabelled)
6) Electrophoretic separation and analysis of amplified DNA fragments
7) Scoring and interpretation of the data.

According to Paun & Schönswetter (2012) the success of AFLP assay depends, among other factors, on a robust and reliable electrophoresis platform. Capillary electrophoresis, also referred to as high-performance capillary electrophoresis (HPCE) makes use of very narrow-bore tubes, typically 50 µm and 300 µm internal and external

diameter respectively, to separate biological molecules electrophoretically (Walker 2005). The microscale nature of the capillaries used, combined with the capacity for on-line detection of even femtomole level sensitivity makes capillary electrophoresis the method of choice for numerous assays (Walker 2005). Capillaries are advantageous because they reduce problems emanating from heating effects (Walker 2005). Relative to gel electrophoresis methods, capillary electrophoresis has numerous other advantages such as a high separation efficiency, short analysis time and low waste generation. Hence in this study the AFLP assay was coupled with capillary electrophoresis.

3.2. Uses and advantages of AFLP markers

AFLPs are applicable to all organisms (Arif et al. 2010). AFLP markers have been extensively used for phylogenetic analysis and determining the genetic diversity for the conservation of numerous neglected or endangered plant species (Arif et al. 2010). AFLP markers use random primers to amplify fragments of DNA. Hence, the strength of AFLP markers is in their potential to quickly reproduce several sets of marker fragments for organisms, without prior knowledge of the genomic sequence (Freeland et al. 2011). Furthermore, as a dominant marker, AFLPs entail a lesser development time and they are a more convenient way to obtain data compared to codominant markers (Freeland et al. 2011). Mba and Tohme (2005) suggested that the ideal genetic marker for utilisation in diversity studies needs to be robust, accurate, reproducible, highly informative, and cost-effective. Furthermore, the ideal marker should produce data in a manner that can be easily put into databases and is suitable for automation (Mba & Tohme 2005). Mba and Tohme (2005) suggested that AFLP markers' increasing popularity is largely due to their ability to meet all these attributes. The capacity to create numerous polymorphic bands for each assay is one of the greatest strengths of the AFLP technique (Mba & Tohme 2005). However, Freeland et al. (2011) proposed that their greatest strength lies in that they do not need prior knowledge of the species' genome, while at the same time enabling a relatively wide assessment of the genome. In contrast to RAPDs and ISSRs, AFLPs have higher reproducibility (Paun & Schönswetter 2012). AFLP is a relatively labour-intensive method, however, it can be easily multiplexed and is frequently used to amplify hundreds of genomic fragments from hundreds of individuals in the same batch (Larranaga & Hormaza 2016).

AFLP markers have a wide use in plant DNA fingerprinting. In their use of AFLP markers to investigate the genetic diversity of *Guazuma crinita*, Tuisima-Coral et al. (2020) described the technique as an effective tool for assessing the genetic diversity of indigenous tree populations at varying stages of domestication. Other indigenous fruit tree of the tropics which have been studied with by utilising AFLP markers include *Swietenia macrophylla* (Lowe et al. 2003), *Simarouba amara* Aubl (Hardesty et al. 2005), *Bulbophyllum occultum* (Jaros et al. 2016), *Calycophyllum candidissimum* (Dávila-Lara 2017), and *Canarina eminii* (Mairal et al. 2017). These, and other numerous well-documented global applications of AFLPs in the assessment of intraspecific genetic diversity, interspecific diversity, examinations of population-level phylogenies and biogeographic patterns, creation of genetic maps and investigations of similarities among cultivars (Paun & Schönswetter 2012), make AFLP method an ideal tool to assess the genetic diversity of *Garcinia kola* populations in Central Cameroon.

4. *Garcinia kola* Heckel

4.1. Botanical description

Garcinia kola Heckel, Synonyms; *Garcinia bergheana* Spirlet, *Garcinia akawaensis* Spirlet, *Garcinia giadidii* De Wild (World Flora Online (WFO) 2020), is most commonly referred to as Bitter kola, False kola or Male kola within its range states. *G. kola* belongs to the Clussiaceae family (JSTOR Global Plants (JGP) 2020). *G. kola* is a medium-sized tree which grows to a maximum height of about 40 m (Maňourová 2017) however, it generally grows to a range of 12 to 15 m (Dah-Nouvlessounon et al. 2016; JGP 2020). *G. kola* is a spreading tree with a dense crown; the bole is straight; and as shown in Figure 1, the bark is greenish-brown, thick, and smooth (JGP 2020). Typical of other species of the Clussiaceae family, when damaged the tree trunk exudes a creamy-white latex (JGP 2020). *G. kola* has broad leaves, five to ten cm long, elongated elliptic to broadly elliptic, acute or shortly acuminate, cuneate, leathery, with very distinct resinous canals. *G. kola* has ten pairs of lateral veins that run parallel to the margin but not forming a marginal nerve; the midrib is prominent at the underside; the stalk is stout, finely hairy in young leaves, and about eight mm long (Iwu 2014; JGP 2020).

Bearing male and female puberulent flowers separately, the tree flowers either once or twice a year, with the exact period varying with the nature of the local environment (Dah-Nouvlessounon et al. 2016; JGP 2020; WFO 2020). Female flowers are yellow and fleshy, globose, 1.5 cm wide; male flowers are smaller but with more prominent stamens (four bundles), four sepals, and four greenish-white petals (Iwu 2014; WFO 2020). *G. kola* fruits are characteristically large (approximately six cm in diameter), reddish, yellowish or orange berries, globular to pyriform and contain two to four brown seeds which are embedded in an orange-yellow coloured sour-tasting pulp (Ebomoyi & Okojie 2012; Iwu 2014; Dah-Nouvlessounon et al. 2016; WFO 2020). The seeds are ellipsoid, 2-4 cm long, surrounded by a quite sticky brown seedcoat, with branched lines, the kernels light-coloured and penetrated with pockets of resin (WFO 2020). Despite research indicating that *G. kola* can be propagated through vegetative means, Maňourová (2017) reported that vegetative propagation methods are currently not widely practised, and they still require further development.
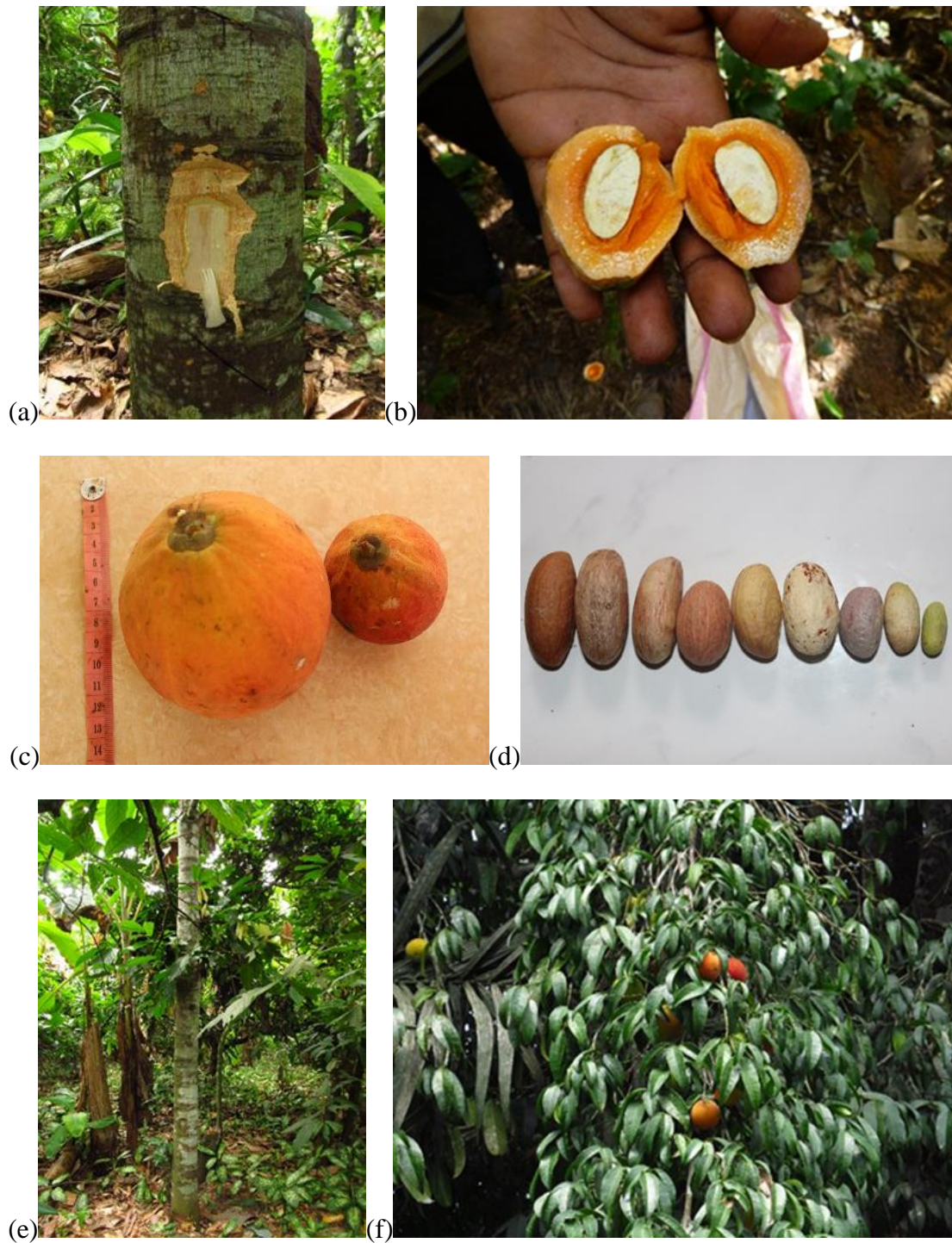
Figure 1. *Garcinia kola* plants parts and tree habit; (a) tree trunk with bark incision; (b) a cross-section of the fruit and seed; (c) mature ripe fruits; (d) seeds at varying stages of maturity; (e) tree in a homegarden (f) tree crown. Source: Maňourová Anna (Private collection).

