Czech University of Life Sciences Prague

Faculty of Tropical AgriSciences

Study Programme: Tropical Crop Management and Ecology

Diversity and nutritional characterization of *Garcinia kola* Heckel in Southwest Cameroon

M.Sc. thesis

Prague 2017

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Declaration

I hereby declare that this thesis, submitted in partial fulfilment of requirements for the master degree in Faculty of Tropical AgriSciences of the Czech University of Life Sciences Prague, is wholly my own work written exclusively with the use of the quoted sources.

Prague, 28th April, 2017

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Anna Maňourová

Acknowledgements

First, I would like to thank my supervisor doc. Ing. Bohdan Lojka, Ph.D. who allowed me to work in Cameroon for a second time and guided me throughout the thesis compilation. This thesis would not be achieved without my co-supervisor Ing. Olga Leuner, Ph.D, who introduced me to the laboratory work. Thank you for your undying enthusiasm and support! Consequently, I have to mention Ing. Dana Homolková, my mentor in laboratory and future collaborator for upcoming African projects. I must also thank to Prof. dr. ir. Patrick Van Damme for his hand notes and the fastest email replies I have ever seen. And finally, I have to mention my friend Ondřej Přibyl for his adventurous spirit, bold decision to join me to Cameroon and tremendous effort in peeling of *G. kola* seeds!

Big thanks belong to family Tchana from Yaoundé, Cameroon, who provided us not just a place to sleep but open their hearths to receive new family members. Merci beaucoup! I am also grateful to all ICRAF Cameroon members, headed by my dear dr. Zac Tchoundjeu. Sygnola, Josephine, Fred and Urcil, hope to meet you soon again! During the field trips in Southwest region, I have met a lot of wonderful people: first Cameroonian vegetarians - Jacob (RECODEV) and Daniel (Key Farmers Cameroon), terrain master Ashu (Elena NGO), the best field guide Elong (ERuDeF), my twin Andy and friend Juliette from Kumba. Thank you for your kindness and hospitality.

Last but not least, I must extend gratitude to my dearest brother Šimon for his data analysis support and 'magic' performed in Python programming language. The whole family and my partner were very patient and of supportive spirit, thank you!

This study was financially supported by 'Podpora mobility studentů' under auspices of Faculty of Tropical AgriSciences, CULS, by foundation "Nadání Josefa, Marie a Zdenky Hlávkových" and by World Agroforestry Centre (ICRAF).

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Abstract

Garcinia kola (Clusiaceae) is fruit tree species indigenous to West and Central Africa. The tree is frequently called 'wonder plant' because all its parts have medicinal use. In Cameroon, seeds of *G. kola* are widely used by locals for treatment of gastric problems. Studies on *G. kola* have mostly focused on its bioactive substances. However, information on intraspecific diversity and the exact nutritional values of the seeds remain unclear. Therefore, the objective of this study was to assess and describe morphological diversity and nutritional status among different populations of *G. kola* from Southwest region of Cameroon. For examination of the species' management and utilization, we visited 50 farms and interviewed 48 farmers. Further, morphological characteristics of 759 fruits, 1,821 seeds and 402 leaves coming from 81 individual trees were examined and botanical descriptors were developed based on the species morpho-types. Seeds of each tree were subjected to nutritional analyses for: ash, moisture, crude fat, fibre, protein and NFE content. The nutritional values were determined as follows: ash 0.33 %, moisture: 42.3 %, crude fat: 1.48 %, crude fibre: 2.27 %, crude protein: 6.48 % and NFE: 47.19 %. As we did not find any significant differences in-between our study sites, the results suggested that morphological diversity within a single population is much higher than the diversity among populations from different sample sites. Process of *G. kola* domestication seemed to be at its very beginning, though we have identified efforts in terms of species targeted cultivation and selection. It is expected that this study may provide basics for the first steps of *G. kola* domestication. In the future, research on species population genetics as well as secondary metabolites content should be conducted to complement our data and fasten the domestication process.

Key words: bitter kola, Central Africa, domestication, indigenous fruit trees, phytochemistry

Abstrakt

Garcinia kola (Clusiacea) je původním ovocných druhem dřeviny pocházející ze Západní a Střední Afriky. Stromu se přezdívá „zázračná rostlina“, protože každá jeho část má léčivé účinky. V Kamerunu místní obyvatelé běžně používají semena *G. koly* na léčbu žaludečních problémů, přípravu tradičních směsí proti malárii nebo je žvýkají pro jejich typickou hořkou chuť a stimulující účinek. Studie publikované na druh *G. kola* se zatím zaměřily hlavně na bioaktivní látky obsažené v semenech. Nicméně informace o vnitrodruhové diverzitě a přesných nutričních hodnotách jsou zatím poměrně nejasné. Proto bylo cílem naší studie posoudit a popsat morfologickou diverzitu a nutriční status různých populací *G. koly* v Jihozápadním regionu Kamerunu. Kvůli zjištění informací o tom, jak se daný druh pěstuje a používá, jsme navštívili 50 farem a vyzpovídali 48 farmářů. Dále jsme určili morfologické charakteristiky 759 plodů, 1821 semen a 402 listů z celkem 81 stromů. Na základě určených morfo-typů jsme navrhli botanický deskriptor pro daný druh. Semena z každého stromu byla analyzována pro obsah: popelovin, vlhkosti, hrubého proteinu, tuku, vlákniny a také BNLV. Výživové hodnoty vyšly následovně: obsah popelovin: 0,33 %, vlhkosti: 42,3 %, hrubého tuku: 1,48 %, hrubé vlákniny: 2,27 %, hrubého proteinu: 6,48 % a BNLV: 47,19 %. Vzhledem k tomu, že jsme nenašli žádné významné rozdíly mezi studovanými lokalitami, výsledky naznačují, že morfologická diverzita byla vyšší v rámci jedné populace než mezi populacemi z různých míst sběru. Ačkoliv jsme zaznamenali snahy o cílené pěstování i selekci *G. koly*, proces její domestikace se zdá být teprve na začátku. Očekáváme, že naše studie poskytne základní údaje pro první kroky domestikace tohoto druhu. Zároveň také doporučujeme, aby byly v budoucnu provedeny další výzkumy, zaměřené hlavně na populační genetiku druhu a obsah sekundárních metabolitů v semenech, které by doplnily naše výsledky a urychlily tak proces domestikace.

Klíčová slova: bitter kola, domestikace, phytochemie, původní ovocné druhy stromů, Střední Afrika

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List of abbreviations

2,4-D – 2,4-dichlorophenoxy acetic acid

BAP – Benzylaminopurine

BNLV – Bezdusíkaté látky výtažkové

CULS – Czech University of Life Sciences

CFA – Central African Franc

DBH – Diameter at breast height

ERuDeF – Environment and Rural Development Foundation

IBA – Indole-3-butyric acid

ICRAF – World Agroforestry Centre

IRAD – Institute of Agricultural Research for Development

NAA – Naphthalene acetic acid

NFE – Nitrogen-free extracts

PCA – Principle component analysis

RECODEV – Regional Center for Conservation and Development

INTRODUCTION

From the outset of humankind, forests have provided people with edible fruits, vegetables and medicine (Onyekwelu et al. 2015a). 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. However, their role is crucial in local food security, health and nutrition or income generation (Termote and Van Damme 2010; Dansi et al. 2012). Especially in Sub-Saharan Africa, local population still depends on obtaining various fruits and other products from wild or not fully domesticated tree species.

In several regions of Africa, World Agroforestry Centre (ICRAF) has started a program of domestication of those highly valuable tree species. In 1997, researchers from ICRAF Cameroon decided to focus their attention to local, indigenous fruit tree species for germplasm conservation and controlled domestication. After years of investigations, six species were selected as priority species: *Allanblackia* spp., *Cola nitida, Dacryodes edulis, Garcinia kola, Irvingia gabonensis* and *Ricinodendron heudelotii* (Tchoundjeu et al. 2006; Asaah et al. 2011, Franzel and Kindt, 2012). Among them, recently the most advanced species in terms of domestication is *Dacryodes edulis* (african plum, safou) (Youmbi et al. 2010). Whereas less attention has been paid to *Garcinia kola* Heckel from family Clusiaceae.

The species, also called bitter kola, plays an important role in African ethno-medicine or traditional ceremonies and it is also one of the most commercialized fruits in West and Central African countries (Jouda et al. 2016). The species is sometimes referred as 'wonder plant', suggesting that each part of the tree can be used as medicine (Usunomena 2012; Onasanwo and Rotu 2016). Its seeds, the most valued product of the tree, are commonly chewed by local people to avoid gastric problems or simply for their typical astringent taste (Leakey 2012).

The kernel contains a wide range of useful phytochemicals. Typically, tannins are present in high quantities and therefore commonly used for treatment of intestinal disorders such as diarrhoea or dysentery (Usunomena 2012). Other compounds such as flavonoids are rich sources of antioxidants (Ijomone et al. 2012). Attention has been recently paid to biflavonoid complex kolaviron, which was reported to possess neuroprotective, anti-inflammatory, antimicrobial, antioxidant, antigenotoxic and hepatoprotective properties (Adegboye et al. 2008; Ijomone et al. 2012; Adaramoye et al. 2014). Some of its compounds are further characterized by antimalarial (Tshibangu et al. 2016) and wound healing properties (Chinaka et al. 2015). Moreover, the extract from bitter kola seeds was reported to halt the deadly disease caused by Ebola virus in its tracks (Iwu 1993).

Despite the species importance and popularity among local people of West and Central Africa, information is generally lacking in terms of its diversity and nutritional characteristics. Insights into morphology, chemical composition and genetics of *G. kola* are generally considered as necessary stepping stones for species domestication. Knowledge of those components can be used as guideline in superior individuals/populations identification and selection. Therefore, the aim of this study was to assess morphological diversity and nutritional status of the species in Southwest region of Cameroon.

LITERATURE REVIEW

2.1. Tree domestication and diversity

Domestication is generally seen as a complex of evolutionary processes in which human use of plant or animal species leads to morphological and physiological changes that distinguish domesticated taxa from their wild ancestors (Purugganen and Fuller 2009). The domestication of crop species started around 10,000-13,000 years ago (Barazani et al. 2016). More specifically, some of the earliest efforts in tree domestication were reported 2,800 years BC for genus *Ficus* (Simons and Leakey 2004). However, great majority of the world's more than 80,000 tree species are still essentially wild or in an early stage of domestication (Ofori et al. 2014). The process of domestication starts with characterisation of naturally available intra-specific diversity of the species, identification and selection of individual mother trees with superior traits. This is followed by vegetative propagation of the mother trees, dissemination of the planting material to farmers and finally cultivation of these improved materials on farms (Anjarwalla et al. 2016). The domestication of new tree crops is one means for improving food and nutritional security. More precisely, the aim of domestication of neglected but valuable tree species is to promote sustainable agriculture through diversification of species generating income, improve diets and health, meet domestic needs and restore functional agroecosystems (Leakey and Asaah 2013; Ofori et al. 2014).

One of essential features in the selection of trees for domestication is the identification of elite individuals with superior desirable characteristics (Tchoundjeu et al. 2006). Practical approach is to seek for trees which have particular trait combination – e.g. larger fruits with sweeter pulp (Leakey and Asaah 2013). Therefore, quantification of the variation in nutrient content and fruit morphological traits is one of the most important steps in identifying superior planting material for domestication. This was proved for example by Simbo et al. (2013) on baobab (*Adansonia digitata*) in Mali, where the authors established selection criteria for domestication by choosing various morphological and phytochemical attributes. However, different environmental conditions, such as climate and soil type, along with genetic heritability, may extensively influence the superior traits development (Leakey 2012).

For the future, there will definitely be a need to develop genetic markers to further determine differences within and between the species provenances. Nonetheless, this procedure is rather expensive and phenotypically oriented studies are considered equally valuable, since the genetic researches still need to be linked with the species morphology and phytochemistry (Van den Bilcke et al. 2014).

