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Genetic Diversity of *Garcinia kola* (Heckel) in Southwest Cameroon

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Author: Benson Agyepong, BSc.

Supervisor: prof. Ing. Bohdan Lojka, Ph.D.

Co-supervisor: Ing. Marie Kalousová

DECLARATION

I declare that this thesis was written by myself, that the work "Genetic Diversity of *Garcinia kola* (Heckel) in Southwest Cameroon" contained herein is my own except where explicitly stated otherwise in the text, and that this work has not been submitted for any other degree or professional qualification.

Prague, 23rd April 2021

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Benson Agyepong

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ABSTRACT

Garcinia kola (Heckel) is an indigenous multipurpose fruit tree, originating in Western and Central Africa. It is a highly valued for the medicinal efficacy of the bark, root, seed in the treatment of common ailments such as cough, bacterial infection, etc. Especially seeds of *G. kola* are frequently used in Cameroon for treatment of a wide range of health problems. Despite its frequent usage, the domestication process of this tree is at its beginning and throughout its distribution area. The seeds are mostly harvested from the wild, which can lead to endangering of the species. Moreover, there are various research papers published on this breed's bioactive substance, nutritional content etc, however, the primary information on genetic structure, diversity, and distribution of existing populations crucial for domestication and conservation efforts of the species is still lacking. The aim of this study was to assess the genetic diversity of *G. kola* in Cameroon. In total, 75 individual trees from four populations were sampled in Southwest Cameroon and DNA was extracted from the seed coat. Twenty arbitrary RAPD primers were used for screening, and seven were chosen for final analysis based on polymorphism shown. PCR products were visualized on agarose gels and bands scored for presence or absence. A total number of 135 loci were obtained, all of them polymorphic, showing 100% polymorphism. The overall values of Nei's gene diversity and Shannon's diversity index (h=0.20, I=0.34) indicate high levels of genetic diversity in the analysed individuals. However, the index of Nei's genetic distance between populations reached very low values, showing lack of population structuring. These values are quite typical for natural populations with low selection and domestication pressure. This study contributes to the description of population genetics of *G. kola*. The results, together with morphological and biochemical data, can lead to better management, conservation, and utilization of *G. kola* gene pool.

Keywords: Diversity, Bitter cola, Cameroon, medicinal plants, distribution, RAPD

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LIST OF ABBREVIATIONS

AFLP - Amplified Fragment Length Polymorphism

FAO - Food and Agricultural Organization of the United Nations

bp - base pair

CULS - Czech University of Life Sciences

DNA - Deoxyribonucleic Acid

dNTP - Deoxynucleotide

ISSR - Inter Simple Sequence Repeat

PCR - Polymerase Chain Reaction

PGRCU - Plant Genetic Resources Conservation Unit

PGRU - Plant Genetic Resources Unit

RAPD - Random Amplified Polymorphic DNA

RFLP - Restriction Fragment Length Polymorph

RNA - Ribonucleic Acid

SNP - Single Nucleotide Polymorphic

SSR - Short Sequence Repeat

WMO - World map organization

PGR - Plant genetic resources

ERuDeF – Environment and Rural Development Foundation

IRAD - Institute of Agricultural Research for Development

RECODEV – Regional Centre for Conservation and Development

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1. INTRODUCTION

The focus on plant analysis around the globe has been increased due to the data collected to prove the immense potential of medicinal plants. Through the means of present-day scientific approaches, various types of medicinal plants have been identified and studied. The findings of this research show these medicinal plants as having bright potential, especially in pharmacology (Mariod et al. 2016). Nowadays, large part of the ancient knowledge about those species is about to extinct due to increasing pressure from modernization and western lifestyles. Only a few of the forest tree species have been fully domesticated, whereas some of them have been rediscovered as underutilizes/neglected species.

The species *Garcinia kola* (Bitter kola) is a tree belonging to the family Clussiaceae growing up to 30 m in height with maximum of 100 cm in trunk diameter respectively and the genus is deciduous, flowering annually (Colombe et al. 2020). *G. kola* is a largely cultivated forest tree indigenous to subtropical and tropical Africa specifically the moist lowland forests of Western and Central part of Africa. Every part of this species can also be used for medicinal purposes and this has earned a name for it as "wonder plant'' (Usunomena 2012; Onasanwo and Rotu 2016). The species has been noted for its high socio-economic and medicinal importance, which is commonly used for various traditional ceremonies and used in folk medicine for the treatments of numerous ailments ranging from aphrodisiac, vomiting, induction of insomnia, movement of bowel and nervous alertness (Azeez et al. 2020).

Assessment of genetic diversity in plant breeding programs is useful for determining the difference and distinctness of a phenotype, genetic constitution of genotypes and selection of parents for hybridization (Bretting and Widrelechner 1996). DNA markers have been used to assess genetic diversity in different crop species (Cooke 1995). Molecular markers are practically unlimited in number and are not affected by environmental factors and/or the developmental stage of the plant (Winter and Kahl 1995). The attraction for RAPDs is due that there is no requirement for DNA probes, or for any sequence information for the design of specific primers, as is needed with other types of DNA markers (Williams et al. 1990).

RAPD markers offer many advantages such as higher frequency of polymorphism, rapidity (Fahima et al. 1999; Jeya et al. 2006), technical simplicity, requirement of a few nanograms of DNA, no requirement of prior information of the DNA sequence and feasibility of automation (Subudhi and Huang 1999).

There is a massive economic importance of Bitter cola based on market surveys conducted in this part of Africa because of its strong medicinal influence but unfortunately no scientific research has been done on the genetic diversity of *G. kola*.

Therefore, there is the need to fill this knowledge gap by providing insight into genetic diversity of *G. kola* which are generally considered as necessary steppingstone for specie domestication. After the study, the outcome or the results can be considered as a protocol in exceptional populations selection and identification, and this can provide a basis for production increase. In this regard the aim of this study was to evaluate the polymorphism of *Garcinia kola* using RAPD molecular markers.

2. LITERATURE REVIEW

2.1. Genetic diversity of tropical trees

The variation in living organisms found in an environment is termed as diversity (Yang et al. 2010). Genetic diversity is the variety of genes found among the individuals within species. It is known as one of the three basic levels of biodiversity, the other two being ecological diversity and species diversity (Hunter et al. 2004). Genetic diversity is the raw material available to plant producers (Huttner et al. 2004; Arif 2010). The development of crop genetic resources is reliant on regular infusions of wild relatives, local diversity, and utilization of current breeding techniques. The significance of genetic diversity is well recognized; when a population of a species consist of a large gene pool then it has a greater chance of living and flourishing than a population without genetic variability (NGA 1999). Plant genetic diversity research is very important to maintain genetic material for long period of time. Plant genetic resources may largely be preserved *in situ* (e.g., produced by farmers themselves) or *ex situ* in gene bank, DNA library etc. for further enhancement of food and nutritional security. These preserved genetic resources should help solve food and nutritional security and be a vehicle for crop development. Generally, genetic diversity is recognized as the centre of biodiversity (Govindaraj et al. 2015). The understanding of molecular basis of this fundamental biological phenomenon in plants is essential for the efficient conservation, management, and effective application of plant genetic resources (PGR) (Mondini et al. 2009).

Genetic plant resources contribute immensely to producing different crop varieties with good harvest capabilities or resistance to biotic and abiotic stress. The germplasm of a crop collected from the local sources provides greater genetic variability and can furnish useful traits to broaden the genetic base of the crop species (Bashir et al. 2008; Mondini 2009). Estimating genetic diversity within species is essential for future sustainable breeding efforts. The rate at which genetic advancement could be achieved rely upon the extent of genetic diversity that occur among cultivated accessions as well as their wild relatives (Bainiwal and Jatasra 1980).

2.1.1. Molecular markers

A molecular marker can be defined as a genomic locus, detected through probe or specific starter (primer) which, in virtue of its presence, distinguishes unequivocally the chromosomic trait which it represents as well as the flanking regions at the 3´ and 5´ extremities" (Govindaraj et al. 2015).

Molecular markers play an important role today in all areas of plant development (Alan 2006). Due to high resolutions and reliabilities in plant species detection, it makes it easier for molecular markers to recognize polymorphism efficiently (Oluwayi et al. 2019). Molecular markers rely either on restriction-hybridization of nucleic acids or techniques based on Polymerase Chain Reaction (PCR), or both (Mondini et al. 2009).

Diversity in plant genetic resources (PGR) offer opportunity for plant breeders to come out with new and revised cultivars with desirable attributes, which consist of both farmer-preferred traits (yield potential and large seed, etc.) and breeders preferred traits (pest and disease resistance and photosensitivity, etc.) (Gonvindaraj et al. 2015).

The assessment of genetic diversity within and between populations is continuously functioning at the molecular level using different laboratory-based techniques such as: allozyme or DNA analysis which determines differences directly.

In species identification, phylogenetic studies and in mapping the genetic linkage molecular markers provide a great leverage over the morphological and biochemical markers (Suh et al. 2011).

Both morphological and molecular markers were used to identify and describe species. However, different molecular markers have been used for the genetic diversity research on Garcinia genus. In molecular studies, Internal Transcribed Spacer (ITS) were also used to analyse the traits of mangosteen, assessed the genetic diversity among and within *G. mangostana* L. (mangosteen) trees. And the result was that genetic variation appeared among progenies within one mother plant and among the accessions of mangosteen (Nazre 2014; Mansyah et al. 2013).