4.2.   Origin and distribution

Garcinia kola is mostly found in the understory of tropical forests (Ebomoyi & Okojie 2012; Iwu 2014; JGP 2020), degraded forests and bush fallows (Fondoun & Manga 2000). The natural distribution range of *G. kola* is limited to Africa where it occurs throughout the West and Central part, particularly in the following countries; Benin, Cameroon, Central African Republic, Congo Republic, Côte d'Ivoire, Democratic Republic of Congo, Equatorial Guinea, Gabon, Ghana, Liberia, Nigeria, Sierra Leone and Togo as shown in Figure 2 (Cheek 2004, Iwu 2014), with Cameroon and Nigeria being the hotspots for *G. kola* presence (Maňourová et al. 2019). In tropical forests, *G. kola* trees are usually cut down to enable the harvesting of the medicinal bark, chewing sticks and palm wine production (Agyili et al. 2007), these destructive harvesting methods coupled with poor natural seed germination rates have led to a decline in populations and the species now classified as "Vulnerable" (Cheek 2004). Restoration programmes have therefore been recommended (Agyili et al. 2007).
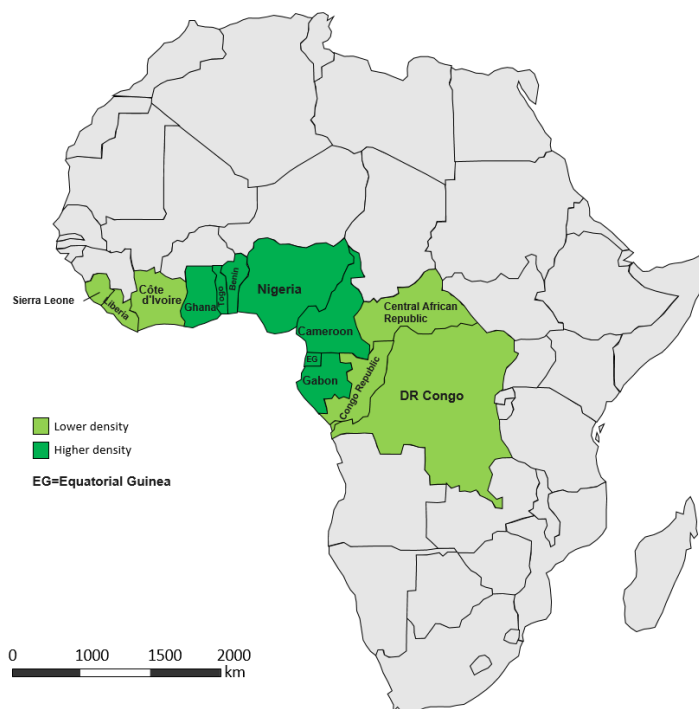
Figure 2. The distribution map of *Garcinia kola*. Source: Maňourová et al. 2019.

4.3.   Uses

Garcinia kola is a multipurpose tree of significant ethnobotanical value and a long history of human use (Iwu et al. 2002). *G. kola* has significant social applications as it is often offered as gifts to visitors (Ayuk et al. 1999). It is widely referred to as the "wonder plant" because almost all the plant parts are known to be utilised in traditional medicine (Ebomoyi & Okojie 2012; Maňourová et al. 2019). *G. kola* seeds, the most valued product of the tree, exhibit a bitter and astringent taste earning its common name "bitter kola" (Ebomoyi & Okojie 2012). The name "male kola" coins the aphrodisiac effect of *G. kola* seeds, especially on men (Farombi et al. 2013). Other plant parts utilised include fruits, bark, twigs and leaves. The younger trees are a source of chewing sticks for dental care (Iwu et al. 2002; Agyili et al. 2007). However, prior to use as chewing sticks, stems and twigs are split (Dawson et al. 2012), a harvesting method which is highly destructive to the trees. Whilst the fruit pulp is edible, it is frequently discarded because of its poor taste. In Cameroon, fruits are harvested from June to November while the other plant parts are harvested continuously throughout the year (Fondoun & Manga 2000).

G. kola is highly valued and extensively utilised for its medicinal properties (Iwu 2014). It is taken to repel emerging flu and sore throat, and as a poison antidote (Iwu et al. 2002). The seeds are an integral component of herbal formulas used by traditional healers, especially in the treatment of respiratory illnesses (Ebomoyi & Okojie 2012). Among the numerous medicinal uses of *G. kola,* seeds are utilised as a remedy for colic of infants (Iwu et al. 2002), headaches, stomach aches and gastritis, jaundice and fever (Iwu 2014), and as a laxative (Iwu 2014). Furthermore, other known ethnomedicinal uses are in the treatment of liver disorders, laryngitis, bronchitis, and gonorrhoea (Iwu 2014). The peeled stems and the cut twigs are sometimes soaked in bottles of water to "mature" over several days and form an aphrodisiac concoction (Iwu 2014). Ethnomedicinal records show that the stem bark is used as a purgative, the powdered bark for treating malignant tumours and the sap for treating parasitic diseases (Iwu 2014). The latex is used to treat gonorrhoea and applied to open wounds (Iwu 2014). Furthermore, *G. kola* has shown potential for future pharmacological uses in several physiological studies. The species has shown varying levels of inhibitory effects on several human viral pathogens such as influenza A, Ebola viruses, malaria-causing *Plasmodia* and *Vibrio cholerae* (Iwu et al. 2002).

Several groups of bioactive natural compounds, such as flavonoids and tannins, are responsible for the medicinal properties of *G. kola*. (Iwu 2014). Therefore, the species exhibits antioxidant, anti-bacterial, anti-viral, anti-fungal, anti-diabetic, bronchodilatory, anti-inflammatory and antihepatotoxic properties (Iwu et al. 2002; Ebomoyi & Okojie 2012). The most significant biomolecule found in *G. kola* is the kolaviron biflavonoid complex (KV) (Maňourová 2019). Besides exhibiting, sedative and anti-inflammatory effect, KV has been reported to possess depressant effects on mice and increasing the life span of *Drosophila melanogaster* through the prevention of inflammation and oxidative stress (Ibironke & Fasanmade 2015; Onasanwo & Rotu 2016; Farombi et al. 2018). *G. kola* therefore appears like a good candidate in the bioprospecting of compounds for the development of new drugs. However, despite the absence of proven harmful overdosing cases, there appears to be some concern with toxicity derived from the long-term consumption of *G. kola* (Farombi et al. 2013). *G. kola* has also applications in the brewing industry where it is used as a ferment in palm wine production and as an alternative to hops in beer (Fondoun & Manga 2000; Ajebesone & Aina 2004).

Generally, the value and income generated from *G. kola* products increases along a market chain from a farm level over rural to urban markets (Onyekwelu et al. 2015). The seeds are not only the most commonly utilised *G. kola* vegetative organ, but they are also among the most traded non-timber forest products (NTFPs) within the Central African region. Reported annual income figures generated either by farmers or traders involved in *G. kola* products tend to vary but they are in the range of 300-1,300 USD (Fondoun & Manga 2000; Onyekwelu et al. 2015). Awono et al. (2016) reported a total annual trade value of around 660,000 USD in Cameroon. Regional trade in *G. kola* plant parts is reported to be quite popular among economic migrants within the region, however, trademarked dietary supplements with *G. kola* extracts already exist in the United States of America and other african markets apart from West and Central African region (Iwu et al. 2002).