2.2. Bitter kola (*Garcinia kola* Heckel, Clusiaceae)

2.2.1. Origin, ecological requirements and distribution

Plants of genus *Garcinia* (family Clusiaceae, formerly *Guttiferae*, order Malpighiales), are widely distributed in tropical Africa, Asia, New Caledonia and Polynesia (Jouda et al. 2016). The genus itself consists of about 200-400 tree species, which can be mainly found in lowland forests of tropical Africa and Southeast Asia (Onayade et al. 1998; Momo et al. 2011). *G. kola* is one of 16 species occurring widespread throughout West, Central and Southwest Africa. More specifically, *G. kola* can be naturally found from Angola to Sierra Leone (Figure 1) (Onayade et al. 1998, Matig et al. 2007). The species prevails in coastal areas and lowland plains up to 300 m above sea level. Average temperature in its ecology usually varies between 21 to 31 °C complemented a by relatively high air humidity of about 76 %. Mean rainfall is commonly at 2,000 – 2,500 mm per year (Blay 2004).

G. kola is highly preferred among African countries and thus also exploited. The species is one of the most important trees in Nigeria and its seeds are mainly collected from wild stands (Anegbeh et al. 2006). In Benin, *G. kola* is regarded as the third most important medicinal plant, therefore is frequently incorporated in numerous traditional healing recipes (Matig et al. 2007). Even though *G. kola* has been identified by ICRAF as one of the most preferred species in Cameroon, only few authors mentioned its natural distribution there (Matig et al. 2006). Among them, 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 (Korup National Park).

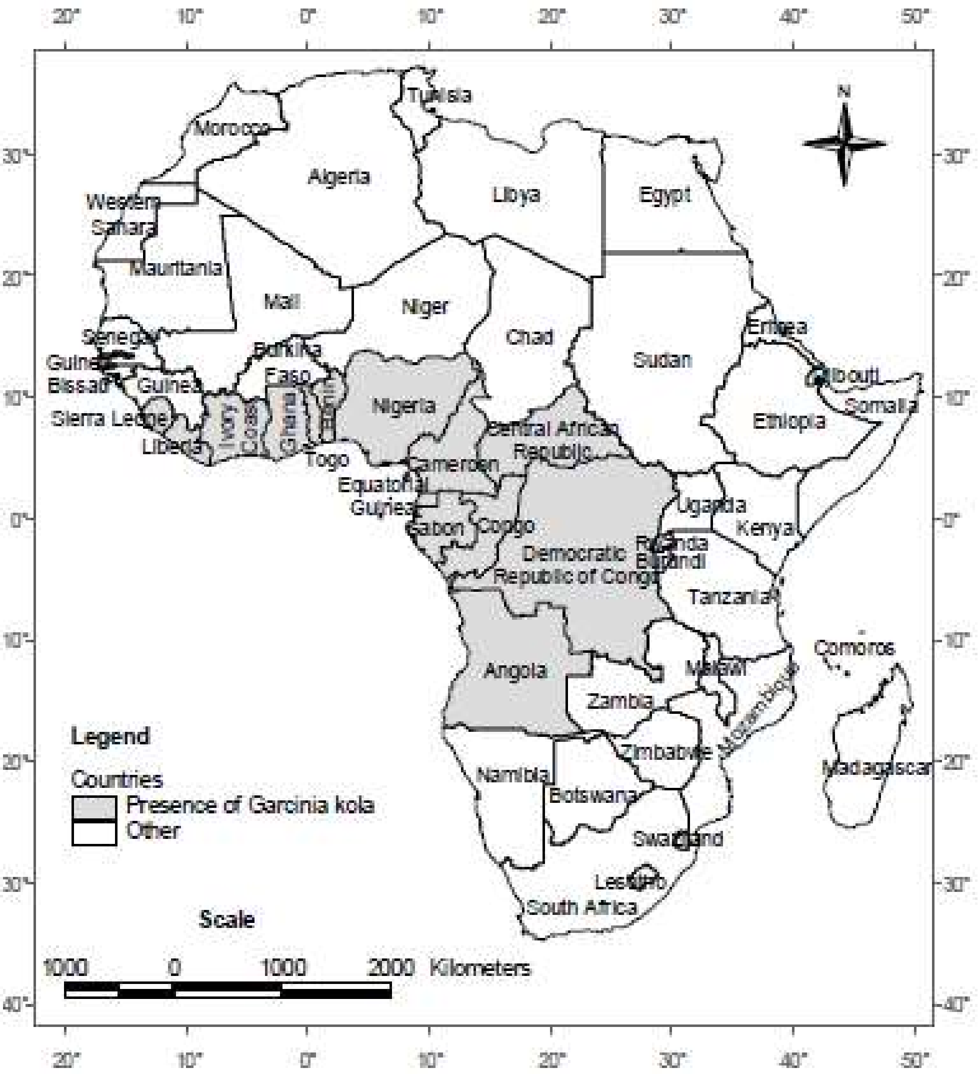


Figure 1. Geographical distribution of *G. kola* among African countries. Source: Matig et al. (2007).

2.2.2. Botanical description

Garcinia kola is a medium-large tree growing up to 40 m height and 100 cm in trunk diameter. According to Anegbeh et al. (2006) the species can reach 12 m in 12 years. The tree has a compact dense crown with erect, slightly drooping branches. The trunk is straight and cylindrical, sometimes lightly buttressed near the ground, with smooth bark, which is dark-brown outside and pinkish inside. When wounded, the bark exudates sticky yellow latex. Leaves are simple, opposite, obovate-elliptic with short acuminate apex (Figure 2). They are usually dark green and can measure up to 20 x 6 cm. Inflorescence is a small terminal umbel with greenish/white flowers (Matig et al.

2006; Matig et al. 2007). The tree is predominantly dioecious but some flowers were reported to be bisexual (PROTA, 2016). Flowering occurs once per year.

Fruits are berries of globular, sometimes slightly flattened, shape having about 6.5 cm in diameter and weight of 120 g. The exocarp is velvety reddish-yellow and the pulp is yellow-orange with apricot odour. One fruit contains about 2-4 seeds (Matig et al. 2006; Matig et al. 2007). The pericarp of the seed is light brown-coloured when fresh but darkens with drying or age. The kernel is white with brownish-red branched lines producing red resinous globules (Onayade et al. 1998). Seed length and width are on average 3.2 × 1.7 cm, mean weight varies around 5.4 g (Table 1). In Cameroon, fruits are, according to various environmental conditions, maturing mainly in: December – February, March – April or July – August (Dosunmu and Johnson 1995; Dah-Nouvlessounon et al. 2015). When ripe, the colour of the fruits changes from green to reddish-yellow. Farmers are harvesting the mature berries by a pole or wait until the fruits fall from the tree. The fruits are then collected and kept in cool dry place until their pericarp and pulpy mesocarp become soft. Once softened, the fruits are pressed to release the nuts, which are thoroughly washed to remove pulp residues. The seeds are eaten fresh or stored for later consumption/market purposes (Adebisi 2004a).

Table 1. Morphological characteristics of *G. kola* fruits and seeds.

Parameter	Mean ± SD	Max	Min
Fruit weight (g)	127.5 ± 47.4	204.5	41.3
Fruit length (cm)	6.5 ± 0.9	7.9	4.4
Fruit width (cm)	6.2 ± 0.8	7.3	4.4
Pulp weight (g)	112.9 ± 40.4	170.5	38.6
Seed length (cm)	3.2 ± 0.5	4	2.2
Seed width (cm)	1.7 ± 0.3	2.5	0.9
Seed weight (g)	5.4 ± 2.1	11.5	1.8

SD = Standard deviation, Source: Onyekwelu et al. (2015a)

G. kola is usually grown from seed and bears fruits after 7-12 years. Its seeds have hypogeal type of germination. However, due to dormancy it can take up to 18 months for the seeds to successfully germinate (Anegbeh et al. 2006; Matig et al. 2007). For the overall view of the tree habitus, see photos in Appendix B.

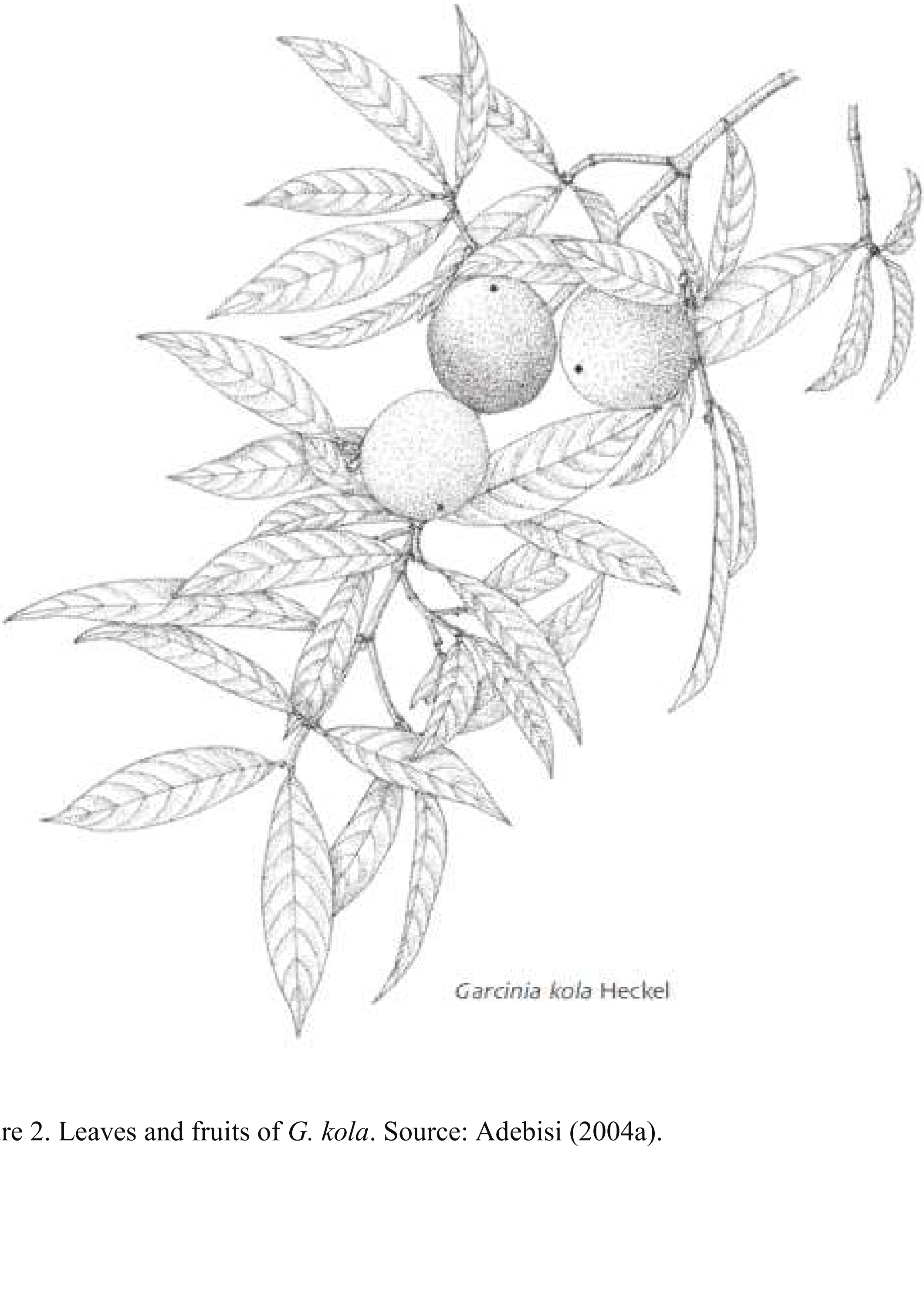


Figure 2. Leaves and fruits of *G. kola*. Source: Adebisi (2004a).

2.2.3. Propagation

Fruits of *G. kola* are frequently harvested from wild stands. In Nigeria, 70 % of fruits are taken from primary or secondary forests. Ripe fruits are usually collected from beneath the tree or harvested manually by using a special pole (Matig et al. 2006). They are kept for 5-7 days until the skin softens, which makes the seed removal easier (Adebisi 2004a). Sometimes the tree is purposely preserved during forest clearing and thus introduced to farmer's compound (Matig et al. 2006). The species is frequently grown in agroforestry systems, e.g. planted with cocoa, oil palm, cassava or as a part of homegarden (Adebisi 2004b). Generally, there is a shortage of available good quality planting stock, consequently wildlings are mostly used for planting (Nzegbule and Mbakwe 2001).