2.1.2. Random Amplified Polymorphic DNA (RAPD)

Genetic variations are largely estimated based upon information at the DNA level by various molecular techniques such as Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), RFLP and microsatellites (SSR) (Lynch 1998; Stephens et al. 1992). Among them, RAPD, markers generated by Polymerase Chain Reaction (PCR) which was introduced since 1990's to assess intra specific genetic variation at nuclear level (Welsh and McClellan, 1990). This method has been used mainly in applied research, e.g. plant breeding but has become increasingly popular also in studies of natural plant populations (Bartish et al. 1999a; Bussell 1999).

RAPD is a PCR based approach for assessing genetic variation. This technique requires the use of random single primer in a PCR reaction, occurring in amplification of various detached DNA. This technique produces an immediate and active screen for DNA sequence-based polymorphism at a very large number of loci. The main reason this RAPD has become more popular than the other ones is that it does not need pre-sequencing of DNA. Genetic diversity and variations within and between the taxa of interest are examined by the presence or absence of each product, which is imposed by changes in the DNA sequence at each locus (Liu and Cordes 2004).

Because of its simplicity and rapidity characterize by the cultivars/ hybrids, Random amplified polymorphic DNA (RAPD) is highly acceptable in scientific research (Amrapali et al. 2008). The PCR based RAPD's profiling have become well-known in recognition and utilization because it has provided a tool for genetic assessment in biological systems (Welsh and McClelland 1990). The results achieved through germplasm analysis using RAPD is widely used for the acceptance of germplasm, screening of duplicates, genetic diversity analysis and managing the genetic strength of conserved germplasm (Amrapali et al. 2008).

The vast range of potential primers that can be used, give the technique great diagnostic power (Datta et al. 2011). Duplication of RAPD bands is recognized by accurate selection of primers, optimization of PCR condition for target species and replication to establish that only duplicated bands are scored. RAPD assessment has been largely used for various reasons which include description and categorization of

18 accessions, recognition of breeds, and assessing genetic diversity (Liu and Cordes 2004).

Due to base substitutions at the primer binding sites or to indels in the regions between the sites RAPD polymorphisms can appear. The potential power to discover polymorphism is relatively high; especially, 5 –20 bands can be formed using a given primer pair, and various sets of random primers can also be utilized to check the entire genome for differential RAPD bands (Liu and Cordes 2004).

RAPD markers are inherited as Mendelian markers in a dominant fashion and scored as present/absent. Homozygotes as well as heterozygotes are formed by RAPD bands though the strength of band may be different. This difference in PCR effectiveness makes scoring of band intensities difficult. Differentiating between homozygous dominant and heterozygous individuals is generally not achievable. Furthermore, it is a bit challenging to determine whether bands serve as different loci or other alleles of a single locus, so that the number of loci under research can be accurately assessed. This is especially true if the RAPD is caused by deletion or insertion within the locus rather than at the primer binding sites (Liu and Cordes 2004). RAPD proved to be reliable, rapid, and practical technique of revealing relationship among sorghum varieties.

RAPD markers have been accurately used to access genetic diversity on these species of *Garcinia,* namely *G. cambogia, G. cowa, G. hombroniana, G. indica, G. mangostana* and *G. xanthochymus* (Odunayo et. al, 2019). After using RAPD markers to detect an intra- and inter-species genetic relationship, the search revealed that cambogia species had high genetic diversity (Rao et al. 2003). However, information on molecular studies of *Garcinia kola* in Southwestern part of Cameroon is limited. Therefore, the focus of this research was to institute genetic diversity among populations of Garcinia kola using RAPD markers.

RAPD technology offers these main advantages; (i) applicability to problems where only limited quantities of DNA are available, (ii) suitability for work on anonymous genomes, (iii) efficiency and low cost (Liu and Cordes 2004). The number of fragments formed is very high. Arbitrary primers used for this technique can be easily purchased and there is no need for initial genetic or genomic information and the unit cost per assay is low. RAPDs have the advantage that they can be purchased at a

moderate cost and will generally amplify a range of fragments of most DNA and show polymorphisms. Primers would create unrelated patterns between unrelated species and identical ones for very closely related species. Apparently, primer sites are randomly dispersed along the specified genome and flank both conserved and highly variable regions. Wide variation in band intensity can be shown to replicate between research, which could be the result of duplicated copies of the amplified regions in the template or the efficiency with which regions are amplified. The polymorphic bands retrieved from RAPDs can also be reproduced for further analysis (Liu and Cordes 2004).

2.2. Genus *Garcinia*

The genus *Garcinia* L. is a member of the family Clusiaceae circulated all through tropical Asia, Africa, New Caledonia, Polynesia, and Brazil. *Garcinia* plants contain an expansive scope of naturally dynamic metabolites which, over recent years, have gotten extensive consideration because of the chemical composition of their concentrates, with compounds which have been revealed to have gainful impacts on various ailments (Espirito et.al 2020). *Garcinia* is a broad genus with more than 250 species of mostly dioecious woody plants that are typically common in humid tropical forest and has been grouped into an African and Asian category and some parts of South America. About 21 species of this genus is common to Western and Central parts of Africa where *Garcinia kola* is one of them which has received a lot of attention (Maňourová et al. 2019). The species are a rich and relevant source of bioactive compounds with valuable therapeutic composition, such as analgesic and anti-inflammatory properties. It is also composed of large range of biological metabolites, which has earned it an extensive consideration over many years for its richness in polyisoprenyl, benzophenones, polyphenols, bioflavonoids, and xanthone (Espirito et al. 2020; Maňourová et al. 2019). Economically *Garcinia* is probably best known for the highly valued fruit of mangosteen (*G. mangostana*), moreover, mangosteen and other species (e.g., *G. gummi-gutta* or *G. cambia*) have become the focus of pharmacological research (Sweeney, 2008), and a lot of natural-supplement industry has formed around these species. There are another *Garcinia* species endemic to tropical

Africa besides *Garcinia kola*. These species are *G. lucida*, *G. mannii*, *G. polyantha* and *G. epunctata* which are domestically useful in West Africa.

Garcinia lucida (Figure 1) is a highly valued non-timber forest product (NTFP) from the Clusiaceae family. The specie is popularly known in South Cameroon as Essok or Boulou (Guedje and Fankap 2002). It is ever-green dioecious tree with a height of 12 - 15 m high in tree stands and 25-30 cm in trunk diameter, which grows in Atlantic: primary rain forest zones of West and Central Africa. *G. lucida* are of various features, well-branched and evergreen. When the dark brown bark is removed stem exhibits yellow and resinous exudates (Solefack et al. 2017). The seed of the specie germinates in few weeks after falling, flowers and provides fruits throughout the year. It is grown in high-density stands around hilly moist forests or mature forests at altitudes over 500 m asl in Cameroon. It has a medium size fruit (13 cm × 11 cm), globular or ellipsoidal form and green to green-golden in colour. *G. lucida* is highly rated for its nutritional and medicinal properties (Omode et al. 1995). The seed, fruit and the bark are the most useful parts in folk medicine to ward off food poisoning and as a remedy to stomach problems. Furthermore, the stem bark is locally used by forest inhabitants in the fermentation of traditional alcohol retrieved from palm or raffia trees (Guedje 2003; Momo et al. 2011).

Figure 1. The fruit of *Garcinia lucida* (left) and *G. livingstonei* (right) (Maňourová 2017).

Garcinia livingstonei (Figure 1) is a shrub or small evergreen tree to 10 – 16 m high; crown dense, spreading, or conical; trunk short, often twisted. It flowers are white or pale to yellowish green, 6-14 mm diameter, borne in small groups in axils of older branches. The male and female flowers are separated, but with some bisexual flowers. Fruits are ovoid to round berries, 2.5-3.5 cm long and 2.5-3 cm broad; orange yellow, reddish, or purple; 1-2 seeded. It is distributed in Angola, Botswana, Cameroon, Ethiopia, Kenya, Namibia, Senegal, South Africa etc. The juicy fruit pulp is acid-sweet, has a pleasant taste and is rich in carbohydrates (Orwa et al. 2009). *G. livingstonei* is a rich source of different phytochemicals and displays anticancer, antiviral, antimicrobial and anti-parasitic activities. Its fruits are useful in traditional medicine as an aphrodisiac, also as a remedy for abdominal pain during pregnancy and after childbirth, or as an antibiotic (Yang et al. 2010; Joseph et al. 2016).

Garcinia mangostana L. (Clussiaceae) popularly known as mangosteen, is a tropical evergreen fruit tree which originates from Southeast Asia with various components such as flavonoids, benzophenones, xanthones and triterpenoids (Espirito, et. al 2020**)**. This species can also be called the "queen of fruits" due to its special and delightful tropical taste. *G. mangostana* is grown in the tropical rainforests of Southeast Asian countries like Indonesia, Malaysia, Sri Lanka, Philippines, and Thailand (Pedraza-Chevarri et al. 2008). In many years, pericarp of this fruit has been used in folk medicine to cure ailments such as trauma, skin infection, abdominal pain, dysentery, and wounds (Ibrahim et al. 2016). Products developed from *G. mangostana* fruit are now highly popular because of the biological activity of phytochemicals (Chaivisuthangkura et al. 2008).

2.2.1. Bitter kola (*Garcinia kola* Heckel)

Every part of this tree can be used for medicinal purposes and this fact has earned it the name "African wonder plant'' (Usunomena 2012; Onasanwo and Rotu 2016).