4.4. Domestication of *Garcinia kola*

The genetic diversity of *G. kola* populations is largely threatened by unsustainable harvesting methods and poor natural regeneration of the species. Jusu and Sanchez (2014)

reported that in some cases, marketed *G. kola* products are transported for distances exceeding 100 km, which is a strong indication of depletion in surrounding areas, including forest reserves. Agyili et al. (2007) pointed out that there is an apparent pressing need to domesticate *G. kola*, secure a resource base for planting programmes, and preserve its genetic diversity. Therefore, the tree is among the species chosen for immediate conservation action in the sub-Saharan forest genetic resources (SAFORGEN) programme (Sacande et al. 2004). Participatory methods are currently being implemented in *G. kola* domestication in Cameroon, and the species is regarded as incipiently domesticated (Maňourová et al. 2019). According to Clement et al. (2010), this implies that the cultivated population has gone through a founder effect as humans selected lines from the wild *G. kola* lines with features desirable to them.

Fondoun and Manga (2000) reported that in all regions of Cameroon, farmers who protected *G. kola* in natural stands constituted the majority compared to those who established seedlings in homegardens. However, FAO (2013) reported that the majority of these trees were ageing and in need of regeneration. Onyekwelu et al. (2015) recommended that domestication efforts should aim to reduce the tree height, increase fruit production, increase seeds/kernel size and improve the taste. Earlier domestication efforts were to some extent stalled by the slow and irregular germination of seeds (Agyili et al. 2007). However, the situation may improve with recent studies suggesting the storage of seeds at 25 °C and the soaking of dehulled seeds in water for 72 hours to improve seed viability and break dormancy respectively (Dadjo et al. 2019). Onyekwelu et al. (2015) also recommended the use of vegetative propagation through grafting of stems from trees which possess desirable traits onto slow-growing high-producing rootstocks. The need to further *G. kola* domestication efforts further emphasises the importance of closing the existing information gaps, especially regarding the genetic diversity. The selection of the best suitable ideotypes for the use in future breeding programs will need to take into consideration the species' genetic diversity. There is a need to identify alleles for different traits. When identified and preserved in the cultivated population, alleles for traits currently less popular with farmers such as rootstock production potential, may find future use especially in plant breeding programmes. According to Maňourová (2017), Bitter kola traits favourable to farmers such as fruit size

or tree height still need to be improved. However, credible studies on *G. kola* with the prerequisite genetic diversity information are still very difficult to find.

5.   Objectives

The main objective of the study was to determine the genetic diversity of *Garcinia kola* populations in the Central region of Cameroon.

The specific objectives of the study were:

1)   To evaluate the levels of genetic diversity within and among populations of *G. kola* with AFLP molecular markers

2)   To assess the potential effects of the first stages of the domestication process on the genetic diversity of *G. kola* in Central Cameroon

3)   To propose strategies for adoption in the domestication efforts of the species.

6.   Materials and Methods

6.1.   Study site

The study was conducted in the Central region of Cameroon. The study area belongs to the zone around 3°05´ to 6°06´ N and 10°16´ to 13°13´ E at its furthest point in each of four cardinal points. The elevation ranged from 400 m in Bot-Makak, to 700 m in Nkelikok. The climate is characterised as tropical by the Köppen-Geiger classification system (Beck et al. 2018). This implies that it experiences high rainfall and humidity. Generally, the weather conditions are not too diverse across the Central region. However, the rainfall tends to decrease from the Southern section towards the North. While the mean annual temperature is about 24 °C, the mean annual precipitation ranges from 1,000 mm to 2,000 mm (Beck et al. 2018). The landscape is largely forested and undulating, with characteristic hills and valleys.

Following the same sampling pattern used in 2016 in the Southwest region (Maňourová 2017), the samples for this thesis were collected in September 2018 in the Central region of Cameroon. Altogether, 96 different trees (Table 1) were sampled from agroforests, homegardens and forest habitats. The sampling sites were marked with GPS (Figure 3), while two fresh mature leaves per individual tree were taken for further laboratory analysis at CZU, laboratory of Molecular Biology, FTA. Before the transportation, the leaves were dried in silica gel to prevent mould infestation. In accordance with outcomes of discussions with ICRAF experts, eight study sites were selected for the sampling: Akok, Bokito, Ebogo, Lekiasi, Ebolowa, Bot-Makak, Nkelikok and Saa; where wild, semi-managed and managed populations were represented. The elevation ranged from 410 masl (Bot-Makak) to 700 masl (Nkelikok) and the mean number of samples per study site was 12 (Table 1).
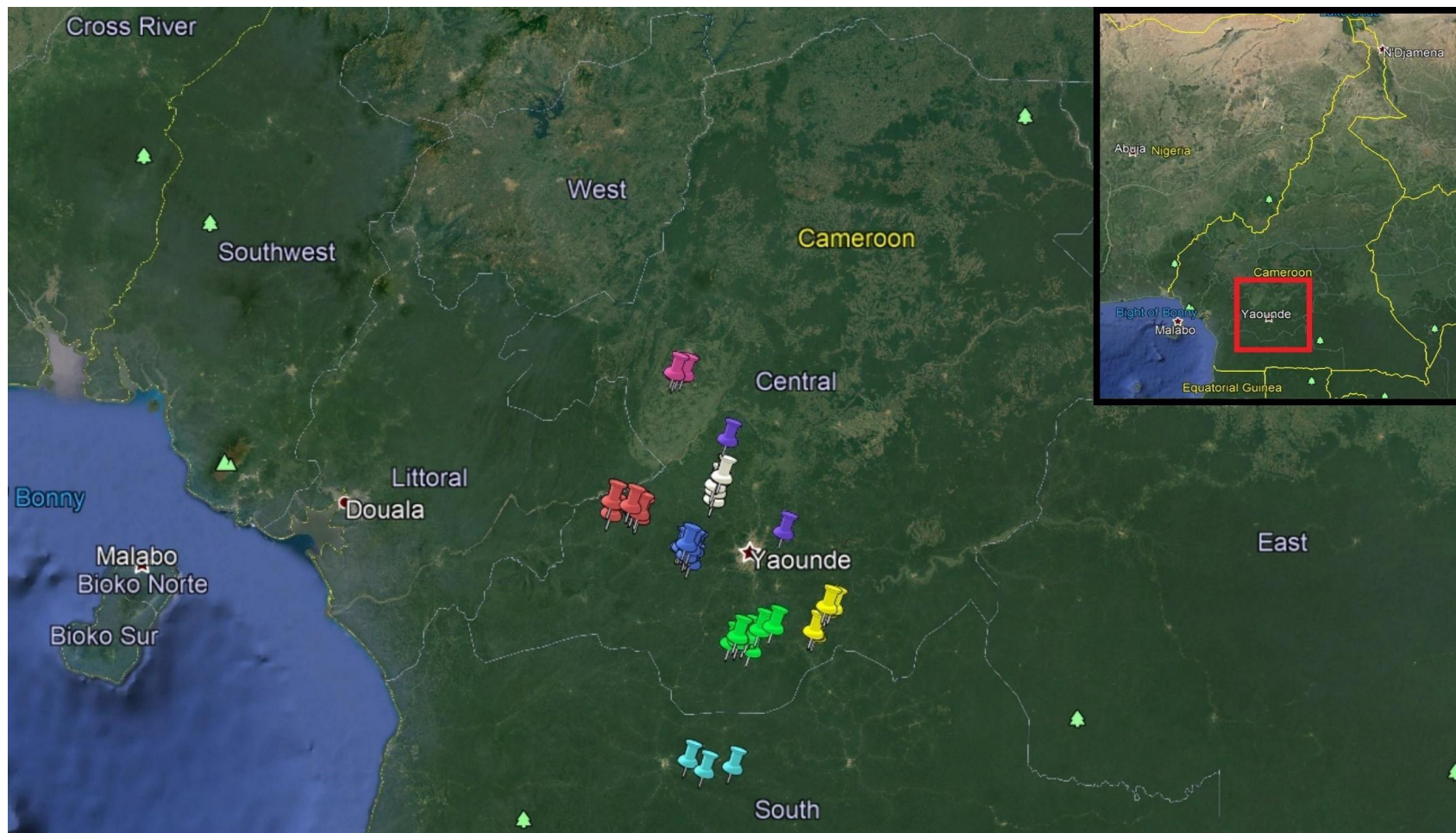
Figure 3. Study area and sampling sites. Sampling locations in Cameroon; Pink-Bokito; Purple-Saa, Red-Bot-Makak; Dark Blue-Nkelikok; Green-Ebogo; Light blue-Ebolowa; White-Lekiasi; Yellow-Akok.

Table 1. Study sampling areas and corresponding samples.