Vegetative methods of propagation are not yet fully developed or practised. However, study of Kouakou et al. (2016) revealed that *G. kola* can be propagated by stem cuttings. According to their results, IBA (indol-3-butyric acid) treatment promoted shoot and root production and accelerated the emergence of shoots and leaves. The best results were achieved by cultivation of softwood cuttings with an aqueous application of IBA in non-mist poly-propagator. Nevertheless, further research is necessary to determine the best planting conditions and optimize the process of vegetative propagation. Development of efficient propagation method is an essential step towards domestication of *G. kola*. In the future, micropropagation might be a solution leading to a large scale production (Kouakou et al. 2016).

2.2.3.1. Dormancy

The major difficulty in *G. kola* cultivation, as for several species in *Garcinia* genus, is related to seed germination. Due to dormancy, seeds can take from six weeks to 18 months to germinate (Matig et al. 2007). This is mainly attributed to embryo dormancy (Kanmegne and Omokolo 2008). However, literature provides contradictory information concerning the seed germination. Some authors describe the seed as easily germinating, others find it difficult (Kanmegne and Omokolo 2008; Kanmegne et al. 2010). Therefore, it is rather problematic to prescribe a standard procedure for enhancing the germination.

It was found that *G. kola* seeds are very sensitive to desiccation, which may influence their viability. Matig et al. (2007) revealed that the species germination rate decreased with lowering of seed moisture content. Therefore, the authors suggested dormancy-breaking through seed coat removal and soaking in cold water. Nzegbule and Mbakwe (2001) proposed another method: pre-treatment of freshly harvested seeds by cold water followed by incubation in a transparent polyethylene bag. Anegbeh et al. (2006) suggested nicking the seeds before sowing as the most successful way for enhancing germination. Kangmene and Omokolo (2008) tried to break embryo dormancy by pre-treatment of the seed with auxins, cytokinins and giberelins but none of the phytohormones significantly increased the germination rate or reduced the dormancy period. However, they also revealed a regeneration potential for *in vitro* cultures because the treatment by NAA, BAP and 2,4-D induced formation of multiple roots, shoots and callus. Their results also showed significant differences in seed germination rate among six studied collections, indicating that the trait may vary within the species.

2.2.4. The use

Garcinia kola is a multipurpose tree species valued in most parts of West and Central Africa. In Cameroon, the species is among the main shade trees for cocoa plantations (Fondoun and Tiki Manga 2000). Because of its dense rounded crown, *G. kola* has also a great agroforestry potential as a wind breaker (Anegbeh et al. 2006). Apart from the service functions, the species offers a lot of products and by-products. Its hard wood is used for tool handles and, at time of scarcity, it can be also burned as firewood (Adebisi 2004a, Anegbeh et al. 2006). Branches and roots are sold in bundles as traditional chewing sticks, important for local dental hygiene. In Ghana, 90 % of chewing sticks come from *G. kola*, *G. epunctata* and *G. afzelii* (Blay 2004; Adebisi 2004a). Bark of bitter kola is used for tanning leathers and palm wine production. It is believed that the bark enhances flavour as well as alcohol content in the traditional drink (Onayade et al. 1998; Leakey 2012). Interestingly, Eyebe et al. (2012) reported that sap and stem of oil palm (*Elaeis guineensis)* together with roots and bark of *G. kola* are used in ethnomedicine for treatment of impotency.

The most important part of the tree are seeds. They are known as 'bitter kola', pointing out their typical astringent taste. Less common expression is 'male kola', which arises from aphrodisiac effect of the nuts on men (Fondoun and Tiki Manga 2000). The seeds are sometimes chewed as an alternative to true cola nuts of *Cola nitida* and *C. acuminate,* and therefore also called 'false kola' (Vivien and Faure 1995; Adamaroye et al. 2014). The nuts were traditionally chewed as masticatory substance, today they are widely consumed as a snack (Adebisi 2004b). The seeds are used in folklore remedies for a treatment of liver disorders, hepatitis, headaches, diarrhoea, laryngitis, bronchitis or gonorrhoea (Adegboye et al. 2008; Iljomonoe et al. 2012). Particularly the seed extract is used as a cure for various types of inflammation or liver cirrhosis (Onayade et al. 1998). Dried ground nuts are mixed with honey to create a traditional paste against cough (Adebisi 2004b). The seeds also play an important role in folk ceremonies such as childbirth, marriage or chieftaincy 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). Some studies also reported a possibility to use bitter kola seeds instead of hops in brewing industry (Dosunmnu and Johnson 1995; Leakey 1999; Nzegbule and Mbakwe 2001; Afolabi et al. 2006).

In one season, a single tree can produce up to 500 fruits, which stands for 1,700 seeds per tree on average (Adebisi 2004b). One kernel may be sold for 10 - 50 CFA (1 USD = 615 CFA), however the price varies widely depending on the season and fruit abundance. The seeds are perceived as a versatile and useful product also due to their relatively easy and good storage possibility (Matig et al. 2006). Income generated from the sale of bitter kola is particularly important in times of financial scarcity such as payment of school fees or medical bills (Fondoun and Tiki Manga 2000).

2.2.5. Seed chemical composition

2.2.5.1. Nutrient content

Although there is considerable interest in the bioactive compounds of *G. kola* seeds mainly from the medicinal point of view, there are few reports on the chemical composition of the kernels and its potentials from a nutritional perspective (Eleyinmi et al. 2006).

In the literature, we can find a lot of variations among published results. Mainly, there are wide differences in terms of proximate and mineral composition of the seeds (Table 2). The published results vary as follows: moisture: 7.2 – 92.7 %; ash: 0.33 – 5.9

%; crude protein: 0.58 – 7.8 %; crude fat: 0.19 – 14.5 %; crude fibre: 1.23 – 20.51 %; NFE: 10.85 – 91.35 %.

Overall, the studies agree on relatively high amount of moisture in the seeds, around 70 %, which is a crucial aspect for the kernel preservation. The seeds are easily losing moisture, shrink and lose their market value. Dosunmu and Johnson (1995) observed that the quick water loss is leading to a change in texture as well as in sensory and nutritional attributes. Consequently, the seeds need to be eaten fresh or stored properly in order to maintain high moisture content (Onyekwelu et al. 2015b). There are various methods for the kernel storage. One of the most popular and easiest way is to air-dry the seeds and store them in a cool and dry place (Adebisi 2004b). Another possibility is to the wrap the nuts in leaves and store them in a basket with jute bag material (Adebisi 2004a). Some farmers also store the kernels in between layers of soil or in dust/ash piles (Dosunmu and Johnson 1995).

Carbohydrates, also described as nitrogen-free extracts (NFE), form the biggest part of the kernel proximate composition. The amount varies around 65 %. On the other hand, content of ash, the result of complete sample burning to inorganic substituents, is very low - about 1.5 %. Mean value for crude protein is 3.5 %, crude fat varies around 6.2 % and crude fibre content is 9.4 %. Compared to proximate content of cola nut (*Cola* spp.), which is also a popular masticatory stimulant in West and Central Africa, Arogba (2000) revealed following: bitter kola kernels contained twice the amount of protein but were twice lower in fat, amounts of ash and NFE were mostly similar.

Table 2. Mean nutritional composition of *G. kola* seeds reported by various authors.

	Odebunmi et al. 2009 (%)	Ibekwe et al. 2007 (% dry wt. basis) *	Esiegwu and Udedibie, 2009 (% dry wt. basis) *	Eleyinmi et al. 2006 (g/100 g)	Dosunmu and Johnson, 1995 (g/100 g)	Onyekwelu et al. 2015b (% fresh wt. basis)	Asaolu, 2003 (% fresh wt. basis) *	Arogba, 2000 (% dry wt. basis)	Adesuyi et al. 2012 (%)
Hulls	Included	Not spec.	Not spec.	Excluded	Excluded	Included	Not spec.	Excluded	Excluded
Moisture	60.48±0.06	14.6	92.7	9.73	84.1±1	71.97±0.00	75.5	10±0.2	7.2±0.08
Ash	0.79±0.005	5.0	1.07	1.14	2.4±0.2	0.33±0.03	5.9	3.1±0.1	0.47±0.09
Crude protein	2.48±0.10	0.58	2.64	3.95	7.8±0.8	1.74±0.00	4.25	7±0.2	1.86±0.15
Crude fat	4.51± 0.56	3.0	9.47	4.33	8.7±0.3	0.95±0.12	14.5	9.9±0.3	0.19±0.32
Crude fibre	5.23±0.16	10.0	20.51	11.40	13.9±0.3	3.22±0.19	NE	NE	1.23±0.15
NFE	35.64	91.3	57.54	69.45	67.2±1	21.79±0.36	10.85	70±1.4	88.3±0.08

± Standard deviation, NFE – nitrogen-free extracts, NE – not examined, * - published in Esiegwu et al. (2014)

More specifically, the dominant fatty acids in the seeds are oleic (38 mg/kg), linoleic (36 mg/kg) and palmitic acid (32 mg/kg). The prevalent essential amino acids are lysine (2.4 g/kg), leucine (1.9 g/kg) and valine (1.7 g/kg), while glutamic acid (6.8 g/ kg) and arginine (5.5 g/kg) are the predominant nonessential amino acids (Eleyinmi et al. 2006). The seeds are low in anti-nutrients such as phytate or oxalate and thus not threat human organism even when eaten in bigger doses (Onyekwelu et al. 2015b).

Regarding mineral and vitamin content of *G. kola* seeds, there is not much information available. However, relatively high amount of vitamin C with 23.1 mg/100 g was recorded (Table 3). Moreover, Onyekwelu et al. (2015b) reported even higher value – 69 mg/100 g. In comparison, apple (*Malus domestica*) possess much lower value of 7.7 mg/100 g (Varming et al. 2013), on the other hand fruit of baobab (*Adansonia digitata*) bears much higher amount of 300 mg/100 g (Gebauer et al. 2002). Potassium and phosphorus are the most abundant minerals in the seeds their values vary between 25 – 722 mg/kg for K and 3.3 – 720 mg/kg for P (Table 4).

Especially in Nigeria, the kernels are often peeled and hulls discarded as a waste. However, results of Eleyinmi et al. (2006) proposed a feeding potential of the seed coats, due to their high protein content (9.92 g/100 g) for domestic animals. Livestock and small ruminants are generally lacking high-protein fodder in developing countries, hence bitter kola hulls might provide an easily obtained solution for this problem. However, bioavailability of the protein should be firstly proved in-vivo before use in feed formulation.

Table 3. Content of vitamins in *G. kola* seeds.

Vitamins	Amount (mg/100 g)
Thiamin (B1)	0.54±0.30
Riboflavin (B2)	0.22±0.01
Niacin (Nicotinic acid)	1.6±0.01
Ascorbic acid (Vit. C)	23.1±0.02

± Standard deviation, Source: Okwu, (2005)

Table 4. Mineral composition of *G. kola* seeds.

	Odebunmi et al. 2009 (mg/kg)	Eleyinmi et al. 2006 (mg/kg)	Dosunmu and Johnson, 1995 (mg/100g)	Okwu, 2005 (mg/100g)
Hulls	Included	Excluded	Excluded	Included
Na	NE	86.4	1.8	0.72±0.10
K	722.10±0.00	335	499	2.50±0.10
Ca	67.07±0.12	34.1	100	1.80±0.40
Mg	114.83±3.47	28.1	166	0.42±0.30
Fe	6.10±0.43	NE	4.2	17.75±0.30
Zn	2.30±0.08	NE	3.5	2.30±0.01
P	188.57±0.37	243	720	0.33±0.10
Cu	NE	38.4	1.3	0.78±0.20
Co	NE	102	NE	0.55±0.20
Cr	NE	ND	0.2	ND

± Standard deviation, NE – not examined, ND – Not detected

2.2.5.2. Secondary metabolites

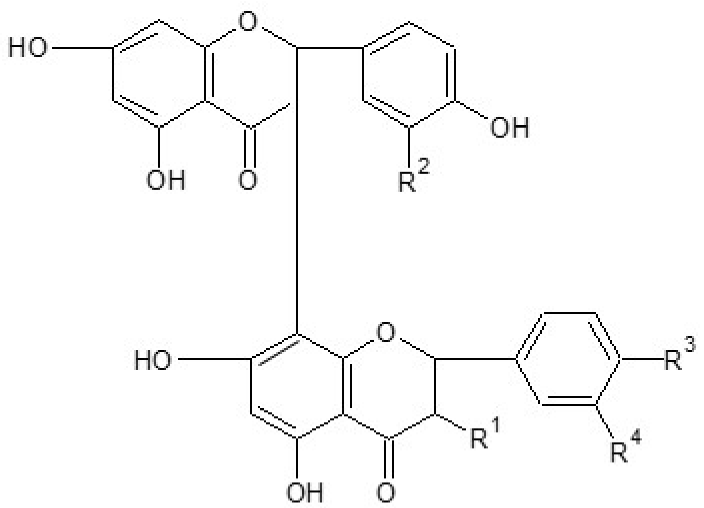
Likewise other members of genus *Garcinia*, *G. kola* contains a wide range of bioactive compounds in its tissues, which makes the plant very popular for traditional remedies. These phytochemicals include: oleoresins, tannins, saponins, alkaloids, cardiac glycosides or flavonoids (Ijomone et al. 2012; Usunomena 2012). The most abundant phytochemicals in the seeds are flavonoids (1.98-2.041 mg/100 g) and saponins (11.48-2.471 mg/100 g) (Table 5). Flavonoids are known as antioxidants, having an ability to scavenge free radicals and transform them into harmless substances for human body. Those compounds are also proposed to play a useful role in protecting central nervous system against oxidative and excitotoxic stress (Ijomone et al. 2012). Currently, one of the most studied compound in *G. kola* is a biflavonoid complex kolaviron, which consists of biflavanones GB1, GB2 and kolaflavanone (Figure 3).