2.2.2. Taxonomy

Garcinia kola, often known as Bitter kola, is also known locally in most African countries as Agambo, Akara, Akbatuwe, Akilu, Aouolie, Bolele, Tweapea. *Garcinia kola* Heckel is scientifically classified as follows:

Kingdom:	*Plantae*
Division:	*Magnoliophyta*
Class:	*Magnoliopsida*
Order:	*Theales*
Family:	*Clusiaceae*
Genus:	*Garcinia*
Species:	*G. kola*
Binomial name:	*Garcinia kola, Heckel*

2.2.3. Origin, ecological requirements, and distribution

Garcinia kola is endemic in the humid lowland rainforest vegetation of the West and Central African subregions. It is found in coastal areas and lowland plains up to 300 m above sea level with an average of 2,500 mm of rainfall per annum. The trees are abundant in densely populated areas of natural and secondary forests where the predominant land use system is tree crop plantation farming (Aiyelaagbe and Adeola 1993).

More specifically, *G. kola* can be naturally found from Angola to Sierra Leone (Figure 2) (Onayade et al. 1998, Matig et al. 2007). *G. kola* is highly preferred among African countries and thus also exploited. This wonder plant, in Benin, is considered as the third most valuable medicinal plant. Therefore, it is often used in numerous traditional healing recipes (Matig et al. 2007). ICRAF, considered it as one of the most acceptable species in Cameroon and only few writers promote its natural distribution there (Matig et al. 2006). Among the species in Cameroon, for example Vivien and Faure (1985) had identified three major natural stands of the species; two of them are in East region (Nki National Park, Bertoua site) whereas one is present Southwest region.

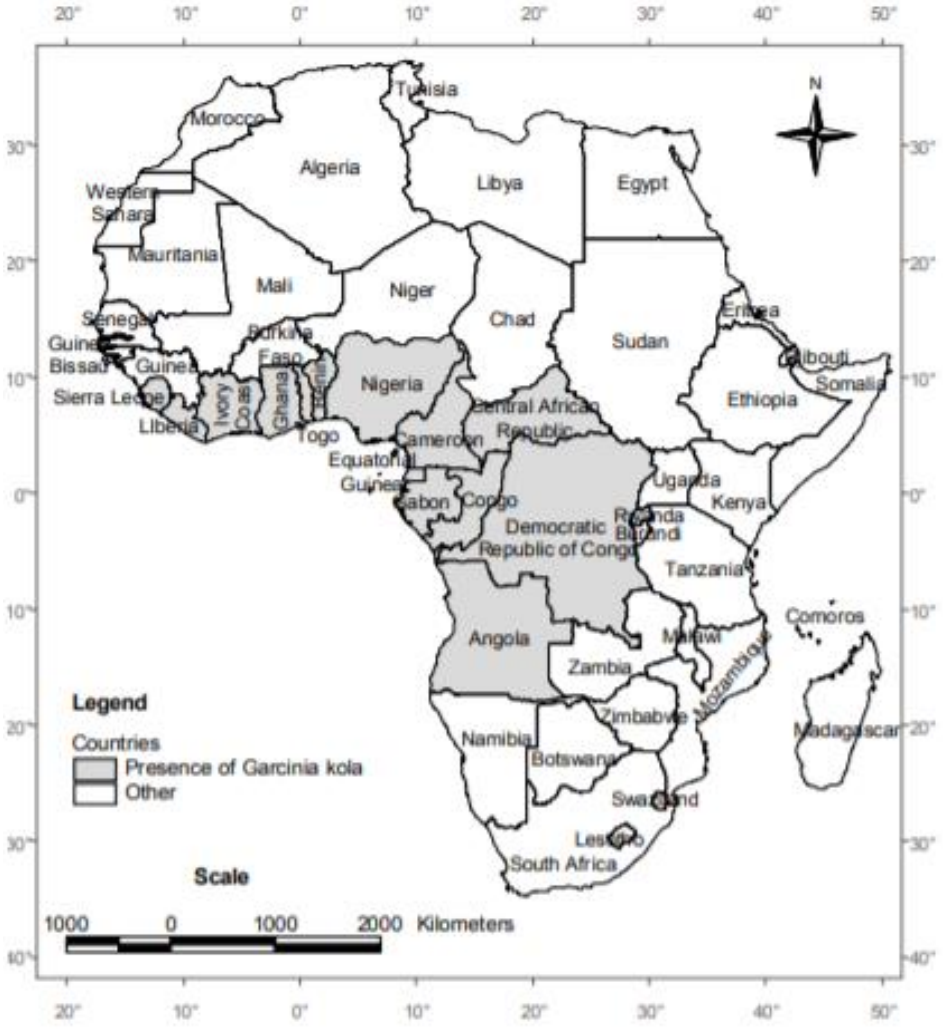

Figure 2. Distribution of *Garcinia kola* (Matig et al. 2007).

2.2.4. Botanical description of *Garcinia kola*

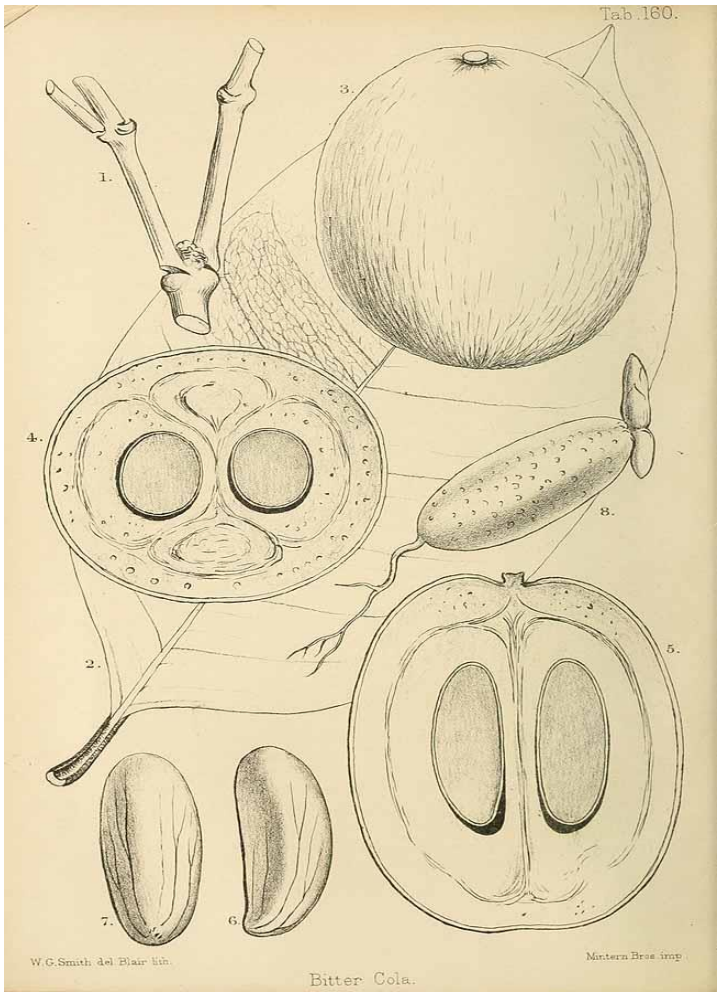

Figure 3. *Garcinia kola* (Heckel) (www.plant illustration.org).

G. kola (Heckel) is a multipurpose tree with traditional and medicinal value to domestic indigens (Dadjo et al. 2020). The trunk is straight with brown bark. The leaves are leathery. The flowers are greenish white, arranged in an umbel like inflorescences and are covered with short, red hairs. The table below also shows the physical characteristics of the seeds. The fruit is a drupe of 5-10 cm in diameter and weighs 41.3 g minimum and 204.5 g maximum. The reddish-yellow fruits are extremely sour but is edible. It is a dioecious species, both male and female forms need to be grown if fruit and seeds are required (Sosef and Dauby 2012). The fruiting generally takes place towards the dry season between September and December (Iwu 1993; Burkill 1994).

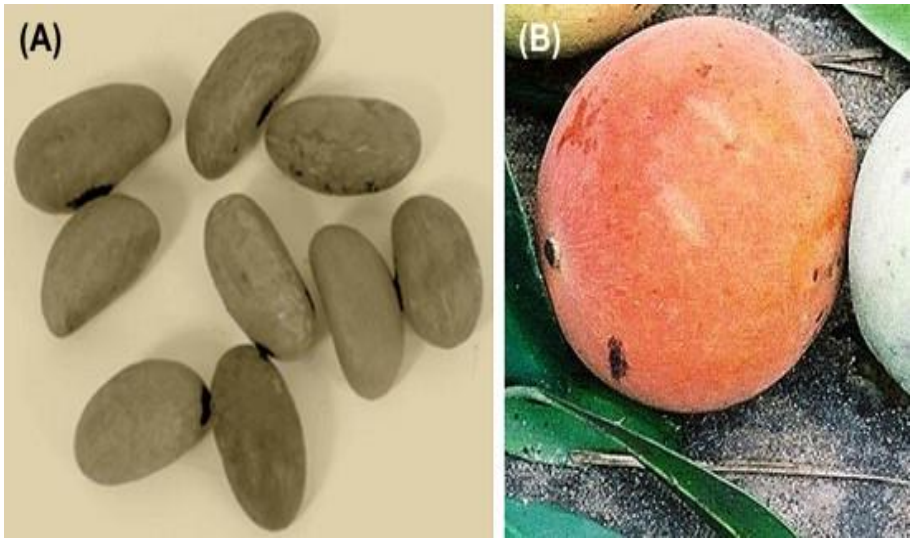

Figure 4. a) *Garcinia kola* seeds, b) fruits of *Garcinia kola* plant.

Table 1. Summary of selected physical characteristics of *Garcinia kola* fruits and seeds (Sosef and Dauby 2012).