Provenance	Altitude (masl)	Number of Samples	Samples (Code)	Location Coordinates	
				East	North
Akok	690	10	CABD1; CADA1; CADA2; CAMR1; CAMT1; CAMT2; CAMT3; CAMT4; CAOM1; CARS1	11°47'50"	3°26'53"
Bokito	510	7	CBBA1; CBBA2; CBBI1; CBGL1; CBJR1; CBMA1; CBVA	11°09'21"	4°35'14"
Ebogo	710	21	CEBA1; CEBA2; CEBC1; CEBO3; CEBO1; CEBO2; CEBO4; CECE1; CEFX1; CEHE1; CEHE2; CEHE3; CEHE4; CEML1; CEML3; CEML2; CEMT1; CESO3; CESO4; CESO1; CESO2; CESO5	11°29'50"	3°24'23"
Lekiasi	579	15	CLJH2; CLJM1; CLJN1; CLJN2; CLME3; CLME4; CLME5; CLME6; CLNL1; CLNL2; CLNO1; CLNO2; CLOG1; CLTE1	11°20'07"	4°03'53"
Bot-Makak	410	12	CMBE1; CMDS1; CMEL2; CMEL3; CMEL1; CMFC2; CMFC1; CMGM1; CMMC1; CMMC2; CMMC3; CMSI1	10°53'43"	4°00'40"
Nkelikok	700	16	CNAN1; CNCE1; CNEL1; CNEL2; CNJH1; CNJM1; CNJM2; CNJP1; CNJP3; CNJP2; CNNA1; CNSE1; CNSE2; CNUN2; CNUN1; CNZI1	11°13'08"	3°48'15"
Saa	600	3	CSGF1; CSMA1; CSRD1	11°36'34"	4°09'32"
Ebolowa	620	12	SEAO1; SEFE1; SEFE2; SEFE3; SEFE4; SEJL1; SEJL2; SEJL3; SEJS1; SESN1; SESN2; SESN3	11°18'33"	2°48'36"

## 6.2.	Genetic analysis

### 6.2.1.	DNA extraction

DNA extraction was carried out at the Czech University of Life Sciences in Prague (CZU), Czech Republic, in the Laboratory of Molecular Genetics of the Faculty of Tropical AgriSciences. The DNA was extracted from the dried leaves using modified Cetyl trimethylammonium bromide (CTAB) method (Doyle & Doyle, 1987; Faleiro et al. 2002). Dry leaf matter was homogenised with a mortar and pestle with the addition of fine sand. The resultant ground mass was transferred into a 2 ml Eppendorf tube, 800 µl of extraction buffer (Table 2) added and the solution mixed by vortexing. 5 µl of Proteinase K (20 mg/ml) was then added, the solution kept at 65° C for 1 hour with regular mixing at 10-minute intervals and left to cool at room temperature. 700 µl of chloroform:Isoamyl alcohol (IAA) (24:1) was added and the mixture vortexed for 10 minutes. The resulting solution was then centrifuged for 10 min at 14,000 RPM and 4 °C. The supernatant was transferred into a new 2 ml Eppendorf tube, 55 µl of 7 % CTAB added and mixed for 5 minutes. To achieve higher purity, the steps of washing the sample in chloroform: IAA mixture and centrifuging for 10 minutes at 14,000 RPM and 4 °C were repeated. The supernatant of the resultant solution was transferred into 1.5 ml Eppendorf tubes, 700 µl of isopropanol added, the mixture vortexed for 5 minutes and then stored either at -20 °C for one hour or at 4 °C overnight. The solution was centrifuged at 14,000 RPM for 10 minutes. The supernatant was decanted from the white pellet which formed at the bottom of the Eppendorf tube. The pellet was washed by adding 400 µl of 96 % ethanol, leaving at 37 °C for 3 minutes and discarding the ethanol. The pellet was washed again by adding 400 µl of 70 % ethanol, leaving it at room temperature for 5 minutes, centrifuging for 3 min and then discarding the ethanol. The pellet was then left to dry at room temperature before 100 µl of water and 5 µl of RNase were added and left at 37 °C until the pellet dissolved.

The samples were then purified by adding one tenth of their volume of 3 M sodium acetate, pH 5.2 and 2 to 3 times their volume in 100 % Ethanol. The solution was mixed and frozen overnight at -20 °C. The samples were then spun in a centrifuge for 30 minutes at 4 °C and the supernatant decanted. The pellet was air dried by opening the Eppendorf

tube caps for approximately 15 minutes and resuspended in 50 µl of sterile water. The concentration of DNA was determined using a NanoDrop 2000 (Thermo Scientific, USA) spectrophotometer and all samples were diluted to concentration of 500 ng/µl.

Table 2. Composition of extraction buffer.

Reagents	Final Concentration	Quantity for 15 samples
CTAB 7 %	2.8 %	6 ml
NaCl 5 M	1.3 M	4 ml
EDTA 0.5 M	20 mM	0.6 ml
TRIS-HCl (pH 8.0) 1 M	100 mM	1.5 ml
PVP 40	1 %	0.15 g
Mercaptoethanol	0.2 %	30 µl
Water	-	Add to 15 ml

6.2.2. AFLP

6.2.2.1. Restriction-Ligation

The PCR was carried out in the Faculty of Tropical AgriSciences, CZU Prague, in the Laboratory of Molecular Genetics. The composition of the restriction-ligation mixture was as summarized in Table 3. A master mix for all the samples was prepared by mixing the reagents listed in Table 3 in relative proportions and briefly vortexed. 19 µl of master mix was added to individual wells on a PCR plate and followed by 1 µl of DNA and the plates were sealed with adhesive film and briefly mixed by vortexing. The mixture was incubated at 37 °C for 4 hours followed by 65 °C for 20 mins and finally stored at 4 °C. The efficiency of the restriction reaction was tested by gel electrophoresis on a 2 % agarose gel stained by EtBr (Ethidium Bromide) and run at 90 V for 1 hour. The product was diluted tenfold, and the mixture stored at -20 °C for further processing.

Table 3. Composition of the restriction-ligation mixture.

Reagent	Volume (µl)	Concentration
DNA template	1	500 ng
T4 Ligase	0.17	67 U
T4 Ligase buffer	2	
*Eco*RI	0.25	5 U
*Mse*I	0.1	1 U
Cut smart Buffer	4	
*Mse*I adaptors	1	50 pmol/µl
*Eco*RI adaptors	1	5 pmol/µl
H$_2$O	10.48	

6.2.2.2.　　　Preamplification

From the initial stock solution, the preselective primers were diluted and mixed with the Qiagen Multiplex PCR Master Mix (Qiagen, Germany) in the proportion shown in Table 4 and 5 µl of the restriction-ligation product added. The primer sequences are shown in Table 5. The mixture was gently vortexed and put in a thermal cycler whose profile is illustrated in Table 6. In the end, the cycler kept the reagents at 4 °C until they were removed. The efficacy of the preselective amplification was tested by running 5 µl of several samples on 2 % agarose gel stained by Ethidium Bromide (EtBr), at 90 V for 1 hour. The preselective reactions were diluted tenfold and then stored at -20 °C.

Table 4. Preamplification mixture composition.

Reagent	Volume (µl)
R-L product	5
Eco+1 primer	1.5
Mse+1 primer	1.5
Qiagen Master mix	10
Q-solution	2

Table 5. Primer sequences.

Primer	Sequence
*Mse*I+C	GATGAGTCCTGAGTAA**C**
*Eco*RI+A	GACTGCGTACCAATTC**A**

Table 6. Cycler profile.

Temperature (°C)	Time (min)	Touchdown	Repeats
95	15		
95	0.5		10x
62	0.5	-1 °C/cycle	
72	2		
95	0.5		20x
52	0.5		
72	1		
72	10		
4	∞		

6.2.2.3. Selective Amplification

A combination of reagents shown in Table 7 was prepared and 5 µl of the preamplification product was added. The mixture was gently mixed by vortexing and then put in a thermal cycler whose profile is illustrated in Table 9. The selective amplification products were then stored at -20 °C until they were analysed by capillary electrophoresis.

Table 7. Composition of the selective amplification mixture.

Reagent	Volume/µl
PRE-AMP product	5
Eco+3 primer (6-FAM)	1
Mse+3 primer	1
Qiagen Master mix	7

After initial screening of 24 combinations of selective primers, four primer combinations were selected based on amplification results (Table 8). The Eco selective primer was fluorescently labelled at 5' end with 6-FAM dye.

Table 8. Selective amplification primer sequences (selective nucleotides are in bold).

Eco+3 (labelled with 6-FAM)	Mse+3
GACTGCGTACCAATTC**ATT**	GATGAGTCCTGAGTAA**CCT**
	GATGAGTCCTGAGTAA**CTA**
GACTGCGTACCAATTC**AAT**	GATGAGTCCTGAGTAA**CGA**
	GATGAGTCCTGAGTAA**CAT**

Table 9. Selective amplification cycler profile.