Table 5. Phytochemical composition of *G. kola* seeds on a dry weight basis.

Constituents	Amount (mg/100 g)[a]	Amount (mg/100 g)[b]
Phenols	0.11±0.2	0.147±0.0
Alkaloids	0.36±0.1	0.647±0.2
Tannins	0.26±0.2	0.342±0.0
Flavonoids	1.98±0.2	2.041±0.3
Saponins	11.48±0.1	2.471±0.0

± Standard deviation, Source: a - Okwu (2005), b – Adesuyi et al. (2012)

It was proved that this complex possesses neuroprotective, anti-inflammatory, antimicrobial, antioxidant, antigenotoxic, hypoglycemic, hepatoprotective and nephroprotective activities (Ijomone et al. 2012; Adamaroye et al. 2014; Chinaka et al. 2015). Apart from that, kolaviron exhibited a significant reduction of *Plasmodium berghei*, parasite causing malaria, which may prove a reasonability of traditional anti-malarial mixtures containing bitter kola (Adaramoye et al. 2014, Tschibangu et al. 2016). Kalu et al. (2016) also reported a therapeutic potential of kolaviron on benign prostatic hyperplasia treatment.



	R1	R2	R3	R4
GB1	OH	H	OH	H
GB2	OH	H	OH	OH
Kolaflavanone	OH	H	OCH_3	OH

Figure 3. Biflavonoid complex kolaviron and its components. Source: Adedara et al. (2015).

Antimicrobial properties of the species are attributed to the benzophenones and flavanones (Anegbeh et al. 2006; Kanmegne et al. 2010). Those active components have been already successfully extracted in petroleum ether, ethanol, methanol and water. Poly-iso-phenyl benzophenone, called kolanone, showed great anti-inflammatory effect against both gram positive and gram negative bacteria. Its results were comparable to salicylic acid (aspirin) or phenyl butazone (Aderibigbe 2012).

Typical astringent taste of *G. kola* kernels is caused by tannins, secondary metabolites known as a natural treatment of intestinal disorders such as diarrhoea and dysentery. Apart from their microbial activities, tannins were observed to have a remarkable potential in cancer prevention (Adegboye et al. 2008; Usunomena 2012). Finally, both cardiac glycosides and steroidal compounds were found in *G. kola* extract. This coincides with the fact that the plant is traditionally used against chest pain or cardiac infection and men are commonly chewing the seeds as an aphrodisiac (Fondoun and Tiki Manga 2000; Usunomena, 2012).

Even though seeds of *G. kola* are frequently sold side by side to cola nuts (*Cola* spp.), their chemical composition is rather different. Unlike in cola nuts, bitter kola seeds contain higher level of phenolic compounds but caffeine, theobromine or catechin were not detected (Niemenak et al. 2008).

2.2.6. Related species

Genus *Garcinia* comprises of variety of medicinal species. Different plant parts are globally used in ethnomedicine for treatment of several disorders such as inflammation, oxidative stress, microbial infection, cancer and obesity (Hemshekhar et al. 2011). One of the most popular species is mangosteen (*G. mangostana*) from South-East Asia, also known as “the queen of fruits” which refers to its tastiness. The species also plays an important role in Ayurvedic medicine (Pedraza-Chaverri et al. 2008).

Beside *G. kola*, we can find many other *Garcinia* species indigenous to tropical Africa. Matig et al. (2006) specified *G. lucida, G. mannii, G. polyantha* and *G. epunctata* as locally important in West Africa. Adebisi (2004a) added *G. livingtonei, G. gnetoides, G. staudtii, G. smeathermannii, G. ovalivolia* and *G. brevipediellata*. And Blay (2004) identified *G. epunctata* with *G. afzelii* as popular chewing sticks in Ghana.

Among the species listed above, the most described or publicly known are *G. lucida* along with *G. livingstonei*.

Garcinia lucida Vesque is a typical understorey evergreen dioecious tree reaching 25–30 cm in diameter and 12–15 m in height. It grows in high-density stands around hilly moist forests of South Cameroon, Equatorial Guinea and Gabon (Guedje et al. 2002). In Cameroon, the tree is known as Essok or Boulou (Sylvie et al. 2014). *G. lucida* flowers and produces fruits regularly at 5 cm of DBH. Important fruiting peak occurs between July and December, during the main rainy season. Fruits are of medium-size (13 cm × 11 cm), ellipsoidal or globular form and green to green-golden in colour (Figure 4). They contain one to four seeds wrapped with a thin and white-yellowish pulp (Guedje et al. 2002). Bark and seeds, both dried or fresh, are widely used for medicinal purposes to prevent food poisoning and to cure stomach ache or gynaecological pains, as well as to cure snake bites. Bark of this species is also popular as additive to palm wine production (Momo et al. 2011; Sylvie et al. 2014).

Garcinia livingstonei T. Anderson, called African mangosteen, is a small evergreen slow growing tree reaching 10–16 m in height. It has leathery, waxy leaves and orange-yellow fruits with edible pulp of slightly acidic and sweet flavour (Figure 4). The species is a rich source of various phytochemicals and exhibits anticancer, antiviral, antimicrobial and anti-parasitic activities. Fruits of *G. livingstonei* are used in traditional medicine as an aphrodisiac, for treatment of abdominal pain during pregnancy and after child birth, or as an antibiotic (Yang et al. 2010; Joseph et al. 2016).

Other species mentioned in literature are for example *G. epunctata* and *G. afzelii*. Whereas the young shoots of *Garcinia afzelii* Engl. are used as chewing stick in Ghana or Cameroon and its leaves and flowers exhibit antibacterial properties (Waffo et al. 2006), *G. epunctata* Stapf is known mainly for its leaves, fruits and bark. In Cameroon, the fruits are used as food ingredient, bark serves as antidote against snake bites and a decoction from leaves is prepared to relieve tooth ache (Anatole et al. 2013).

Figure 4. Fruits and seeds of *G. lucida* (left), fruits of *G. livigstonei* (right); Source: Alain Tsobeng (ICRAF) – *G. lucida*, Christopher Hind (Wikimedia Commons) – *G. livingstonei.*

2.3. Study area

Cameroon is a country situated in Central Africa sharing borders with Nigeria, Chad, Central African Republic, Equatorial Guinea, and Gabon. From south to north its borders lay between longitudes 8° and 16° E and latitudes 1° and 13° N, from Atlantic Ocean and Lake Chad. Estimated land area is 475,400 km^2 (Pamo 2008) with a total population of about 23.3 million with annual population growth rate 2.5 % between years 2013-2015 (World Bank, 2016). The country is divided into 10 different regions. Two of them are anglophone (Northwest, Southwest) and the rest is francophone (South, East, Centre, Littoral, West, Adamawa, North and Extreme North) (Pamo 2008) (Figure 5).

Cameroon is sometimes titled as “Africa in miniature” and known to be Africa’s fourth richest country in terms of biodiversity. This is mainly due to richness in forest area which is a part of Congo Basin, the world’s second largest tropical forest, and the country’s diverse climate including hot, moist and dry conditions (Pamo 2008; Eyebe et al. 2012). Even though this diversity favours a variety of economic and agricultural activities, 70 % of the population depends on agriculture and pastoral activities (Essama-Nssah and Bassolé, 2010). Cameroon consists of five different agro-ecological zones (Figure 5): Sudano-Sahelian upland, Guinean savannah, Western highlands, Rainforest with monomodal rainfall pattern and Rainforest with bimodal forest pattern (FAO, 2005).

Arable land covers around 13 % of Cameroon and agronomy employs 50-70 % of the whole population (FAO, 2014; World Bank, 2014). Traditional cropping systems of smallholders are based on shifting cultivation, producing food crops for home consumption and cash crops for marketing. Main staple 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*). They are frequently intercropped with local types of leafy vegetables and fruit trees such as: mango (*Mangifera indica*), citruses (*Citrus* spp.) or avocado (*Persea americana*). The most important cash crops are: 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). For example, cocoa is commonly planted together with fruit and forest species, from which indigenous trees cover a substantial part (Jagoret et al. 2011).

Those native species are crucial for farmers because of their high-level adaptation to local conditions and provision of valuable products such as fruits, nuts or medicine. Apart from that, the species may also play an important role in family income diversification. Selling of the indigenous tree products can help for example during cocoa prize fluctuations or to cover relative high expenses, such as school fees and tuitions (Schreckenberg et al. 2006). Therefore, ICRAF Cameroon started a programme for domestication of tree species producing indigenous fruits and nuts: *Allanblackia* spp., *Cola nitida, Dacryodes edulis, Garcinia kola, Irvingia gabonensis* and *Ricinodendron heudelotii* (Franzel and Kindt 2012).

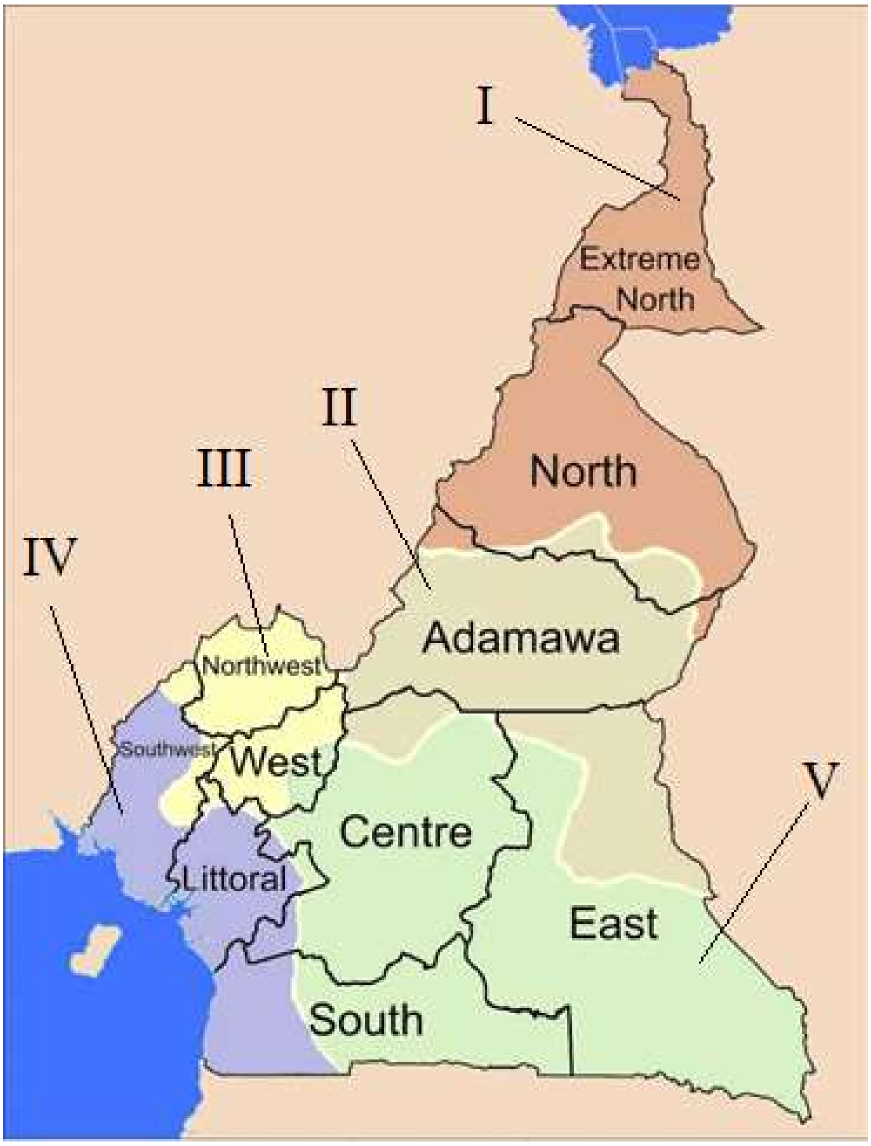


Figure 5. Ten different regions and five agro-ecological zones of Cameroon. I – Sudano-Sahelian upland; II – Guinean savannah; III – Western highlands; IV – Rainforest with monomodal rainfall pattern; V – Rainforest with bimodal rainfall pattern. Modified from: Wikimedia Commons and Ndoumbe-Nkeng et al. (2004).