Physical characteristics	**Fruit**	**Seeds**
Colour	Reddish-brown	Brown
Shape	Spherical	Ovoid
Average weight	123 g	7.06 g
Skin appearance	Smooth	Rough
Width	80-85 mm	20-21mm
Length	80-85 mm	35-40 mm

2.2.5. Nutritional and chemical composition

Garcinia kola is known for its wide range of biological and pharmaceutical constituents such as anti-inflammatory, anti-bacteria and anti-fungal components. The active composition contributing to the protective effects are phytochemicals, vitamins, and minerals (Okwu, 2005).

Chemical analysis of composition of Garcinia kola proved that main elements consist of calcium, phosphorous, sodium, potassium, magnesium, and traces of elements

like iron, zinc, copper, manganese, chromium, cobalt and cadmium (Okwu 2005). *Garcinia kola* which is popularly called the "African wonder plant" is a product which constitutes a lot of vitamins, minerals, proteins, and carbohydrates in large quantities (Tables 2 and 3).

Table 2. Vitamin composition of *Garcinia kola* seeds on a dry weight basis.

Vitamins	**Content (mg/100g±SD)**
Thiamin (Vitamin B1)	0.5±0.30
Riboflavin (Vitamin B2)	0.22±0.0
Noacin (Vitamin B3)	1.60±0.01
Ascorbic acid (Vitamin C)	23.10±0.02

SD: Standard deviation

Table 3. Mineral composition of *Garcinia kola* seeds on a dry weight basis (Maňourová 2017).

Mineral	**Content (mg/100g±SD)**
Macro Elements	
Magnesium	0.42±0.30
Calcium	1.80±0.40
Potassium	2.50±0.10
Phosphorous	0.33±0.10
Sodium	0.72±0.10
Micro elements	
Iron	17.75±0.30
Zinc	2.30±0.01
Copper	0.78±0.20

SD: Standard deviation

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are natural bioactive compounds found in plant food, leaves or other parts of plants that interact with nutrients and dietary fibre to fight against diseases. They can protect humans against diseases as well as, curtailing the risk of various chronic or inflammatory conditions (Middleton et al. 2000). *G. kola* contains phytochemical compounds such as alkaloids, flavonoids, tannins, phenols, and saponins which provide its attributes nutritional and pharmaceutical properties (Okoli et al. 2014) (Table 4).

Table 4. Phytochemical constituents of *Garcinia kola* seeds on dry weight basis.

Constituents	**Content (mg/100g ±SD)**
Phenols	0.11±0.20
Alkaloids	0.36±0.10
Tannins	0.26±0.20
Saponins	11.48±0.10
Flavonoids	1.98±0.20

SD: Standard deviation

Research has proved the wide spectrum of organic compounds contained in *G. kola* seed such as flavonoids which confer some antimicrobial and antifungal actions against gram negative and gram-positive microorganisms. The biological reaction of flavonoids include action against allergies, inflammation, free radicals, and hepatoxins (Terashima et al. 2002). Most phytochemicals have antioxidant reactions that safeguard cells against oxidative destruction and cuts down the possibility of developing certain types of cancer. These are some of the phytochemicals that possess antioxidant reactions; carotenoids (found in fruits) flavonoids (found in fruits, vegetables), polyphenols (tea, grapes) Adesuyi et. al. 2011). In this regard the protective impact of *G. kola* on human being and animals is because of the active content of phytochemicals, vitamins, and minerals (Okwu and Ekeke, 2003) (Table 5).

Table 5. Approximate chemical composition of *Garcinia kola* (Manourova 2017).

Parameters %	
Moisture content	7.2 ± 0.08
Crude protein	1.86 ± 0.15
Crude fiber	1.23 ± 0.15
Ash	0.47 ± 0.09
Crude fat	0.19 ± 0.32
Carbohydrate	88.3 ± 0.08

SD: Standard deviation

2.2.6. Cultivation

The tree is cultivated in home gardens as well as fruits are harvested from natural stands found in forest, specifically in the Western part of Africa (Daramola and Adegoke 2011). Stem cuttings are used for propagation (Kouakou et al. 2016). In the tropical moist forest throughout West and Central Africa the tree takes about 8 years to fully mature (Kamegne et al. 2010). The fruit mature during rainy season between June and August annually. The process of harvesting is done either by collecting the ripe fruit from under *G. kola* tree or by using a harvesting tool (Matig et al. 2006)**.** The tree is rarely grown, due to poor natural regeneration and slow growth of seedling of the species. As a result, production of the seed is very low during harvesting as such *G. kola* is considered in most part of Africa as a rare species. Even though the seed of the tree is highly valued, its propagation is not well known because of the adversity in seed germination (Adebisi 2004). Seed dormancy has become a major problem for *Garcinia kola* propagation as for various species of *Garcinia* genus. *Garcinia kola* seeds can take about 18 months to germinate due to seed dormancy. Therefore, it is imperative to develop means of breaking this dormancy for early germination (Aduse-Poku 2003).

Farmers consider *G. kola* as one of the useful domestic trees in West and Central Africa (Anegbeh et al. 2006), but the high diversity of the species makes it challenging to recommend the basic method of improving its germination.

Several approaches have been used to affect tree dormancy and to improve seed germination in Nigerian, Cameroonian, Ivorian, and Ghanaian collections (Emmanuel and Roy 2001; Agyili et al. 2007; Kanmegne and Omokolo 2008). With *G. kola* cultivation most of West African indigenous tree producers have identified easy way of propagation by leafy stem cuttings (Leakey 2004). In addition, vegetative propagation studies have investigated the grafting of juvenile seedlings. Figure 5 shows stem cuttings used for propagation (Kouakou et al. 2016). The results stated that within the period of the experiment there was an appreciable increase in height of grafted materials from the first week to 12th week (Yakubu et al. 2014).

Figure 5. Stem cuttings used for propagation. a) leafless semi-hardwood cuttings; b) leafy softwood (Kouakou et al. 2016).

2.2.7. The use of *Garcinia kola*

The seeds are recognized as the most important part of the tree. They are known as ‘bitter kola’, pointing out their typical astringent taste. On chewing, *G. kola* seed has

a bitter astringent and resinous taste, somewhat resembling that of raw coffee, followed by a slight sweetness (Eisner 1990). This bitter astringent taste after chewing the seed is considered as a stimulant and aphrodisiac for men (Yakubu et al. 2014). This has earned the seed a common name 'male kola', (Fondoun and Tiki Manga 2000). In addition, the sap and stem of oil palm (*Elaeis guineensis*) together with roots and bark of *G. kola* are used in ethnomedicine for treatment of impotency (Eyebe et al. 2012). *G. kola* is believed to be an important source of new chemical substances with potential therapeutic benefits (Eisner 1990). Because of the therapeutic composition, the seed extracts can be used to prevent or cure bacterial infection (Stanley et al. 2014; Denloye et al. 2009).

The seed/kernels also contain a chemical called 'kolaviron', a bioflavonoid which has antimalarial properties (Murray et al. 2012; Oluwatosin et al. 2014). *G. kola* seed is composed of a wide spectrum of organic constituents such as flavonoide which confer on it some antimicrobial and antifungal effects against gram negative and gram-positive microorganisms. The biological reaction of flavonoids includes fighting against allergies like; inflammation, free radicals and hepatoxins. It can also be used in curing of liver disease, diarrhoea, diabetes, bronchitis, and throat infection (Yakubu et al. 2014). It is a hard wood which is used for tool handles and, when there is shortage of firewood, it can be also burned as firewood (Adebisi 2004; Anegbeh et al. 2006). It is considered important for local dental hygiene; therefore, the branches and roots are sold in bundles as traditional chewing sticks. In Ghana, 90 % of chewing sticks come from *G. kola*, *G. epunctata* and *G. afzelii* (Blay 2004; Adebisi 2004). Because of the tannin content in bark of bitter kola it is used for tanning leathers, and for palm wine production. The taste is considered to improve the flavour and alcoholic constituent in the local drinks (Onayade et al. 1998; Leakey 2012). Many of the plant part extracts are useful remedy for laryngitis, mouth infections, cough, heart burns, liver disorders, chest colds, hoarseness, and other inflammatory diseases. The seeds can also be used for treatment of throat troubles, bronchitis, urinary tract infections etc. (Kanmegne and Omokolo 2008). It is consumed by the three main traditional groups in Nigeria (i.e., the *Yurobas*, the *Agbilu* and the *Hausas*) and this has earned local names for the tree as, *Agbilu* in *Igbo* and *Orogbo* in *Yuroba* languages (Okoli 2014). The seeds also play an important role in traditional gatherings such as childbirth, marriage, or chieftaincy the seed is being served as part of the occasion and people sometimes believe that the nuts

may work as a snake repellent when placed around their compounds (Anegbeh et al. 2006; Eyebe et al. 2012).

The process of production and consumption of *G. kola* has an immense positive effect on various homes and especially its trade has contributed to enhancing the living standards of the people close to the forest reserve areas. The processing is usually a household affair and the income made through trading of the seed is used to fulfill social, family, and educational obligations (Yakubu et al. 2014).

2.3. Study area

Cameroon is a lower-middle-income country with a population of 23.3 million people and a growth rate of 2.5% per annum. It is situated in Central Africa, bounded clockwise (from the west) by the Gulf of Guinea, Nigeria, Chad, Central African Republic, Congo, Gabon and Equatorial Guinea and it covers total land area of 475,442 km^2 (World Bank 2016). From the Atlantic Ocean and Lake Chad where the southern and northern part borders, lay between longitudes 8º and 16º and E and latitudes 1º 13ºN. The country comprises ten regions: Adamaoua, Centre, Coastal, East, Far North, North, North-West, South, South-West and West (Pamo 2008).