Temperature (°C)	Time	Touchdown	Repeats
95	15 min		
95	30 sec		10x
62	30 sec	-1 °C/cycle	
72	2 min		
95	30 sec		20x
52	30 sec		
72	1 min		
72	10 min		
4	∞		

The selective amplification products were separated by capillary electrophoresis on a 3500 Series Genetic Analyzer (Applied Biosystems, USA), in the Laboratory of Molecular Biology in the Faculty of Environmental Sciences, CZU, Czech Republic. The results of fragment analysis were visualized using Geneious Prime 2020.1.1 software (https://www.geneious.com 2020).

6.3.   Data analysis

The data set was inserted into Microsoft Excel spreadsheets and then converted to binary data which indicated the presence or absence of alleles by scoring for band presence (1) or absence (0). Each AFLP band was considered as a single bi-allelic locus with an amplifiable (dominant) and a null (recessive) allele. The data was then analysed using AFLP-SURV 1.0 (Vekemans et al. 2002). AFLP-SURV was used to estimate genetic diversity and the population genetic structure of the analysed population samples. Assuming there are only two alleles, dominant and recessive null alleles (indicated by the presence or absence of a band at a given position respectively), the AFLP-SURV program computed allelic frequencies at all marker loci within each population following the method by Lynch & Milligan (1994). The program further computed the genetic diversity and generated population genetic structure matrices. Genetic diversity was described by the AFLP-SURV program using the following indices;

1) *Hj*: expected heterozygosity under Hardy-Weinberg genotypic proportions (Nei's gen)
2) *F_{ST}*: the proportion of the total genetic variance contained in a subpopulation. Termed the Wright's fixation index, it measures the genetic correlation between pairs of genes sampled within a population relative to pairs of genes sampled within the overall set of populations (also interpreted as the proportion of the total gene diversity that occurs among, as opposed to within, populations)
3) *PLP*: proportion of polymorphic loci, as a percentage, at the 5 % level
4) *#loc_P*: number of polymorphic loci at the 5 % level, i.e., loci with allelic frequencies lying within the range 0.05 to 0.95 (Vekemans 2002).

6.3.1.   Structure Analysis

To infer the genetic structuring of the sampled populations, a model-based Bayesian clustering method was used, implemented by software Structure version 2.3.4 (Pritchard et al. 2000). The program was run with 10,000 burn-in steps, followed by 100,000 MCMC iterations for a number of clusters K = 1 to 8, with six repetitions for each K. The model assumed correlated allele frequency for the populations and mixed

ancestry (admixture) for the individuals. Because weak structuring was assumed, the LOCPRIOR model was implemented, which uses sampling locations as prior information to assist the clustering (Hubisz et al. 2009). Due to the uneven sampling across geographical populations, the parameter alpha was set to 1/K, following the recommendations of (Wang 2017).

The output files were analysed by STRUCTURE HARVESTER, an online program for organising results generated by the program Structure (Earl & vonHoldt 2012), which implements the Evanno method (Evanno et al. 2005), to detect the optimal number of K, that best fits the data. The resulting replicate q-matrices for the optimal K were aligned in CLUMPP (Jakobsson & Rosenberg 2007) and a bar graph was visualised using the program DISTRUCT (Rosenberg 2004).

7.   Results

A total of 96 samples, drawn from eight sampling areas of the Central Cameroonian region were evaluated using AFLP markers. As shown in Table 10, a total of 1176 fragments were generated using four different combinations of primers. The primer combinations in this study produced a mean of 262 fragments per individual. The expected heterozygosity under the Hardy-Weinberg genotype proportions (Nei's gene, Hj) ranged from 0.16851, for Ebogo, to 0.21307, for Bokito, and had a mean value of 0.1894. This implies therefore that Bokito and Ebogo were the most and least genetically diverse populations respectively.

Table 10. Population data of *G. kola* groups sampled in the Central region of Cameroon.

Population	*n*	*#loc.*	*#loc_P*	*PLP*	*Hj*
Akok	10	1176	744	63.3	0.19504
Bokito	7	1176	745	63.4	**0.21307**
Ebogo	21	1176	631	53.7	**0.16851**
Lekiasi	15	1176	661	56.2	0.19933
Bot-Makak	12	1176	816	69.4	0.19223
Nkelikok	16	1176	619	52.6	0.18360
Saa	3	1176	433	36.8	0.19119
Ebolowa	12	1176	723	61.5	0.17250
Mean Value	12	**1176**	**671.5**	**57.11**	**0.18943**

n= number of scored individuals; *#loc*= number of loci scored; *#loc_P*= number of polymorphic loci at 5 % level; *PLP*= proportion of polymorphic loci at the 5 % level, expressed as a percentage; *Hj*=expected heterozygosity under the Hardy-Weinberg genotype proportions (Nei's gene diversity=*H/He*).

As shown in Table 11, overall, the obtained mean within-population expected heterozygosity under Hardy-Weinberg genotypic proportions (*Hw*) (Nei's gene diversity within populations) (analogous to *Hs*) was 0.1894. The computed total gene diversity (*Ht*) was 0.1922. A low F_{ST} value of 0.0145 was obtained (Table 11), implying that altogether the eight populations of *G. kola* sampled in the Central region of Cameroon were closely related. The average gene diversity among populations in excess of that observed within

populations (*Hb*) was 0.0028. The highest differentiation was at the level of individuals, and not population level. The greater level of genetic differentiation was within populations and not among populations. There existed very little genetic differences among the populations the sampled eight genetic cluster groups.

Table 11. Genetic structure of *G. kola* populations in the Central region of Cameroon.

n	*Ht*	*Hw*	*Hb*	F_{ST}
8	0.1922	0.1894	0.0028	0.0145
	S.E	0.005102	0.000912	0.323893
	Var	0.000026	0.000001	0.104907

n=number of scored populations; *Ht*= total gene diversity; *Hw*= mean gene diversity within populations; *Hb*= average gene diversity among populations in excess of that observed within populations; F_{ST}=Wright's fixation index; *S.E*= Standard Error; *Var*= Variance.
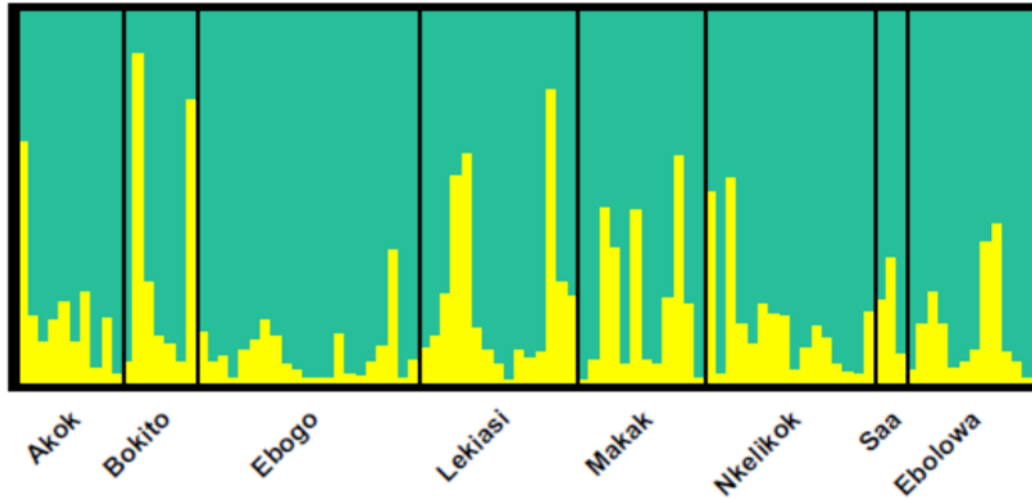
Figure 4. Histograms showing the Bayesian clustering of individuals within populations (STRUCTURE) for a K value of 2. The x-axis represents an individual samples as identified in Table 1, whereas the y-axis (each colour) represents the estimated membership coefficient of the individual's assignment into each inferred Bayesian group. Yellow represents Cluster I, Green represents Cluster II. (*Makak = Bot-Makak).

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A K value of 2 (Figure 6) was revealed to be the optimum number of statistically distinct genetic clusters within the study area, which will now be denoted Cluster I (yellow) and II (green). As shown in Figure 4, there was some structuring within populations, however it was weak. The results did not reveal some complete dominance of either individual samples or sampling groups by either one of the two Cluster groups. However, Cluster II appeared to be predominant in the majority of the individual samples, and in all cluster groups.

Resampling statistics based on 1000 random permutations of individuals among populations revealed an observed F_{ST} value of 0.0145 (Table 12). The table implies an overall low total gene diversity among the eight sampling areas.