2.3.1. Southwest region

Southwest region, one of the two anglophone territories in Cameroon, is the smallest one in terms of surface area (25,410 km^2) and number of inhabitants (1,153,000). On the other hand, Southwest is also considered to be the most developed region because of its highly-advanced infrastructure and industry. Local capital city Buea, situated at the foot of West African highest mountain Mt. Cameroon (4,040 m), is also known for hosting the first anglophone university in the country. Main business partner for this region is neighbouring Nigeria (Republic of Cameroon, 2016). Generally, tropical climate there provides nine months of rains and three months of dry season every year (Sama et al. 2007). Southwest region consists of six divisions: Fako, Kupe-Manengouba, Lebialem, Manyu, Meme and Ndian (Figure 6).

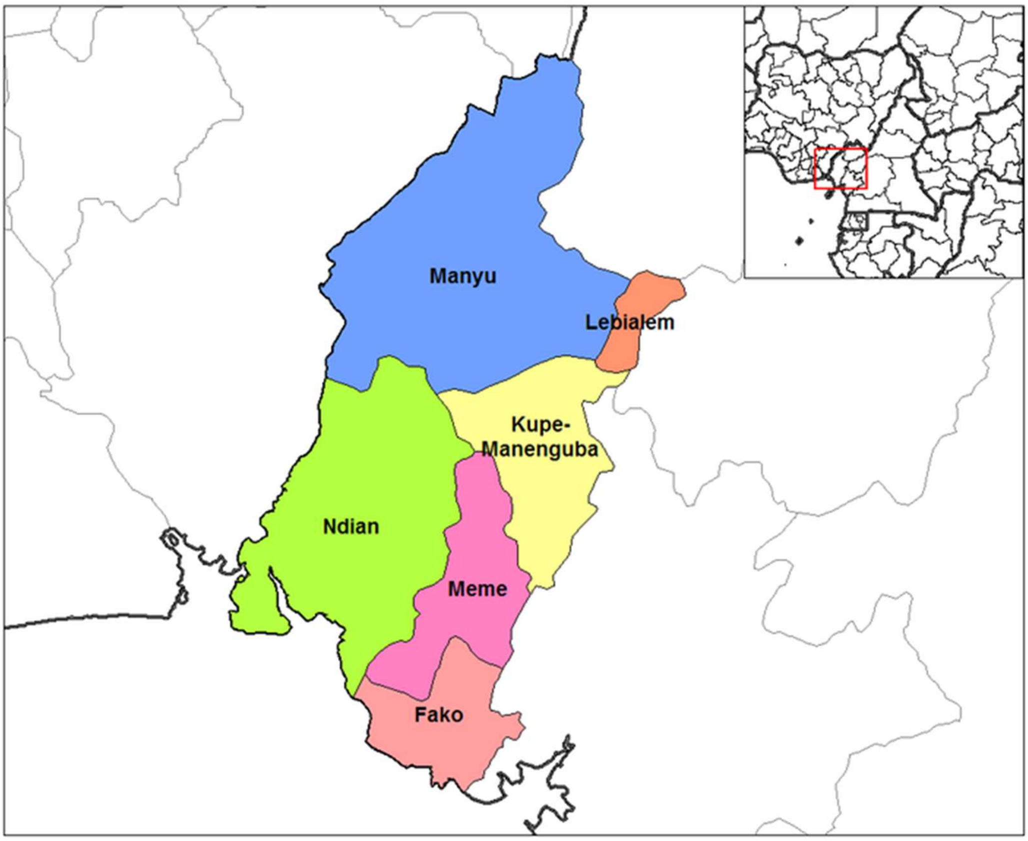


Figure 6. Divisions of Southwest region in Cameroon. Source: Wikimedia Commons.

OBJECTIVES

Main objective of this study was to assess and describe morphological diversity and nutritional status among different populations of *Garcinia kola* from Southwest region of Cameroon. Therefore, we have stated following specific objectives:

1) To examine utilization, cultivation and harvesting of *G. kola* by local population.
2) To characterize phenotype of *G. kola* from different study sites in Southwest region.
3) To evaluate nutritional values of *G. kola* seeds from diverse localities of Southwest region.
4) To assess correlation between morphological and nutritional traits, and to determine differences within individuals as well as among diverse localities.

Based on previous knowledge and information, we set following research questions:

- How is *G. kola* utilized, cultivated, harvested and what are the methods practically used for seeds storage?
- How morphological features vary among different study sites of *G. kola* in Southwest region of Cameroon?
- Are there significant differences in nutritional composition of *G. kola* seeds among individuals in different localities?
- Are there correlations between nutritional content and morphological characteristics of *G. kola* trees?

MATERIALS AND METHODS

4.1. Study site

According to advises provided by ICRAF employees and literature pointing out the main harvesting periods of *G. kola* (Fondoun and Tiki Manga, 2000), 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. Consequently, local institutes and NGO's were contacted in order to provide support and assistance 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 K-Town, 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 m a.s.l. (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 m). Average daily temperature in Tombel is 24.6 °C, mean rainfall per year is 3,090 mm whereas average altitude there ranges about 450 m a.s.l. (Climate Data, 2016).

Lebialem division is characterized by diverse climate and hilly landscape, which is ranging from 200 m up to 2,400 m of altitude. A semi-evergreen tropical broadleaf forest dominates the lower altitudes. Data were collected in Menji, divisional headquarters with average altitude of 730 m, 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, where most of the farmers were interviewed, is the daily mean temperature around 26.7 °C along with average annual rainfall of 2,753 mm and elevation usually below 200 m a.s.l. (Climate Data, 2016).

4.2. Sampling and data collection

All data were collected during June-July 2016. In total, 48 farmers from 50 farms were selected by snowball method and interviewed using simple semi-structured questionnaire (Appendix A) to get background knowledge about cultivation and utilization of the tree and its products, e.g. information on best harvesting period, seeds usage or storage possibilities were recorded. Eleven respondents were found in Kumba, 10 people were questioned in Tombel, 12 farmers came from Lebialem and 15 from Mamfe. In total, 81 mature fruiting trees were found on the farms, measured and examined (Figure 7). Then, 8 - 10 fruits along with 4 - 5 leaves were taken per each tree for further morphological analysis. After that, seeds were extracted from the fruit pulp and measured as well. The exact number of samples taken per each study site is presented in Table 6.

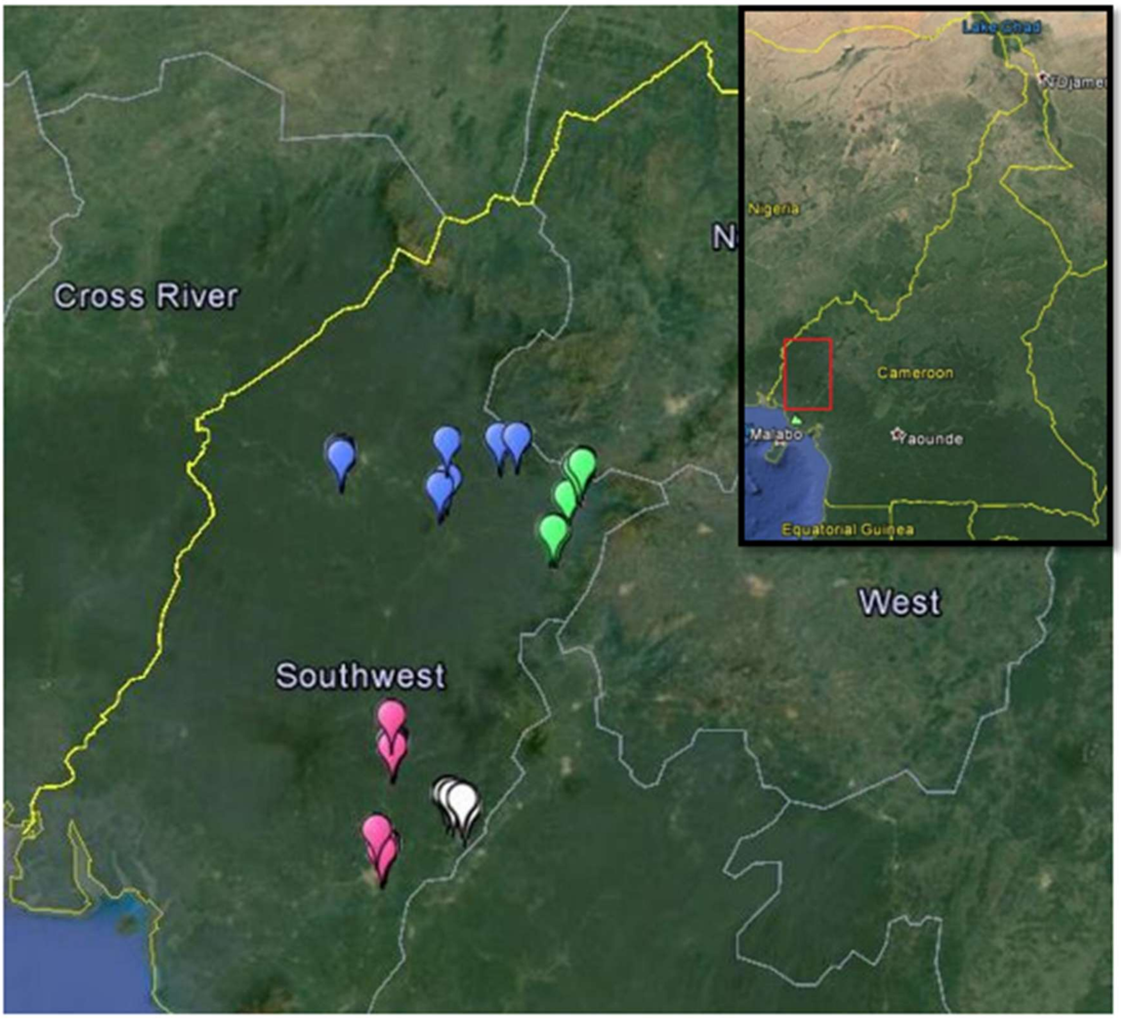


Figure 7. Locations of sampled trees. Pink – Kumba, Meme division, White – Tombel, Kupe-Manengouba division, Green – Lebialem, Lebialem division, Blue – Mamfe, Manyu division; modified from Google Earth.

Table 6. Data collection details from different study sites.

Study site/ population	Villages	No. of farms	Trees	Leaves	Fruits	Seeds
Meme	Kumba	8	13	65	122	298
	Konye	4	8	39	80	192
		12	**21**	**104**	**202**	**490**
Kupe-Muanenguba	Tombel	10	20	100	194	452
		10	**20**	**100**	**194**	**452**
Lebialem	Menji	3	5	25	50	115
	Banti	4	4	19	40	117
	Bechati	3	9	45	80	183
	Nkong	2	2	10	20	42
		12	**20**	**99**	**190**	**457**
Manyu	Mfuni	8	11	55	95	233
	Etoko	1	1	5	10	26
	Kendem	2	2	10	10	21
	Messeng Bakebe	2	2	10	20	54
	Kepoti	2	3	15	28	59
	Nchemba I	1	1	5	10	29
		16	**20**	**100**	**173**	**422**
Total		**50**	**81**	**403**	**759**	**1,821**

4.3. Morphological evaluation

Overall, 81 trees from which we took 403 leaves, 759 fruits and 1,821 seeds were morphologically characterised. Due to lacking botanical descriptors on *G. kola*, pattern for the study (Appendix C) was modified from mangosteen (*Garcinia mangostana* L.*)* (IPGRI, 2003).