The country is endowed with rich biodiverse forest, which is part of the Congo Basin, ranked as the second largest tropical forest in the world. It is sometimes termed as "Africa in miniature"; therefore, it is considered as fourth richest region in terms of biodiversity (Pamo 2008; Eyebe et al. 2012). The total land cover for cultivation is around 13 % in Cameroon and the population dependent directly on farming is about 50-70 % (FAO 2014; World Bank 2014). Cropping systems of the region are mainly based on long-fallow rotations and produce food crops for home consumption and local markets. The major food crops cultivated in the humid parts of the country are corn (*Zea mays*), beans (*Phaseolus spp., Vigna spp*.), cassava (*Manihot esculenta*), yam (*Dioscorea spp*.) and taro *(Colocasia esculenta*). The food crops are in intercropped with a variety of other vegetables (Gockowski et al. 1998). There are five agro-ecological zones with various agriculture production system in Cameroon (Figure 6): Sudano-Sahelian upland, Guinean savannah, Western highlands, Rainforest with monomodal rainfall pattern and Rainforest with bimodal forest pattern (FAO 2005). In

the north there is the Sudano-Sahelian zone where pastoral system is common, which stretches into the Sahel and contains the North and Far North regions. Most of the drought tolerant species such as sorghum, millet, maize and cotton are grown in this area. Likewise, for the Adamawa region, which lays in the Guinea-Savanah zone (World Bank 2016). Annual rainfall is about 2,815 mm and mean annual temperature is 18.6℃ in the south forest zone (Epule and Christopher 2016), and although the population density is lower, most of the country's cash crops like oil palm and cocoa are cultivated there. However, in the South and East there is a difference between bimodal rainfall zone of the coast and unimodal rainfall. Finally, agro-pastoral system could also be associated with the Plateau in the North West part of the country and coffee is the main cash crop produced there (World Bank 2016).

Cameroon can also boast of important cash crops like cocoa (*Theobroma cacao*), coffee (*Coffea* spp.) and oil palm (*Elaeis guineensis*) (Degrande et al. 2006). Among smallholders, cash crops are usually cultivated in agroforestry systems with varying level of intensification (Akinnifesi et al. 2007).

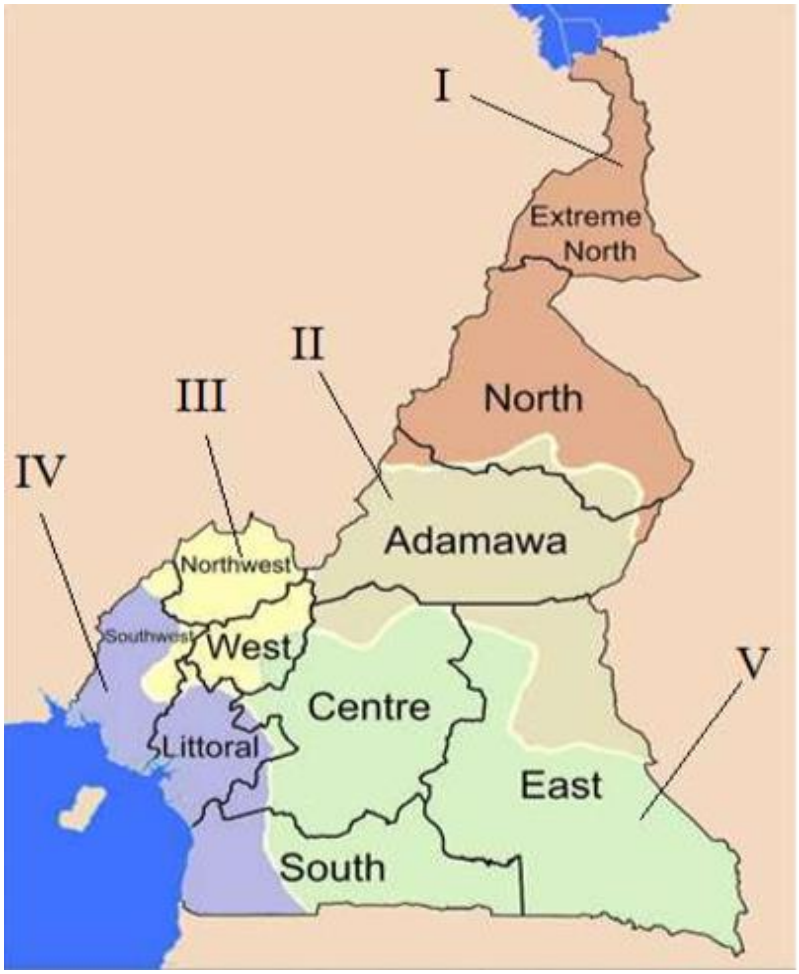

Figure 6. The five agro-ecological zones of Cameroon. I – Sudano-Sahelian up1land; II – Guinean savannah; III – Western highlands; IV – Rainforest with monomodal rainfall pattern; V – Rainforest with bimodal rainfall pattern. Modified from: Wikimedia Commons and Ndoumbe-Nkeng et al. (2004).

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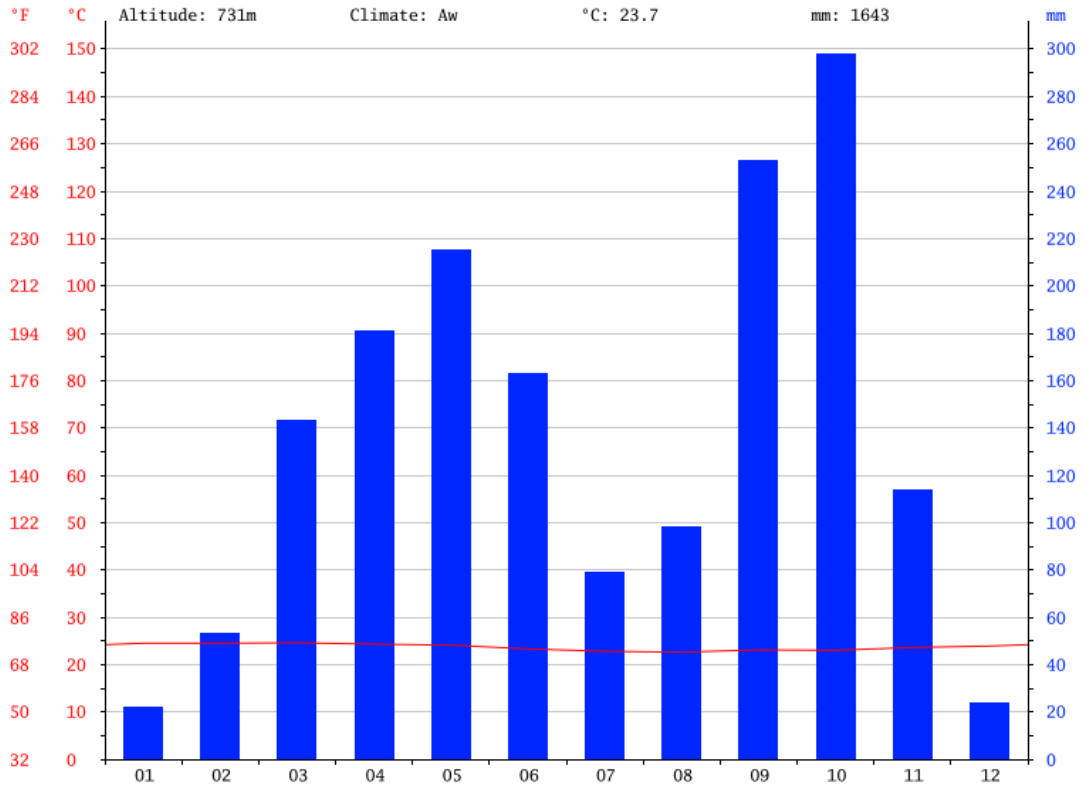


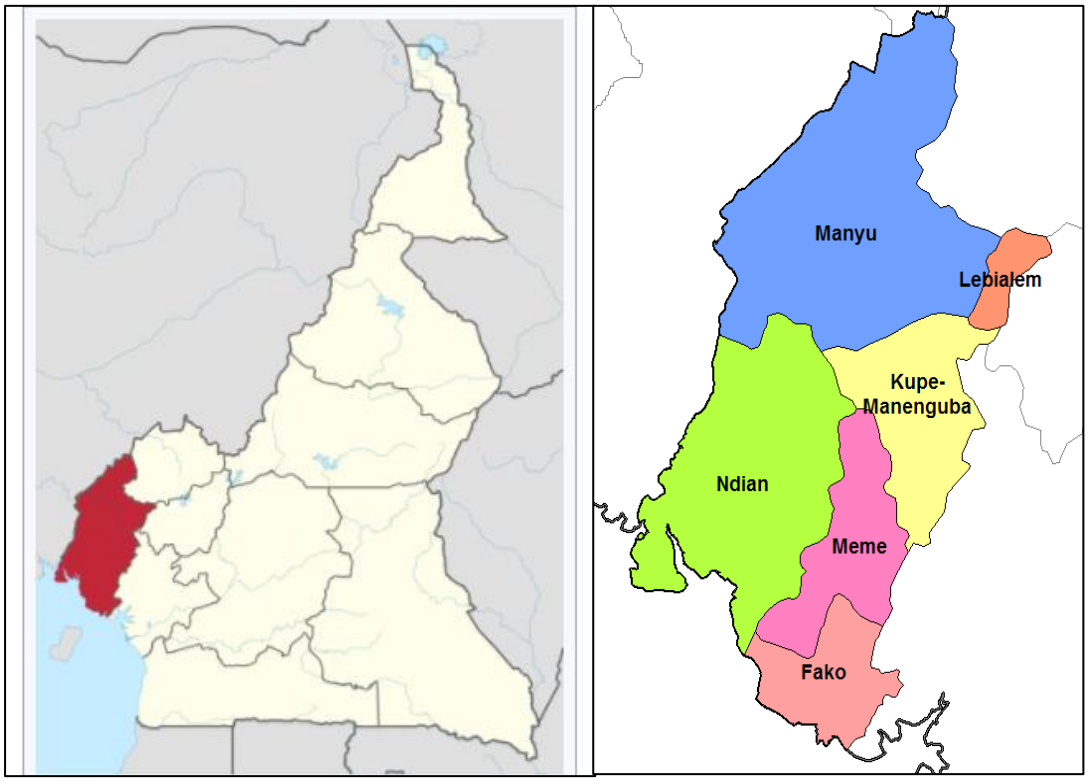
Figure 7. The climate of Cameroon (https://en.climate-data.org).