**Table 12.** Permutation test for genetic differentiation among populations.

Statistic	F_{ST}
Observed	0.0145
Lower 95 % limit	-0.0087
Upper 95 % limit	0.0048
Lower 99 % limit	-0.00098
Upper 99 % limit	0.0076
P value (low)	1.0000
P value (high)	0.0000

Among the eight provenance populations which were assessed for similarity probability percentages, the most closely related were Bot-Makak and Saa, with a similarity percentage probability difference of 1.42 %, whereas the most distantly related were observed to be Bokito and Ebogo, with a difference of 24.35 % as shown in Figure 5, illustrating that while the optimum number of statistically distinct genetic cluster groups of *G. kola* within the study area is two, Cluster II (Green) was predominant in all eight sampling groups. Overall, Cluster II generally appeared to increase in dominance among the sampling groups from North towards the South. Attempts to construct a dendrogram (Figure 7), together with the principal component analysis (PCA) (Figure 8) did not show identifiable differentiation of individuals or genetic cluster groups according to geographic locations.
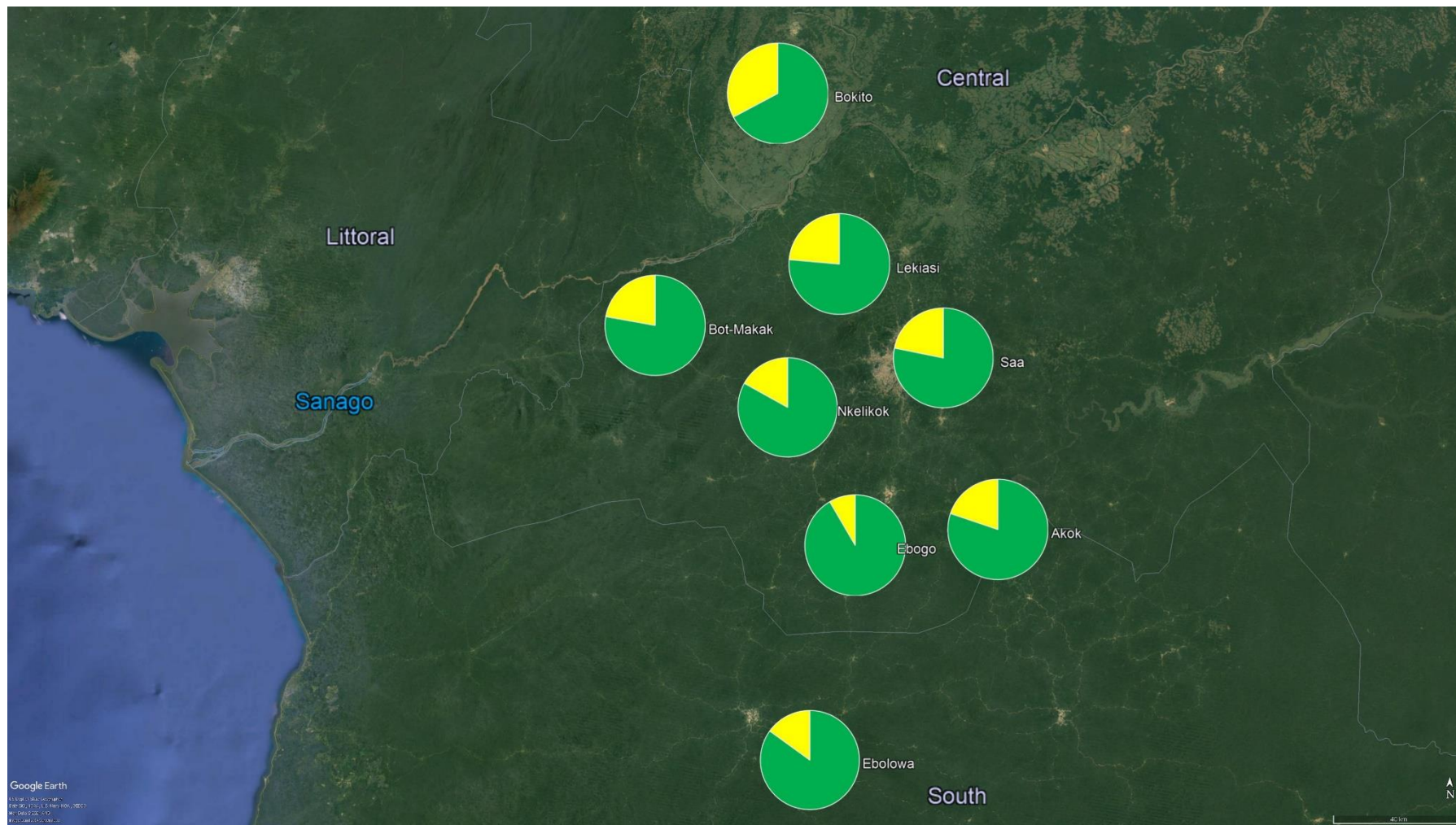
Figure 5. Pie charts showing the Bayesian clustering of individuals among populations (STRUCTURE). Yellow-Cluster I, Green-Cluster II.
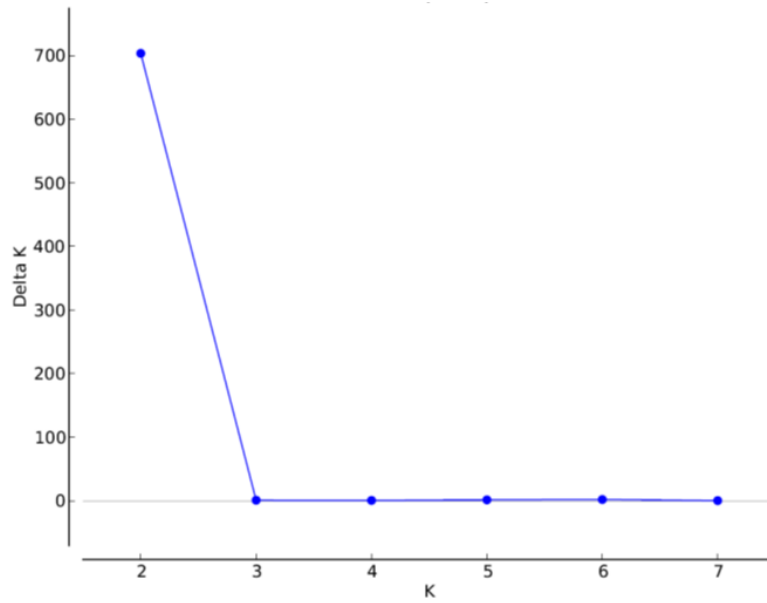
Figure 6. Determination of Δ K based on Evanno method from STRUCTURE HARVESTER.

Figure 7. An Unweighted Pair Group Method with Arithmetic mean (UPGMA) dendrogram constructed using Jaccard's index from the program PAST.

Figure 8. A PCA of the assessed *G. kola* individuals.

8.   Discussion

8.1.   AFLP markers

In this study, a total of 1176 fragments were generated, giving a mean of 261.9 fragments per individual and a 98.6 % level of polymorphism. To date, there are very few properly published studies on the genetic diversity of *Garcinia kola* which are available for comparison purposes. However, a study by Dadjo et al. (2020) using SNP markers on *G. kola* in populations in Benin reported a comparatively related polymorphism level of 97.86 %. Using AFLP markers on a different species, *Guazuma crinita*, and more primer combinations (seven), Tuisima-Coral et al. (2020) amplified a total of 171 fragments and observed a 99.4 % level of polymorphism. Using ten pairs of AFLP primer combinations, Cao et al. (2019) detected 1,046 polymorphic loci on *Pyrus pyrifolia*, with an average of 105 bands per primer. Dávila-Lara et al. (2017) generated 226 fragments on *Calycophyllum candidissimum* (Rubiaceae) in Nicaragua, using three primer pair combinations. In another study, Sing et al. (2015) detected 952 fragments from Ziziphus species using 11 AFLP primer pairs.

The results obtained in this study are comparable to those of other similar studies, which utilised AFLP markers to assess fruit tree genetic diversity. The ease of multiplexing, the ability of AFLPs to amplify hundreds of genomic fragments and their potential to be effectively applied where there is no prior knowledge of the sample's genome were demonstrated in this study. Among the numerous advantageous properties of AFLPs which were raised in the literature review, Mba & Tohme (2005) singled out the capacity of AFLPs to create numerous polymorphic bands for each assay, as one of their greatest strengths. Mba & Tohme (2005), further recommended than an ideal marker should produce data in a manner that can be easily put into databases and is suitable for automation. Besides the resolution and efficiency capillary electrophoresis offers, its incorporation into the research design also helped in ensuring that there was minimal manual handling of data. In contrast to conventional gel electrophoresis methods which require significant manual handling of data, the 3500 Series Genetic Analyzer unit automatically organises and files electronic data (Thermofisher 2020). Further data

processing methods which were employed in this study involved data transfers from one electronic platform to another and the simple execution of relevant commands, which altogether amounted to minimal manual manipulation. This situation helped in minimising the potential of human error emanating from manual manipulation of data.