At first, the trees were selected in a distance of at least 100 m from each other to lower the possibility of inbreeding. Following data were obtained for each tree: GPS position, elevation, estimated age, prevalent soil composition, surrounding, tree condition, crown shape, crown rating, trunk shape and branching pattern. Tree height and trunk height were estimated. DBH was measured in the height of 130 cm above ground by a soft tape, diameter of tree crown corresponds to a distance from the trunk to the farthest branch.

As a next step, length and width of leaf blades as well as petioles were measured. Then overall shape of blade with specific attention for apex and base were recorded. Weight of every single fruit was noted using PTS 3000 scales (Pesola, Switzerland) (± 0.01 g), diameter was measured by a soft tape whereas length was obtained by simple ruler. Shapes of fruits were categorised and colour defined according to British Standard (BS 381). After the fruit characterisation, seeds were manually removed from pulps, washed and left to dry at a room temperature for 24 hours. Subsequently, each seed was weighed and measured in length and width with a ruler. Also shape as well as colour of the seed coat were recorded. After the morphological evaluations, seeds were gathered into 81 groups according to the tree they belonged to.

4.4. Chemical analysis

Dried seeds of all 81 sampled trees were transported to CULS and evaluated in laboratories of Faculty of Tropical AgriSciences (Department of Crop Sciences and Agroforestry) and Faculty of Agrobiology, Food and Natural Resources (Department of Microbiology, Nutrition and Dietetics). Non-peeled seeds of each tree were firstly homogenized using laboratory blender Grindomix GM 100 (Retsch, Germany) and microfine grinder MF 10 Basic (IKA, Germany). Subsequently, content of crude fibre, crude protein, crude fat and ash were determined. After the samples defatting, methanol extract was obtained from the samples and stored for further experiments. All laboratory analyses were performed at least in duplicates based on Commission Regulation (EC) No 152/2009 (European Commission, 2009). The final result is then arithmetic average of those measurements (per tree) in complying with standard deviation.

One of the samples had been later on discarded from the analysis due to mould infestation. Therefore, the final number used in the study stabilised at 80 samples.

List of laboratory devices used for the evaluations:

Crude fibre analysis – ANKOM 200 Fiber Analyzer (ANKOM Technology, USA)

Crude protein analysis – Kjeltec 2400, Digestion unit – MB 442 (FOSS, Denmark)

Crude fat analysis – SER 148 (VELP Scientifica, Italy)

Drying oven –UN55 Single DISPLAY (Memmert, Germany)

Laboratory scales – AE200 (Mettler Toledo, Czech Republic)

Muffle furnace – LH 15/13 (Nabertherm, USA)

4.4.1. Moisture content

For moisture content analysis, all the seeds were dried in Gallenkamp oven (UK) for 5 days at 50 °C to reach constant weight. The moisture content was calculated according to formula:

$$\% \text{ MC} = \frac{w1 - w2}{w1} \times 100$$

w_1 – weight of fresh samples (g)

w_2 – weight after drying (g)

This analysis was the only one performed with contribution of ICRAF in Nkolbisson, Yaoundé, Cameroon. After drying, the seeds were packed in sealable plastic bags with silica gel and transported to CULS for following examination.

4.4.2. Crude fibre analysis

Crude fibre determination was performed using Filter Bag Technology on ANKOM 200 Fiber Analyzer. Crude fibre is an insoluble residue of acid hydrolysis followed by alkaline one, composed of true cellulose and insoluble lignin.

Filter bags were pre-dried at 103.5 °C, after taking account of the bag weight, 1 g of sample was added; the bag was sealed and placed into carrier (three samples in one partition). For each analysis, one "correction" bag was left blank and added into the carrier as a control. Carrier was put into the vessel, loaded with additional weight and poured by two litres of H_2SO_4 (c = 0.1275 mol.dm^{-3}). After 45 minutes of stirring and heating, the sulphuric acid solution was drained and carrier rinsed three times for five minutes with hot distilled water. The same procedure was repeated with NaOH (c = 0.313 mol. dm^{-3}). The filter bags were dried with filter paper and placed in 250 ml of acetone. After three minutes, bags were dried in the oven for four hours (103.5 °C). The weight of each bag was recorded and the samples were put in the porcelain crucibles of known weight. The cups were annealed in muffle furnace under 550 °C for 5.5 hours. The weight of burned content was noted and crude fibre calculated using the formula:

$$CF = \left(\frac{w3+w4-w5-(w1\times c)}{w2}\right)\times 100 \qquad c = \frac{w3}{w1}$$

w_1 – weight of empty filter bag (g)
w_2 – sample weight (g)
w_3 – weight of filter bag after drying (g)
w_4 – weight of empty crucible (g)
w_5 – weight of crucible after annealing (g)
c – correction factor of empty filter bags

4.4.3. Crude protein analysis

Protein content was determined by Kjeldahl method on Kjeltec 2400 apparatus. 0.5 g of sample was put into glass digestion tube together with one mineralisation tablet Kjeltabs CK (3.5 g K_2SO_4, 0.4 g $CuSO_4.5H_2O$), 10 ml of H_2SO_4 (96 %) and two times 5 ml of H_2O_2. After mixing the contents, tubes were inserted into digestion unit for 45 minutes at 420 °C. As a final step, 5 ml of distilled water was added into each tube prior to analysis. The results were provided in % N × 6.25.

4.4.4. Crude fat analysis, methanol extraction

Crude fat determination was done by Soxhlet method performed on SER 148 apparatus. The crude fat content was determined by extracting the fat from 5 g of the sample with 75 ml of petrol ether. The crude fat content was calculated according to formula:

$$F = \frac{w3-w1}{w2}\times 100$$

w_1 – weight of empty extraction glass (g)
w_2 – sample weight (g)
w_3 – weight of extraction glass after the analysis (g)

The defatted sample was directly used for preparation of methanol extract on the same apparatus. The extract, brown - yellow liquid, was stored for further analysis.

4.4.5. Ash determination

At first, 2 g of each sample were put into porcelain crucibles of known weight, dried in the oven for 3 hours and weighed again to obtain dry matter weight. After that, the crucibles were placed in a muffle furnace for calcination under 550 °C for 5 hours. Results were counted by formula:

$$A = \left(\frac{w3-w0}{w2}\right)\times 100$$

w_0 – weight of empty crucible (g)
w_2 – weight of crucible with sample after drying (g)
w_3 – weight of crucible after calcination and drying (g)

4.5. Data evaluation

All the data were firstly entered into MS Office Excel spreadsheets, cleaned and pre-coded. The statistical analyses were performed in software STATISTICA version 12 (STATSOFT, USA). At first, basic descriptive statistics, such as means, standard deviations and medians, were determined. As a next step, ANOVA (analysis of variance) was calculated to determine differences among the selected study sites. Post-hoc Tukey HSD (honest significant difference) test was used for both morphological data and seeds nutritional composition. Then, correlation was assessed among seed and fruit morphological parameters and seeds nutritional values. Software R (R Core Team, 2013) was used for PCA (principle component analysis) to see variability of particular components among study sites or individual trees. More specifically, we used R [stats] package, prcomp command; for visualizing PCA diagrams R, [devtools] and [ggbiplot] packages, ggbiplot command.

RESULTS

5.1. Tree occurrence and farmers survey

At 50 farms, we evaluated occurrence of 81 trees that were further sampled for morphological evaluation. Concerning the agricultural systems, 43 trees were grown in cocoa agroforestry systems, 31 were found in homegardens and 6 were planted in association with oil palm (oil palm agroforestry). Cocoa agroforestry was predominant in study sites Kumba and Tombel, planting in homegardens prevailed in Lebialem and Mamfe. Soil, in general, composed mainly of silt and clay particles, sandy structure was detected just in Lebialem. Topography varied mostly between flat or gently undulating pattern with exception of few steep sites found in Lebialem and Tombel. The highest elevation was measured in Lebialem with 755 m a.s.l., on the other side, the lowest value was observed in Mamfe – 139 m a.s.l. Mean altitude was: 228 m a.s.l. in Kumba, 414 m a.s.l. in Lebialem, 199 m a.s.l. in Mamfe and 453 m a.s.l. in Tombel. According to the topography and altitude distribution, we can divide the study sites into two groups; Tombel and Lebialem represent more hilly localities, whereas Mamfe and Kumba belong to lowland areas. However, just five trees from Lebialem were grown above 700 m a.s.l., other 15 individuals were found in altitude of 300 m a.s.l. on average.

Altogether, 48 farmers (owners of *G. kola* trees) were interviewed among the four study sites. In majority of cases, farmers planted their trees from seeds, as wildlings or save them during forest clearing. Only two respondents tried to propagate their trees vegetatively. The first farmer did marcotting, however the trees had not survived, the second farmer was successfully using cuttings for couple of years already.

The main product derived from *G. kola* are, according to 34 (71 %) respondents, the seeds. Fourteen farmers (29 %) also used bark, which was found to have mostly similar way of utilization but is also specifically used for palm wine production and in remedies against malaria. Focusing solely on seeds, the most important function is relief of gastric disorders. (Figure 8) Apart from that, the seeds are used in treatment of cough, chest pain, malaria, liver disorders or as antibiotics. Besides, some people use them as natural aphrodisiac or to boost energy. Seeds have also an important cultural asset because they traditionally served as gifts for family members and those, who were respected by local community.

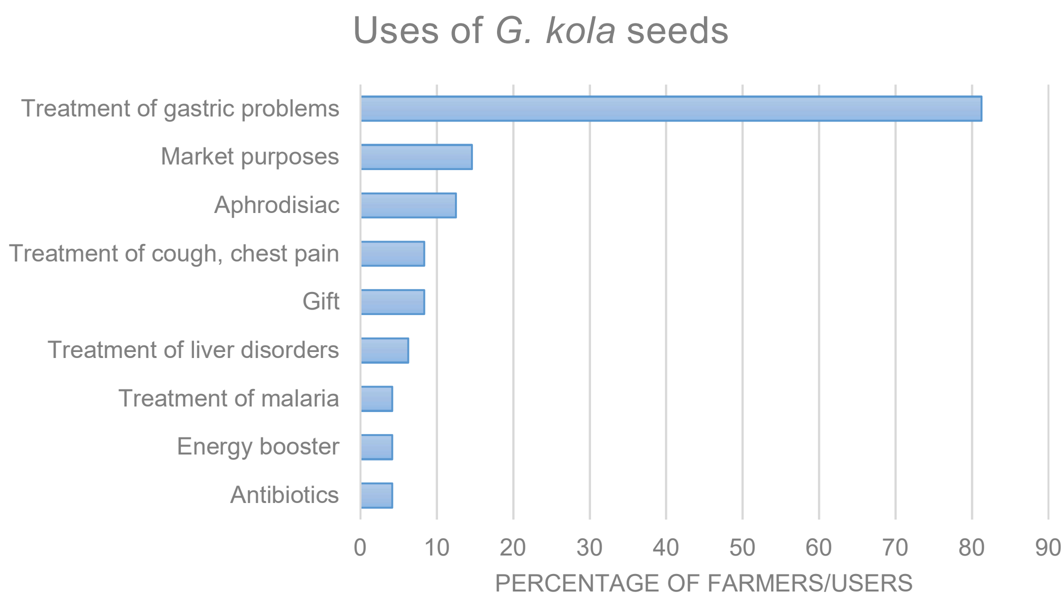


Figure 8. Utilization of *G. kola* seeds in Southwest region of Cameroon.

Fruiting usually occured once per year, just in four cases the farmers mentioned fruiting twice in one year. Based on the answers, second fruiting may appear in December. Harvest was always done manually by climbing the tree or picking the fallen fruits from ground. Among our respondents, 33 (69 %) practised climbing, 13 (27 %) were rather picking the fallen fruits and two (4 %) of them did both options. Harvesting was mostly done once per year (by 46 % of farmers) but sometimes the fruits were ripening unevenly resulting in two (27 %) or three (23 %) harvests per year. Few respondents also mentioned four or five harvests annually. In Southwest region of Cameroon, the best time for harvesting can be generally defined as June-July (Figure 9). However, there were several differences found among the four study sites, e.g. in Mamfe the main period was May-July, on the other side, in Kumba the harvesting was practised equally from June until August and finished in late September.