2.3.1. Southwest region

The study was conducted in the Southwestern part of Cameroon. This region is one of the two anglophone territories in Cameroon, and it is the smallest one in terms of its landmark (25,410 km^2) and number of citizens (1,153,000) which lays between latitude 5.417 and longitude 9.333 where Bue is the Capital city (World index 2018) (Figure 8a). The Equatorial climate of southwest Province of Cameroon experiences a very heavy rainfall, (1,500–2,000 mm) each year; thus 9-months of rainy season and a 3 month of dry season per year (Matyn et al. 2007). Annual mean temperature of about 18.6 °C. The dry season stars from December with rainfall of 29 mm up to August which is the wettest month with rainfall of about 488 mm. The warmest and the coldest month is between March and July with temperatures of 19.7°C and 17.3 °C respectively. About 70% population in southwest Cameroon are essentially made up

of small-scale peasant farmers and all are family members (Epule and Christopher 2016). Because of the country's diverse agro-ecological zones, three main farming systems are dominating; forest based, tree crop based and cereal-root crop-based farming systems (de Graaf et al. 2011). Major food crops cultivated in this area are: maize (*Zea mays*), rice (*Oryza sativa*), cassava (*Manihot esculenta*), and potato (*Solanum tuberosum*) (Genesis and Jonas 2013). There are six divisions in the Southwest province: Fako, Kupe Manengouba, Lebialem, Manyu, Meme and Ndian (Figure 8b).

Figure 8. a) The southwest region on the map of Cameroon b) The map of divisions of



Southwest province (Rarelibra 2006; Wikimedia Commons).

3. OBJECTIVES

The main objective of this study was to assess the genetic diversity of *Garcinia kola* (Heckel) from Southwest region of Cameroon.

Specific objectives were:

1. To evaluate the polymorphism for 75 accessions of *Garcinia kola* using RAPD molecular marker.
2. To analyse the genetic relationship between 75 accessions of *Garcinia kola* using statistical methods.
3. To propose for better management, conservation, and utilization of *G. kola* gene pool.

4. MATERIALS AND METHODS

4.1 Study sites and sampling

During this study four sites, i.e. Kumba, Mamfe, Lebialem and Tombel, in four different divisions of Southwest region, i.e. Meme, Kupe-Manengouba, Lebialem and Manyu, were selected for sampling, according to the advices by ICRAF employees and reviews that reveal the main harvesting periods of *G. kola*. (Fondoun and Tiki Manga 2000). Local authorities and NGOs were consulted to assist and support in data collection: IRAD and Key Farmers Cameroon from Meme division, RECODEV from Kupe-Manengouba, ERuDeF from Lebialem and Elena NGO from Manyu division.

In Meme division, data were sampled around city Kumba, also known as KTown, with about 80,000 inhabitants, and in Konye village. Average daily temperature in Kumba is 25.5 °C with average annual rainfall of 2,751 mm and mean altitude of 239 masl (Sama et al. 2007; Climate Data 2016).

Surrounding of Tombel town was the main site for data collection in Kupe Manengouba division. The town is part of the mountainous Bakossi landscape with the highest peak called Mt. Kupe (2,064 masl). Average daily temperature in Tombel is 24.6 °C, mean rainfall per year is 3,090 mm whereas average altitude there reaches about 450 masl (Climate Data 2016).

Lebialem division is characterized by diverse climate and hilly landscape, which is ranging from 200 up to 2,400 masl of altitude. A semi-evergreen tropical broadleaf forest dominates the lower altitudes. Data were collected in Menji, divisional headquarters with average altitude of 730 masl, along with lowland villages Bechati, Banti and Nkong located about 300 meters above sea level. Mean annual rainfall is reported to be 4,500 mm, average daily temperatures vary between 23-24 °C (Wright and Priston 2010; Climate Data 2016).

In Manyu, the division directly neighbouring Nigeria, villages surrounding its main city Mamfe were explored: Etoko, Kendem, Kepoti, Messeng Bakebe, Mfuni and Nchemba I. In Mfuni, the daily mean temperature is around 26.7 °C along with average annual rainfall of 2,753 mm and elevation usually below 200 masl (Climate Data 2016).

Between June and July 2016, Snowball method were used to select 48 farmers from 50 farms were and interviewed using simple semi-structured questionnaire (Appendix A) to unveil the basic knowledge about the usage propagation of the tree and its products, e.g getting facts on best harvesting seasons, seed utilisation or storage possibilities were also recorded. Eleven respondents were found in Kumba, 10 people were questioned in Tombel, 12 farmers came from Lebialem and 15 from Mamfe. Finally, 81 matured fruiting trees were gathered on the farms, measured, and analysed (Figure 7). Then, 8 - 10 fruits along with 4 - 5 leaves were taken per each tree for further morphological examined. Afterwards, seeds were removed from the fruit pulp and measured as well. The exact number of samples taken per each study site is presented in Table 6.

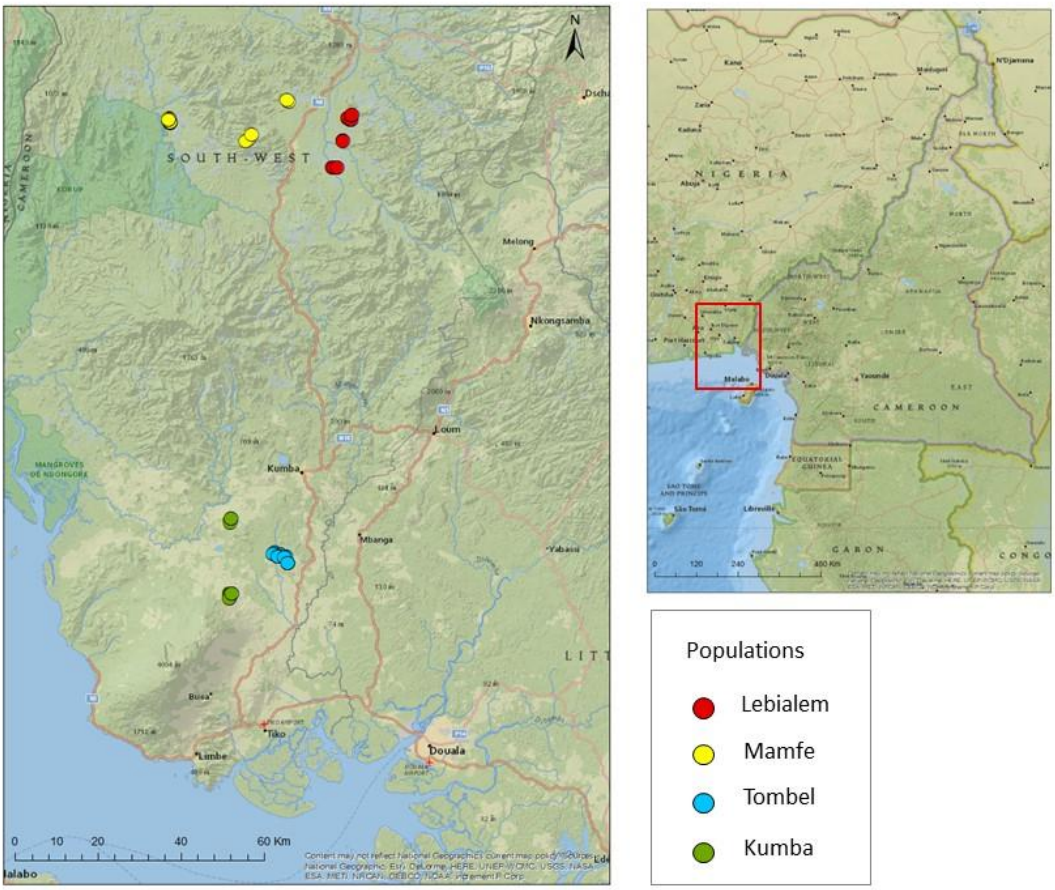


Figure 9. Locations of sampled trees. Red – Lebialem, Yellow – Mamfe, Blue – Tombel Kupe-Manengouba division, Green – Kumba, Meme division

Table 6. Data collection details from different study sites.