The study therefore revealed that AFLPs are a useful tool for assessing the genetic diversity of *Garcinia kola* trees. This study adds one more species to a growing list of fruit trees which have been successfully assessed for genetic diversity using AFLP markers. In response to Maňourová's (2017) recommendation for the development of molecular markers which are suitable for assessing *G. kola*, it is therefore recommended that future genetic studies on *G. kola* consider adopting AFLP markers alongside any other useful molecular techniques in studying other species of the genus Garcinia, and other underutilised fruit trees indigenous to the tropics.

8.2.   Population structure

The Wright fixation (F_{ST}) is a measure of population differentiation due to its genetic structure. The F_{ST} has not only been widely used in diverse research (Ma et al. 2015), but it is also one of the most frequently used indices when assessing population structure (Meirmans & Hedrick, 2011). F_{ST} values vary on a scale from 0 to 1, with values close to 1 implying considerable genetic differences among the sampling areas, whereas values close to 0 imply that there are considerable genetic similarities among the sampling cluster groups. The observed F_{ST} value (0.0145), shown in Table 12 was closer to 0, implying that there are significant genetic similarities among the eight sampling areas. According to Frankham et al. (2010), for plants of the same species, an F_{ST} value of less than 0.05 implies that there exists a low genetic differentiation. The F_{ST} value was also notably higher than the value at lower 95 % (-0.0087), hence it can be assumed that the crossing between the individuals happens randomly and that there exists some level of population structuring, which however is low. This assertion is supported by data from the STRUCTURE analysis and the principal component analysis (PCA) (Figure 8).

In contrast to the traditional population genetic structure analysis approaches such as F_{ST} analysis which rely on predefined populations, STRUCTURE's major strength is its ability to reveal, using purely genetic data, cryptic or hidden population structures

(Pritchard et al. 2000). Wang (2017) stated that STRUCTURE reveals population structures that are difficult to detect using visible characters such as sampling locations or phenotypic traits. The STRUCTURE analysis further took into consideration the situation of unbalanced sampling. Scenarios of multiple sampling groups involving unbalanced sampling numbers may in some cases lead to distorted output values of K. However, according to Wang (2017) this situation can be rectified by STRUCTURE and therefore reveal accurate K values. Wang (2017) recommended STRUCTURE users to choose the population-specific ancestry prior and ALPHA of 1/K in analysing their data. The adjustment was implemented in this study and, according to multiple simulation studies, this remedies the statistical imbalances and therefore results in accurate K values (Wang 2017).

The determination of genetically similar clusters of individuals is an important aspect in population genetics and the STRUCTURE software, through the Bayesian method, enabled us to further assess this phenomenon. STRUCTURE is therefore commonly utilised in the fields of ecology, evolutionary biology, and genetics to detect concealed genetic assemblages (Puechmaille 2016; Wang 2017). STRUCTURE infers the most likely number of genetic clusters (K). Our study revealed an optimum K value of two. The software revealed that there existed some level of population structuring, although weak, and neither of the two clusters I or II was completely fixed in either individual samples or in the assessed sampling cluster groups. Notably, in their study of *G. kola* populations in Benin, Dadjo et al. (2020) reported a similar phenomenon of two principal genetic clusters occurring in admixture. Olawuyi et al. (2019) also reported the occurrence of two genetic clusters in Nigerian *G. kola* populations studied using RAPD markers. In this study, the dominant Cluster (II), appeared to increase in proportions within the sampling groups, from North to South. This phenomenon suggests that adaptation to local climatic factors has a significant role on the genetic diversity. This may infer the existence or slowly ongoing evolution of two different genetic provenances within the study area. According to the F_{ST} index value, there exists significant but weak genetic differentiation in studied populations, a situation similarly reported by Dadjo et al. (2020) in Benin.

A UPGMA dendrogram constructed using Jaccard's index from the program PAST (Figure 7), and the PCA (Figure 8) both failed to reveal a clear relationship between

individuals and geographic locations. This implies that the population structuring was weak. Similarly, after UPGMA analysis of studied populations Dadjo et al. (2020) observed no clustering which was based on geographical locations. Plant population genetic structure results from a multitude of factors such as mutations, selection and drift. However, influential similarities between the two studies include climatic conditions, anthropogenic impacts on species, and the species' natural reproductive biology. Furthermore, according to Loveless and Hamrick (1983) other prevailing features which could have encouraged weak population structuring are, seed dormancy, long-distance seed dispersal by humans, long distance range between individuals and floral phenology.

8.3. Genetic diversity

The expected heterozygosity under the Hardy-Weinberg genotypic proportions (Hj), is an index used to interpret the data with respect to genetic diversity among populations. The Hj ranges in value from 0, null diversity, to 0.5, which indicates the highest level of genetic diversity. Therefore, according to the data in Figures 6 and 7, the most genetically diverse sampling group was revealed to be Bokito, with an Hj value of 0.21307. The least genetically diverse sampling group was Ebogo with an Hj value of 0.16851.

While Bokito is furthest North, Ebogo is the second furthest South within the study area, and in the middle, they are separated by a tropical forest and the capital city, Yaoundé. The long-distance separation of these two sampling groups implies that, outside of originating from similar sources of germplasm, crossbreeding between them is highly unlikely, and therefore the likelihood of them being regarded as completely fragmented populations would be higher. Furthermore, Bokito's natural conditions are known to be notably distinct from those of other surrounding sampling points. Bokito is a transition zone between humid tropical forest and savannah climates. These conditions would thus be expected to encourage the perpetuation of diverse genes for adaptation to the existing diverse ecological and environmental conditions.

From the data in Table 11, among the computed indices, the total gene diversity (Ht) had the highest value. This implies that the highest differentiation is at the individual level, in contrast to population level. According to Hamrick et al. (1992), wood perennials

and outbreeding plant species such as *G. kola* maintain most of their variation within populations. This inference is further supported by the values of the other indices within the table. The value of the mean gene diversity within populations (*Hw*) also indicates that the greater proportion of genetic differentiation is within each population and not among populations. The low value of the genetic diversity among the sampling areas (0.0028) further supports the inference that very little genetic differences exist among them, within the Central region of Cameroon. The low genetic diversity among the sampling clusters is further illustrated by the dendrogram which did not reveal the presence of genetic clusters that corresponded to particular geographical populations.

Overall, this study revealed a relatively low level of interpopulation genetic variation among the assessed eight sampling areas of *G. kola* within the Central Region of Cameroon. Numerous factors have the potential to influence a population's genetic diversity. Freeland et al. (2011) stated that in natural conditions these factors rarely exert their influence in isolation, but rather in combination with others. Therefore, it would be challenging to identify a single factor as effecting the change in genetic diversity, but it is however possible to point out several factors as potentially influencing the genetic diversity.

The gene flow is generally known to be one of the extremely influential forces in population genetics (Freeland et al. 2011). The low levels of interpopulation genetic variation that were revealed by the study may thus be attributed to the spread of the seed germplasm, through trade of vital seed products, over vast areas within the Central Region but all emanating from a narrow range of sources. *G. kola* fruits are known to bear seeds ranging from two to four per fruit. However, seed collectors are reported to prefer trees which bear multiple seeds (especially four) per fruit. If this characteristic of seeds born per fruit is genetically fixed, it is highly likely that it will be selected for by collectors. *G. kola* is considered to be an insipiently domesticated species (Maňourová et al. 2019). Despite domesticating indigenous fruit trees being a route to harnessing the potentials of genetic diversity, one of its major drawbacks is that it results in shifts and/or losses in underlying genetic diversity in cultivated tree populations (Jamnadass et al. 2000; Cornelius et al. 2006).

G. kola is known to exhibit poor natural regeneration properties (Gyimah 2000). This therefore implies that human cultivation is an influential force in its growth. In

contrast to other areas like Nigeria where *G. kola* fruits are reportedly mostly harvested from wild stands, in Cameroon the seeds are reported to be mostly harvested from agroforests and rarely from the natural forest (Guedje et al. 2001). In Cameroon, *G. kola* seed collectors are reported to be proportionally lesser than groups of traders who buy the seeds for resell. These traders then resell the seeds at a higher price in numerous different urban marketplaces, but mostly in urban traffic junctions (Agyili et al. 2007; Přibyl et al. 2017), most likely to long-distance travellers. In Cameroon, *G. kola* seeds are an important source of germplasm. They are not only the most important AFTPs derived from *G. kola* (Maňourová et al. 2019), which are utilised mostly for their medicinal and cultural value, but they are among the most-traded AFTPs in the whole of West and Central Africa. The enormity of their trade value, poor natural regeneration, and the significant human influence in *G. kola* regenerative cycle all indirectly point to a significantly wide distance range of human dispersal.

Natural selection is another way in which a population's overall genetic diversity may be affected through directional and stabilising selection (Freeland et al. 2011). The climatic conditions in most of the Central Cameroon are known to be relatively narrow range therefore, the natural conditions may act to select against species which are less adapted to the local region, resulting in a low genetic diversity.