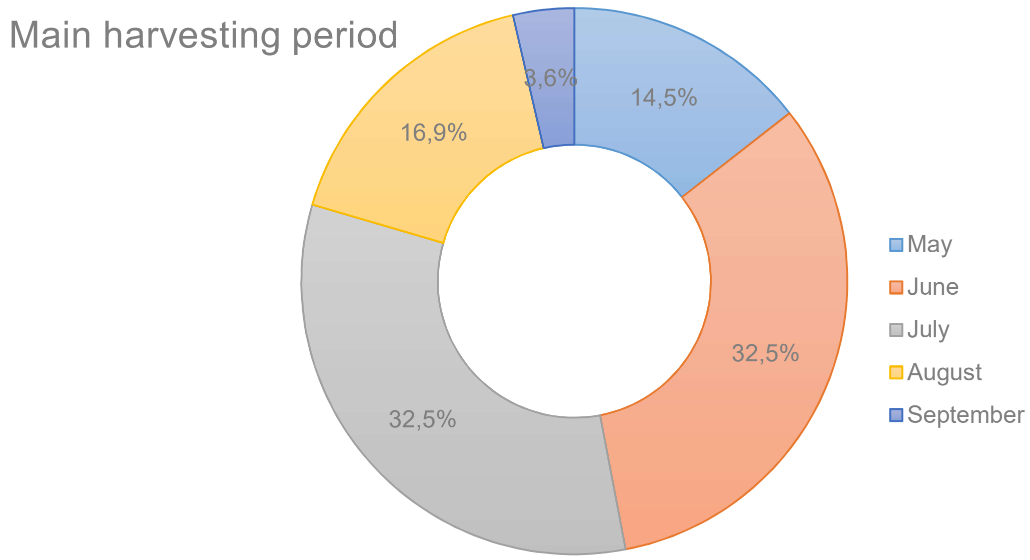


Figure 9. Main harvesting period of *G. kola* in Southwest region of Cameroon.

The seeds were sold by 46 farmers from 48 interviewed. Typically, the main measurement unit used for the sale was 15 l bucket or bag. The price fluctuated widely between 10,000-60,000 CFA (16 – 98 USD) per bucket/bag according to harvesting period. Overall, the highest cost was reported from Lebialem (Table 7).

Table 7. Price fluctuation for 15 l bucket/bag of *G. kola* seeds among different study sites in Southwest region of Cameroon.

Price (CFA)*	Kumba (10 resp.)	Tombel (10 resp.)	Mamfe (14 resp.)	Lebialem (12 resp.)
10,000-20,000	5	4	6	0
25,000-30,000	5	5	5	1
31,000-35,000	0	1	1	3
36,000-40,000	0	0	1	3
50,000-60,000	0	0	1	4

*1 USD = 615.53 CFA (17/4/2017); Resp. - respondents

Interestingly, all farmers practised seed storage to prolong the kernels shelf-life and increase their price value. In total, we have identified seven different ways of storage practised by our respondents (Figure 10). Generally, the first steps were similar: removing the seeds from fruit pulp followed by washing and drying. Then, in method coded as ‘A’, the kernels were simply stored in cool and dry place. In ‘B’, the seeds were stored in plastic bag. Method 'C' represented wrapping in more natural materials such as dry leaves or paper and storing the seeds in cool place. Methods 'D' were identified as the most traditional, consisting of burying the seeds into the ground, sand or dust. 'E' stood for storage in any type of air-tight container. 'F' and 'G' each were practised both by just one respondent. The first way suggested refrigerating the seeds, the second proposed storing the kernels in unopened fruits. According to our results, the methods were almost equally practised among the study sites, without one highly recommended way of storage.

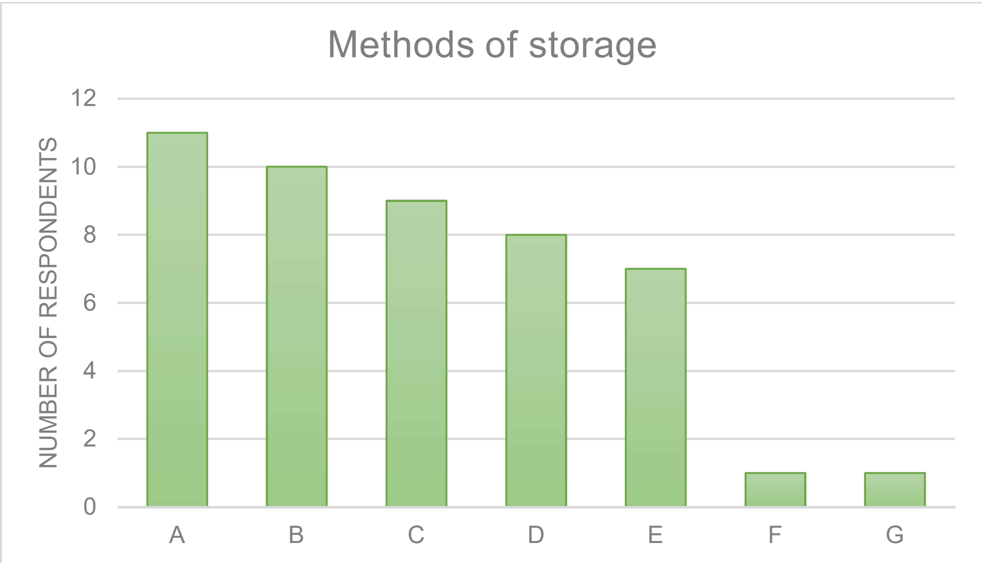


Figure 10. Various methods of storing *G. kola* seeds reported from Southwest region of Cameroon. A – cool and dry place, B – plastic bag, C – wrapping in leaves/paper, D – bury in ground/sand/dust, E – air-tight container, F – refrigerator, G – unopened fruits.

The time for which can be the kernels stored had been usually estimated for one year (Figure 11). We tried to evaluate which method of storage last for the longest time, however the farmers did not have clear opinion on that problematic. Only one clear result came for the method 'A', by which the kernels can be stored for between six months and one year.

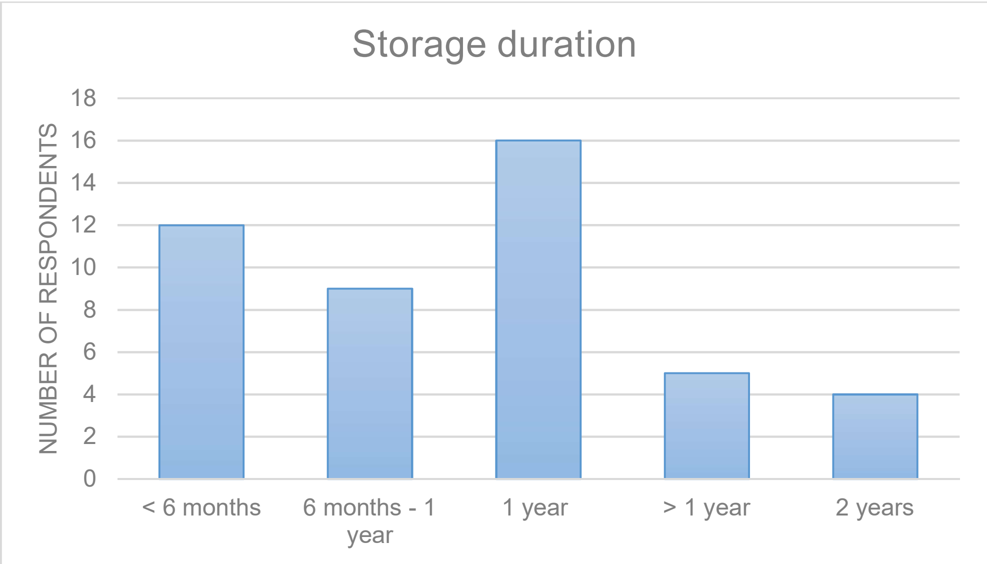


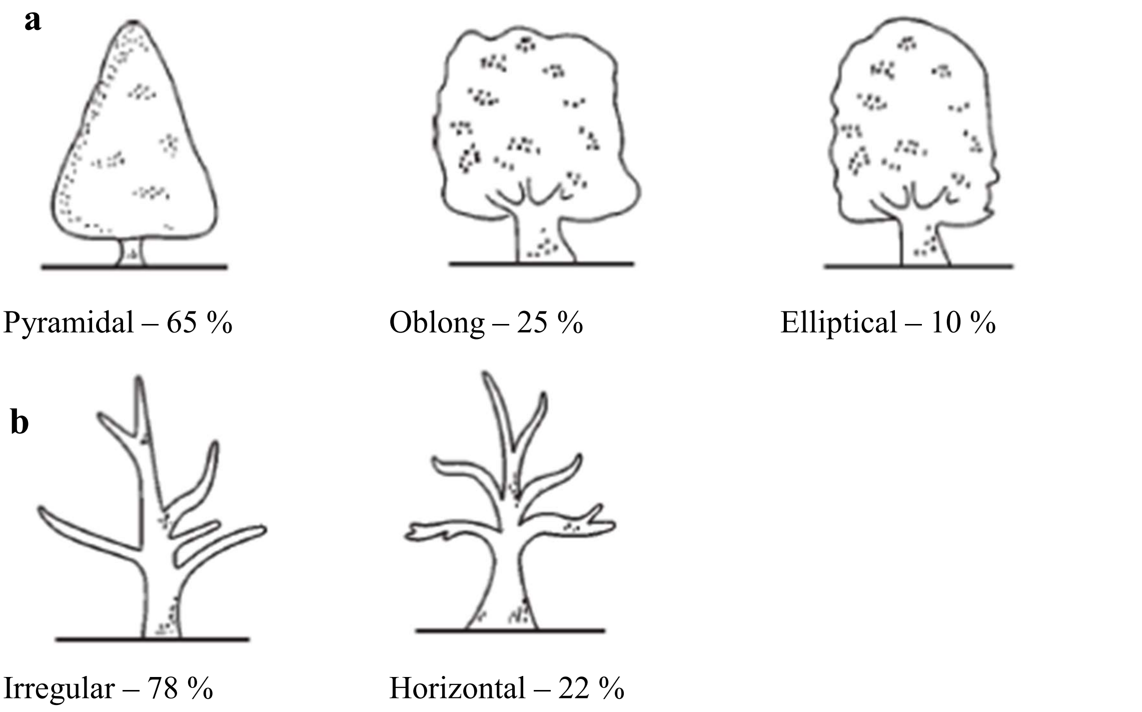
Figure 11. Longevity of storage of *G. kola* seeds reported from Southwest region of Cameroon.

5.2. Morphological evaluation

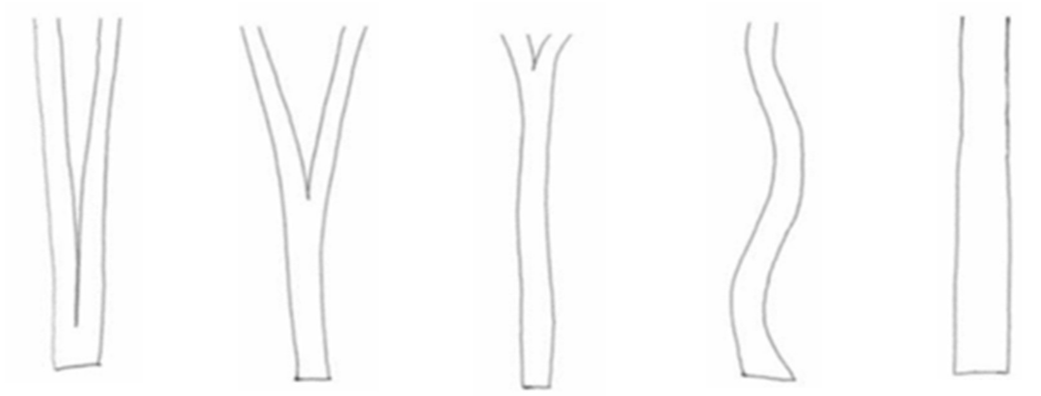
5.2.1. Tree habit

We morphologically evaluated all 81 tree individuals for their growth habit. More than a half (65 %) of the trees showed pyramidal shape of crown, second most frequent pattern (25 %) was oblong (Figure 12a). We did not find any significant differences in the shapes distributions among the study sites. According to prepared descriptor (Appendix C), crowns were generally rated as 'good' or 'perfect', just in the case of Lebialem a few more 'poor' and 'tolerable' ratings were identified. Branching was defined as irregular in 59 (78 %) individuals, the rest 22 (22 %) trees possessed horizontal type (Figure 12b). Shape of trunk was mainly straight, in 35 (43 %) trees, followed by a type in which forking starts above 6 m of height (22 trees, 27 %) and forking in less than 6 m (14 trees, 17 %). Just two individuals showed twisted stem and only eight were forking right from bottom (Figure 12c). Straight trunk was the most common shape in all study sites except Kumba, where forking above 6 m of height dominated.