Study site/population	Division	No. of farms	No. of trees
Mamfe	Manyu	16	17
Tombel	Kupe-Manengouba	10	20
Kumba	Meme	12	18
Lebialem	Lebialem	12	20
Total		40	75

4.2. Laboratory analysis

4.2.1. DNA extraction

DNA was extracted from the dried seed coat using a modified CTAB protocol (Doyle and Doyle 1987; Faleiro 2002). The tissue was manually ground with purified sand using a mortar and a pestle. The cells in the ground sample were lysed by adding 800ul of extraction buffer (CTAB 2.8%, NaCl 1.3 M, EDTA 20 mM, TRIS-HCl 100 mM, PVP 1%, mercaptoethanol 0.2%) and 100 ng of Proteinase K and heating the samples to 65 °C for one hour while mixing them every 10 minutes. The contaminants in the samples were denatured by adding 700 ul of chloroform: isoamyalcohol (24:1) and mixing the contents for 10 minutes and the phases were separated by centrifugation for 10 minutes at 14,000 RPM and 4 °C. The supernatant was transferred to a new microtube, 55 ul of CTAB 7% was added and the chloroform IAA extraction was repeated to remove all contaminants. The resulting supernatant was mixed with 900 ul of isopropanol in new tubes, which were placed at 20 °C for one hour to allow the DNA to precipitate. After precipitation, the tubes were centrifuged for 10 minutes at 14,000 RPM and 4 °C, the supernatant was discarded, and the resulting pellet was washed twice with ethanol to remove remaining salts. Consequently, the pellet was dried at room temperature and dissolved in 100 ul of TE buffer with addition of 30 ng of RNase. The concentration and quality of the extracted DNA was measured by the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). Extracted DNA was stored in

microtubes at -20 °C until further processing.

4.2.2. PCR and fragments visualization

Eight samples were used to optimize the PCR reaction using 20 arbitrary decamer primers (East Port, Czech Republic). Ultimately, seven primers that produced clear polymorphic bands were selected for final RAPD analysis of all samples (Table 7).

Table 7. List of RAPD primers used.

Primer	Sequence 5'-3'	Ta
OPA9	GGGTAACGCC	47 °C
OPA11	CAATCGCCGT	44 °C
OPA12	TCGGCGATAG	44 °C
OPA15	TTCCGAACCC	44 °C
OPA18	AGGTGACCGT	44 °C
OPA19	CAAACGTCGG	47 °C
OPA20	GTTGCGATCC	47 °C

Ta: annealing temperature

The amplification of random fragments by PCR (polymerase chain reaction) was performed using a 10 ul reaction mixture containing 5 ul of Multiplex PCR Kit (Qiagen, Germany), 1 uM of primer, 40 ng of DNA and 2 ul of ddH_2O (Table 8).

Table 8. PCR composition.

Substance	Concentration	Volume
ddH_2O	-	2 µl
Multiplex PCR Kit	2x	5 µl
Primer	10mM	1 µl
DNA	20 ng/ µl	2 µl
Total		10 µl

The PCR reactions were run in T100 Thermal cycler (Bio-Rad, USA) with the following program: one cycle of 15 minutes at 95 °C for initial denaturation, followed by 45 cycles of 30 seconds at 95°C, 1 minute at optimal annealing temperature for the respective primer (Table 7), 1 minute at 72 °C and 10 minutes at 72 °C for final extension (Table 9).

Table 9. PCR profile.

Step	Temperature	Time	Nr of cycles
Initial denaturation	95 °C	15 min	1
Denaturation	95 °C	30 sec	
Annealing	44 °C/ 47 °C	1 min	45
Extension	72 °C	1 min	
Final extension	72 °C	10 min	1
Hold	4 °C	∞	1

Amplified fragments were separated by size by electrophoresis. 5 µl of each PCR product were mixed with loading dye (Thermo Fisher Scientific, USA) and loaded onto 1.5% agarose gel stained with EtBr (ethidium bromide). The length of the amplified fragments for each RAPD primer was assessed based on its migration relative to molecular weight size 100 bp DNA ladder (Thermo Fisher Scientific, USA).

Electrophoresis was run for 90 minutes at 90 V. Subsequently, the gels were photographed under UV light in a transluminator (Figure 10).

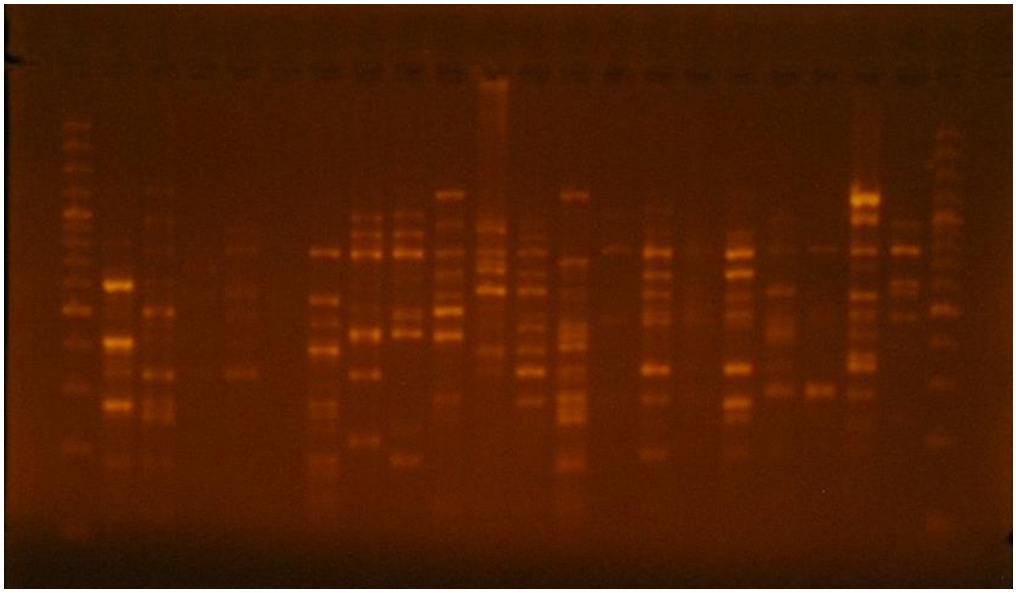


Figure 10. Photographed gel under UV light in a transluminator.

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4.3. Data analysis

The amplified RAPD fragments were scored for the presence (1) or absence (0) of bands on the gel, and a binary matrix was created in a Microsoft® Excel spreadsheet, considering only clear and strong bands.

The data was analyzed by POPGENE 1.32 software (Yeh and Boyle 1997). The binary matrix was imported into the software and used to calculate basic statistics, such as number of scorable bands and number and percentage of polymorphic loci. To assess the population structure of analyzed accessions, POPGENE 1.32 was used to calculate percentage of polymorphic loci per population, as well as Nei's (1973) gene diversity index and Shannon's information index (1972) per population and across all accessions. Further, Nei's genetic distance and genetic identity between pairs of populations was calculated to analyse the relationships. Nei's genetic distance was consequently used to construct a UPGMA (unweighted pair group method with arithmetic mean) dendrogram that was visualized by Fig Tree 1.4.3.

5. RESULTS

5.1. RAPD profile and analysis

The 7 primers that producing clear bands were used to assess the genetic diversity of 75 *Garcinia kola* samples. The length of fragments from selected primers ranged between 100 to 1,500 base pair (bp), but the results proved that fragments out of range between 200 and 1200 were not reproducible. The amplification products ranging from 250 to 1200 bp that yielded only sharp and strong were scored to come up with a binary matrix.

The total number of bands observed were 135 with a mean number of 19.29 scorable bands per primer and with polymorphic scorable bands 135 were identified which means 100%. Meanwhile with monomorphic, no bands were identified out of the total scorable bands and gives 0%.

Table 10. Primers used, and schematic description of monomorphic and polymorphic bands obtained with RAPD markers.

Locus	Total scorable bands	Monomorphic loci	Polymorphic loci	%	Range of amplification
OPA 09	20	0	20	100	150 - 1200
OPA 11	20	0	20	100	200 - 1200
OPA 12	21	0	21	100	150 - 1200
OPA 16	19	0	19	100	150 - 1500
OPA 18	19	0	19	100	150 - 1200
OPA 19	16	0	16	100	150 - 1200
OPA 20	20	0	20	100	150 - 1200
Total	135	0	135		
Mean	19.29	-	-	100	-

5.2. Genetic diversity and population structure

Genetic diversity as estimated by Nei's gene diversity (h), Shannon's Information Index (I) was not significantly different among populations (Table 11). The Nei's gene diversity (h) ranged from 0.1940 (in Kumba) to 0.1889 (in Labielem) with an average of 0.1914 and the overall value of 0.2028 (Table 11).

The Shannon's Information index (Lewontin 1972) (I) ranged from 0.3184 (in Tombel) to 0.3355 (in Mamfe) to an average of 0.3270 and an overall value of 0.3431. The number of polymorphic loci also ranges from 121 (in Kumba) to 129 (in Lebialem) with an average score of 125 and the percentage of polymorphic loci which ranges from 89.63% (in Kumba) to 95.56% (in Lebialem) with an average of 92% (Table 11).

Table 11. Genetic diversity indices per analysed population of *G. kola*.

Population	**nr polymorphic loci**	**% polymorphic loci**	**h**	**I**
Kumba	121	89.63 %	0.194	0.323
Lebialem	129	95.56 %	0.188	0.337
Mamfe	122	90.37 %	0.203	0.335
Tombel	128	94.81 %	0.186	0.318
Overall	135	100%	0.202	0.343

h: Nei's gene diversity index, I: Shannon's information index

The genetic distance was overall low between all pairs of populations, but lowest (0.0055) between Kumba and Tombel populations whereas the largest distance (0.0082) was between Lebialem and Mamfe populations. The genetic identity was generally high between all pairs of populations but the lowest was (0.9918) between Tombel and Mamfe populations and the largest genetic identity (0.9945) was between Kumba and Tombel populations (Table 12).