The revealed overall low genetic diversity among all the eight sampling groups of *G. kola* populations in the Central Region, and the presence of only two distinct clusters, add more evidence to Agyili et al.'s (2007) concern of declining population numbers. It further adds more weight to their call for the establishment of restoration programs. Farmers and researchers would therefore be encouraged to outsource progeny or breeding material from other surrounding ecologically similar areas. The similarities in ecological conditions, increase the chances of obtaining material that is genetically diverse but already adapted to the local conditions. Perpetuating populations with low genetic diversity puts them at risk of genetic drift effects in the future.

The significant level of genetic variation within populations may be explained by the dioecious nature of the *G. kola* trees. Dioecy, by nature of having male and female inflorescences on separate individual trees, encourages cross pollination and higher levels of genetic diversity (Muyle et al. 2020). The high genetic diversity further implies that the purposeful selection by humans has not yet significantly influenced the sampled

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population groups. Genetic diversity is of great importance to PTD programmes. This scenario would therefore be supported by the assertion by Maňourová et al. (2019) who categorised *G. kola* as an insipiently domesticated species. This, according to Clement et al. (2010), implies that there are apparent human attempts to cultivate and select the high-performing individuals of the species, however domestication still remains in its early stages.

Molecular markers can provide vast amounts of information about populations however, the most important issue is how the data can be utilised in practical applications (Freeland et al. 2011). The significant genetic diversity that was observed within *G. kola* population groups is perceived to be a positive scenario not only for the ongoing PTD programmes, but also for the conservation efforts which may be currently directed towards this species. For the conservation efforts, the significant genetic diversity offers the potential of the *G. kola* species to be effectively resilient to future shocks and stresses, especially emerging challenges such as climate change. Due to a decline in *G. kola* populations which is attributed largely to destructive exploitation methods, Agyili et al. (2007) recommended the establishment of restoration programmes. A good gene resource population, which was demonstrated by the results to be in existence in the Central region of Cameroon, is a prerequisite for such a program. For the PTD programmes, the observed significant genetic diversity implies that there exists a "healthy" gene resource population of *G. kola* in the Central region, from which the Selection and Production populations can be derived for the effective development of the program to the final achievement of high-quality end products. In this manner, genetic diversity acts as a valuable resource for the sustainable improvement of production in *G. kola* farming systems and agroforestry in general.

8.4. Implications for domestication and conservation

The results of this study are highly significant as they present an opportunity to advance the participatory domestication program of *G. kola* within the West and Central African region. The *G. kola* PTD programme was reported by Maňourová et al. (2019) to have been stagnated by a lack of genetic information. The study findings now enable researchers to harmonise or match the obtained genetic data of *G. kola* individuals, with

existing data from morphological studies. Consequently, it will enable them to assess and identify the elite trees, which possess superior qualities of specific traits which would be desirable for domestication.

In accordance with the standard ICRAF procedure, after the prioritisation of a species for participatory domestication, the selection of elite ideotypes is the next step, followed by the "development of efficient propagation techniques" for the creation of quality propagules. These propagules of the high performing lines are then distributed to farmers for trials for performance trials. According to Dawson et al. (2012), it is this process which would then allow researchers to "zone in" on the *G. kola* lines that would offer the highest quality of AFTPs to local farmers. Consequently, the participatory domestication can thus be expected to benefit local communities by the provision of higher quality AFTPs (such as superior nutrition in the case of *G. kola*), but at the same time being well-adapted to local ecological and environmental conditions. Summarily, as put by Leakey (2010), the whole PTD programme is now one step closer to presenting opportunities for local inhabitants to harness the genetic potential of this indigenous fruit tree species through the development of various AFTPs and services with enhanced potential, marketability and uses. This study was therefore part of a broader study on the PTD programme of *G. kola* in the Western and Central regions of Africa.

The results of this research study open the way for the genetic diversity of *G. kola* within the study area to be secured through conservation programmes. This conserved diversity can then be utilised in future selection programs or through breeding to broaden the genetic base of the cultivars in the production populations of the PTD programme. This would be quite important should there be a need to breed for resistance to emergent threats of pests and diseases. Conservation of the genetic diversity could thus be carried in three major ways; creation of a gene bank (*ex situ* conservation), use of the genetic resource in PTD programmes (*circa situm* conservation), and through the protection of important individuals within their stands, both wild and cultivated (*in situ* conservation).

The samples used in this study were selected from eight genetic cluster groups. The samples were from the forest, homegardens, farms and agroforests. The samples collected from the forest were thus classified as "Wildly occurring" whereas the rest were classified as "Cultivated". However, the relatively small number of wildly occurring individuals to cultivated individuals (5:91) made it difficult to make a statistically

meaningful comparison of genetic similarities between the major two forms of propagation.

9.   Conclusions

The study proved that AFLP markers are a useful tool for assessing the genetic diversity and the structure of *G. kola* populations. It is therefore recommended that future studies consider adopting AFLP markers alongside other molecular techniques for assessing the genetic diversity of *G. kola*, as well as other species of the genus *Garcinia*.

A relatively low level of genetic diversity among the sampled *G. kola* populations was observed, contrasting to a relatively higher level of genetic diversity within the populations. In the *G. kola* population of the Central region of Cameroon, two distinct genetic cluster groups existed but both showed weak structuring. The dominant cluster group, however, appeared to become increasingly dominant from the north towards the south. The relatively low genetic diversity among populations was most likely influenced either by the long-distance trade in *G. kola* seed from a narrow range of sources, or adaptation of the species to local environmental and ecological conditions.

The high genetic diversity within populations was viewed as a positive scenario for the species. The high genetic diversity was most likely encouraged by the dioecy of *G. kola*. *G. kola* population therefore exhibits attributes of an incipiently domesticated species. The existing high genetic diversity within the sampled groups is valuable for advancing restoration conservation programs as well as for the ongoing tree domestication program.

It is therefore recommended that the obtained genetic data is reconciled with existing morphological data for the assessed individuals, to enable the identification of high performing *G. kola* lines/individuals. The existing high genetic diversity of *G. kola* in the Central region is a valuable resource for local communities, having the potential to ultimately improve their livelihoods, and it therefore needs to be utilised in a sustainable manner. It is recommended that initiatives are established for the conservation of the existing genetic diversity through some or all of the following measures;

1) Establishment of a gene bank (*ex-situ*), or depositing accessions in existing gene banks
2) Sustainable use of the genetic diversity in PTD programmes (*circa situm* conservation)

3) Protection of important individuals within their stands, both wild and cultivated (*in situ* conservation).

In this study, the genetic differences between the cultivated and naturally growing populations could not be established in a scientifically credible manner. Hence, further studies would therefore be recommended, which aim to determine the genetic differences between the two major groups.

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Appendix

Appendix A. Population data of sampled G. kola groups in the Central region of Cameroon. (Lynch and Milligan method).

Population	n	#loc.	#loc_P	PLP	Hj	S.E.(Hj)	Var(Hj)	VarI(Hj)	VarI %	VarL(Hj)	VarL %
Akok	10	1176	744	63.3	0.19504	0.00504	0.000025	0.000005	20	0.000020	80.0
Bokito	7	1176	745	63.4	0.21307	0.00489	0.000024	0.000007	31.2	0.000016	68.8
Ebogo	21	1176	631	53.7	0.16851	0.00492	0.000024	0.000002	10.3	0.000022	89.7
Lekiasi	15	1176	661	56.2	0.19933	0.00485	0.000024	0.000004	16.0	0.000020	84.0
Bot-Makak	12	1176	816	69.4	0.19223	0.00487	0.000024	0.000005	19.0	0.000019	81.0
Nkelikok	16	1176	619	52.6	0.18360	0.00490	0.000024	0.000003	13.6	0.000021	86.4
Saa	3	1176	433	36.8	0.19119	0.00594	0.000035	0.000013	35.9	0.000023	64.1
Ebolowa	12	1176	723	61.5	0.17250	0.00493	0.000024	0.000004	16.2	0.000020	83.8

Where; n= number of scored individuals; *#loc*= number of loci scored; *#loc_P*= number of polymorphic loci at 5 % level; *PLP*= proportion of polymorphic loci at the 5 % level, expressed as a percentage; *Hj*=expected heterozygosity under the Hardy-Weinberg genotypic proportions (Nei's gene diversity=*H/He*); *S.E.(Hj)*= the standard error of *Hj*; *Var(Hj)*= the variance *Hj*; *VarI(Hj)*= the variance component of *Hj* due to sampling of loci; *VarI %*= the proportion of *Var(Hj)* due to sampling of individuals; *VarL(Hj)*= the variance component of *Hj* due to sampling of loci; *VarL %*= the proportion of *Var(Hj)* due to sampling of loci.