Table 12. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) between pairs of populations of *G. kola.*

pop ID	**Kumba**	**Lebialem**	**Mamfe**	**Tombel**
Kumba	****	0.9929	0.9922	0.9945
Lebialem	0.0072	****	0.9923	0.9926
Mamfe	0.0078	0.0077	****	0.9918
Tombel	0.0055	0.0075	0.0082	****

The UPGMA dendrogram based on genetic distances among the four populations of *G. kola* is shown in Figure 11. The populations were divided into two basic clusters, with Kumba and Tombel being in one cluster and Lebialem and Mamfe being in the other cluster. The division based on genetic distance corresponds to the geographical distribution of the populations (Figure 9). The genetic distance between populations Kumba and Tombel which is the lowest (0.0055), looking at the graph though they are dissimilar but closely related whereas (0.0082) which is the largest genetic distance between Labielem and Mamfe accessions, this is because there is a slightly larger dissimilarity among these 2 accessions as indicated on the graph.

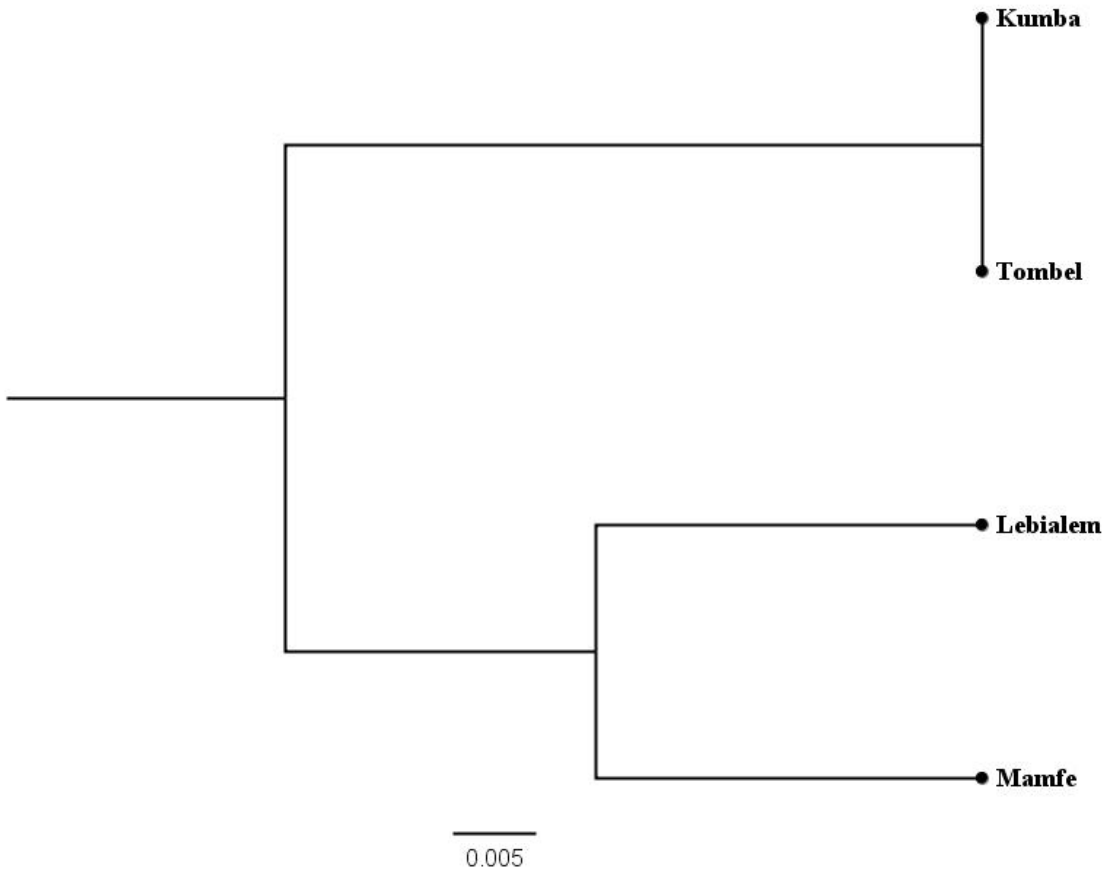

Figure 11. UPGMA dendrogram based on Nei's genetic distance showing relationships between populations of *G. kola.*

6. DISCUSSION

Assessment of genetic variation is important for executing plant domestication, conservation, and breeding programmes (Sreekumar and Renuka 2006). Genetic plant resources contribute immensely to producing different crop varieties with good harvest capabilities or resistance to biotic and abiotic stress. The germplasm of a crop collected from the local sources provides greater genetic variability and can furnish useful traits to broaden the genetic base of the crop species (Bashir et al. 2008; Mondini 2009). In this research, a total number of bands observed were 135 with a mean value of 19.29 scorable bands per primer. Polymorphic scorable bands 135 were identified which means 100% polymorphism were achieved. So far, published research journals on genetic diversity of Garcinia kola for comparison purposes is inadequate. Alejandro (2016) using RAPD on *Sorghum bicolor*, analyse 126 bands and observed 89% of polymorphic bands. 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 %. The study of Irikidzai (2021) using AFLP on garcinia kola, a total of 1176 fragments were generated, and 98.6 % level of polymorphism were recorded.

The high level of polymorphism and ease of scoring of amplified bands proved the utility of the seven selected RAPD primers in assessing the genetic relationships within and among populations of *Garcinia kola*. These values indicate high levels of genetic diversity and are quite typical for wild plant populations that are not highly affected by genetic drift or bottleneck effect (Verma and Rana 2011; Bakatoushi and Ahmed 2017). The values of Nei's genetic distance between populations show low level of interpopulation differentiation, which can indicate low selection and domestication pressure on the species (Gao et al. 2017).

The average percentage of polymorphic loci and Nei's genetic diversity (h) values in the present study show that the diversity in *G. kola* is generally high in Southwestern Cameroon. This information is considered as necessary steppingstone for species domestication and will serve as a guide in selection and identification of exceptional populations which can provide a platform for production increase.

The four studied populations were divided into two groups with obvious regionalism, where the populations Kumba and Tombel were in one cluster and Lebialem and Mamfe in another cluster. Therefore, the division based on genetic distance corresponds to the geographical distribution of the populations, and to the differences in altitudes. Kumba and Tombel are geographically close to each other and the terrain is similar in both sites, whereas the sites where populations Lebialem and Mamfe were collected differ significantly in altitudes (up to 2000 masl and below 200 masl respectively). The steep difference in altitudes between the later populations probably causes a barrier in gene flow between them, thus causing a larger genetic distance than in the former two.

Manourova (2017) assessed and described the morphological diversity and nutritional status among the same populations of *G. kola* from Southwest region of Cameroon. According to the research morphological diversity within populations was higher than the diversity between populations and this must be because of external conditions having influence on morphological diversity rather than by genetics. These results of morphological diversity assessment are supported by the findings about levels of inter- and intra-population genetic diversity from our study, however, the results of present study show that the influence of genotype on morphology and especially biochemical properties should be taken into consideration, as was also demonstrated by other authors (Des Marais et al. 2013; Cheng et al. 2016).

Dadjo, (2020) also accessed the genetic diversity and population structure of *G. kola* in Benin through a genome-wide SNP dataset. The outcome of this study showed low genetic variation among the studied populations and high variation among individuals within populations. The findings of this research also showed very high levels of inbreeding (F_{IS} = 0.781–0.848), and this could be ascribed to self-pollination in *G. kola* populations (Szczecińska, et al., 2016). It was also observed that, *G. kola* is a dioecious species which has the potentials to copulate with half sibs which may have resulted in inbreeding among closely related individuals. In addition, genetic variations were fundamentally found within populations (97.86%). This could be because of the little dispersion scope of *G. kola* in Benin and the short distances between the studied population, which speeds up gene flow between populations. Ultimately, the population structure analysis indicates the studied populations of *G. kola* to be a single population.

Therefore, it is impossible to establish whether these findings and observations could be estimated to the larger distribution range of *G. kola* due to small deliveries of the species in Benin. Selecting from a lager region, within the existing population of *G. kola* in West Africa, would give a much stronger description of genetic diversity and population structure of the species throughout in its locality.

7. CONCLUSION

This study analysed 75 accessions of *Garcinia kola* from four populations collected on 40 farms in Southwestern Cameroon with seven RAPD primers, to discover the levels of within and among populations genetic diversity.

Therefore, considering the high levels of genetic diversity per population with Nei's diversity index, additional studies are required to support the development of this genus with therapeutic properties for the prevention and treatment of various diseases; most importantly, non-transmissible chronic diseases and its conservation. In the same vein, this plant provides a promising potential source of natural metabolites for pharmaceutical and medicinal applications.

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APPENDICES

Appendix A – Questionnaire

Name: Gender: Age:

Village: Estimated size of farm:

Number of bitter kola trees on farm:

1) Which parts of the tree do you use and how?
2) How did you get the tree? (Wildlings, from another farmer, remain after forest clearance, seed, seedling, cutting…)
3) Did your father (ancestors) also use the tree?
4) Are there any traditions, taboos, stories connected with the tree?
5) Does the tree need any special care?
6) Do you propagate the tree? (vegetative/generative)
7) How many times per year is the tree fruiting, how many times do you harvest?