Estimated age of most of the sampled trees was between 20-28 years according to the farmers' information. However, in Mamfe the trees were said to be older, around 35 years on average. This was influenced by the fact that the data collection was done at the end of harvesting period, so the younger trees were already without fruits and mainly old trees prevailed, because they were too high for harvesters to climb. Based on farmers' testimonies, the oldest tree was around 100 years old and still bore fruits. This tree was also the tallest, with 15 m of height, and had the biggest DBH value of 82.4 cm, from the whole data collection. Commonly, the trees were about 13 m in height with average DBH of 30 cm. Mean trunk height was estimated to 3.5 m, while crown diameter measured 10.5 m on average (Table 8). Statistically, there were significant differences in DBH values between Lebialem and Mamfe. In terms of trunk height, Kumba was statistically different compared to the other study sites. We did not find any other statistically significant difference among the trees of different study sites.



c



Forked from bottom – 10 %	Fork at less than 6 m – 17 %	Forking starts above 6 m – 27 %	Twisted stem – 3 %	Straight stem – 43 %

Figure 12. Tree morphological characteristics of *G. kola*. a - frequency of different tree crown shapes by IPGRI (2003); b - frequency of different branching patterns by IPGRI (2003); c - frequency of diverse trunk shapes, provided by Simon Njeudeng Tenku (IRAD).

Table 8. Quantitative data for tree morphology among different study sites in Southwest region of Cameroon; mean ± standard deviation and median. Numbers with the same letters within a same column are not significantly different at 0.05 level.

Study site	Tree height (m) mean		DBH (cm)		Trunk height (m)		Crown diameter (m)	
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median
Kumba	12.67 ± 3.42	13.00	29.3 ± 11.4^{ab}	0.250	3.46 ± 1.72	3.0	9.71 ± 2.84	8.58
Lebialem	14.35 ± 7.69	13.00	27.2 ± 14.8^{a}	0.239	2.88 ± 1.41	3.0	9.79 ± 2.53	9.62
Mamfe	15.85 ± 3.51	15.75	42.7 ± 14.4^{b}	0.455	5.26 ± 3.85	4.0	10.33 ± 2.78	10.61
Tombel	11.83 ± 2.60	12.00	36.0 ± 10.7^{ab}	0.359	3.35 ± 0.95	3.5	11.96 ± 2.67	11.58
Average	13.66 ± 4.89	13.00	33.8 ± 0.14	0.298	3.73 ± 2.41	3.5	10.44 ± 2.81	10.48

5.2.2. Leaf morphology

Altogether 403 leaves from 81 were analysed for both qualitative and quantitative data. The most common type of leaf blade, similarly in all study sites, was oblong. This type was recorded 290 times (72 %), followed by lanceolate shape, described 51 times (13 %), and elliptic shape, which occurred in 37 cases (9 %) (Figure 13c). Apex was predominantly acuminate in 359 instances (89 %) across the collection sites. Leaf base disposed mainly oblique shape, which was detected in 347 (86 %) cases (Figure 13a,b).

Average blade length varied between 11-14 cm and 4-6 cm in case of blade width. Petiole was usually about 12 mm long and 2-3 mm wide. Statistically significant differences were found just in case of Tombel, in which the sampled leaves were generally of smaller size (Table 9).

a

Acuminate – 89 %　Acute – 4 %　Obtuse – 2 %　Retuse – 5 %

b

Cuneate – 3 %　Oblique – 86 %　Rounded – 5 %　Truncate – 6 %

c

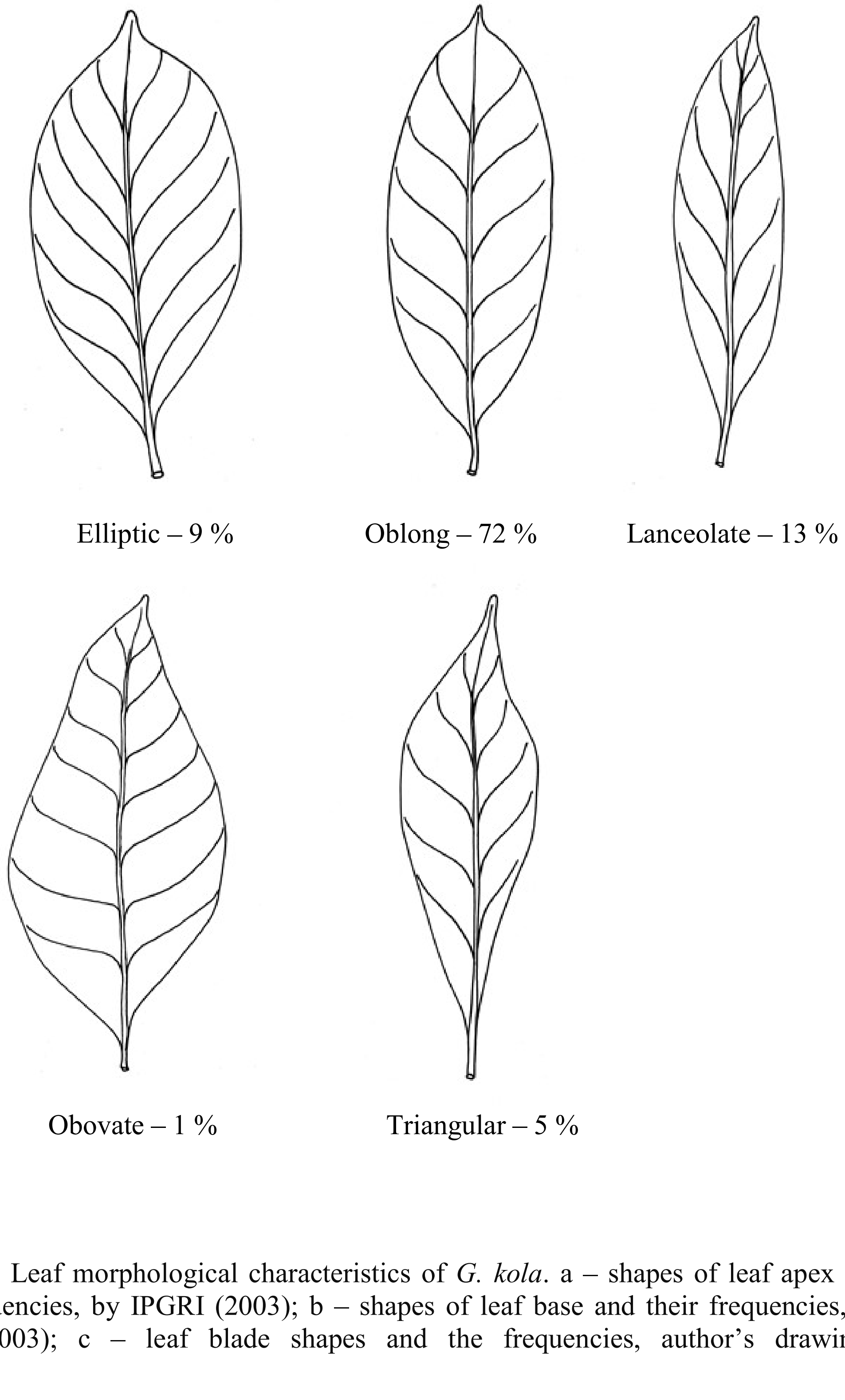


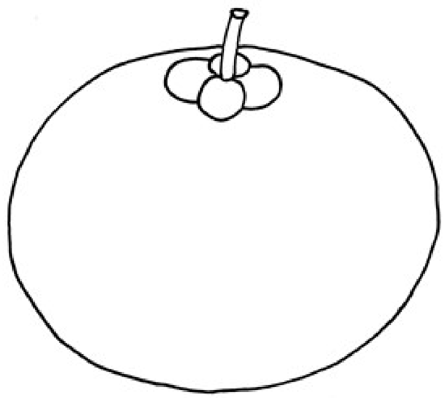
Figure 13. Leaf morphological characteristics of *G. kola*. a – shapes of leaf apex and their frequencies, by IPGRI (2003); b – shapes of leaf base and their frequencies, by IPGRI (2003); c – leaf blade shapes and the frequencies, author's drawings.

Table 9. Leaf quantitative morphological characteristics among different study sites in Southwest region of Cameroon; mean ± standard deviation and median. Numbers with the same letters within a same column are not significantly different at 0.05 level.

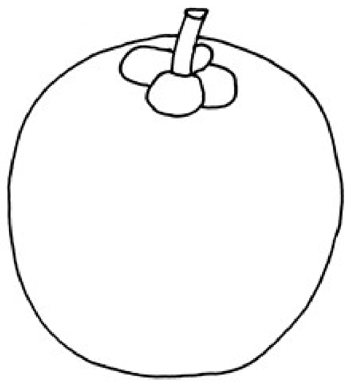
Study site	Blade length (cm)		Blade width (cm)		Petiole length (mm)		Petiole width (mm)	
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median
Kumba	13.56 ± 3.37[b]	13.45	5.29 ± 2.01[b]	5.25	11.94 ± 4.44	12	2.52 ± 0.81[ab]	3
Lebialem	13.58 ± 2.68[b]	13.40	5.29 ± 1.09[b]	5.20	11.90 ± 4.14	12	2.92 ± 0.85[b]	3
Mamfe	12.97 ± 3.33[b]	12.30	5.27 ± 1.43[b]	5.10	11.91 ± 4.22	12	2.71 ± 0.80[ab]	2
Tombel	11.35 ± 3.47[a]	11.50	4.46 ± 1.54[a]	4.10	12.10 ± 4.43	11	2.41 ± 0.78[a]	2
Average	12.87 ± 3.34	12.50	5.08 ± 1.59	5.00	12.01 ± 4.24	12	2.46 ± 0.82	2

5.2.3. Fruit and seed morphology

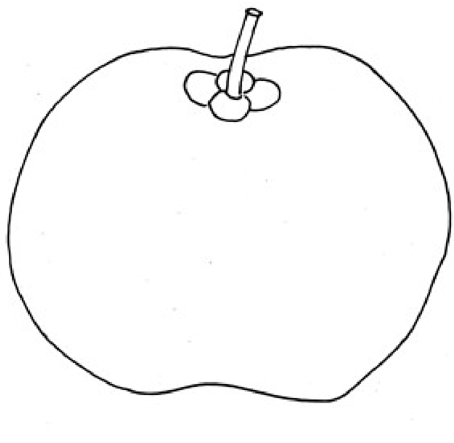
Overall, 759 fruits from 81 trees were examined. The most common shape of fruit was determined as spherical with 262 findings (35 %), followed by flattened shape which occurred in 214 (28 %) fruits and ellipsoid shape found in 132 cases (17 %) (Figure 14). Spherical shape dominated in Kumba and Tombel, flattened prevailed in Lebialem and ellipsoid in Mamfe. Typically, fruit skin varied somehow in-between yellow, orange and red colour tones. According to BS 381, the most common colour was defined as crimson with occurrence in 23.5 % of fruits, followed by post office red in 18.9 % fruits, international orange with 16.3 % and currant red in 13.1 % of fruits. According to the measurements, average fruit weighed about 140 g, had 7 cm in diameter and was around 6.3 cm long (Table 10). There were no significant statistical differences in fruit morphology revealed among the study sites.



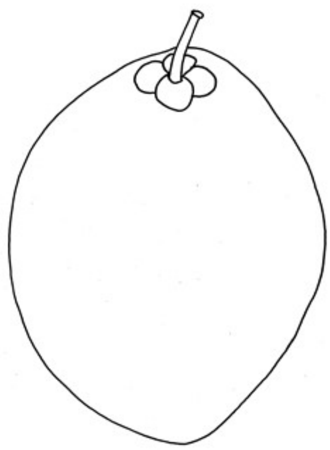
Flattened – 28 %



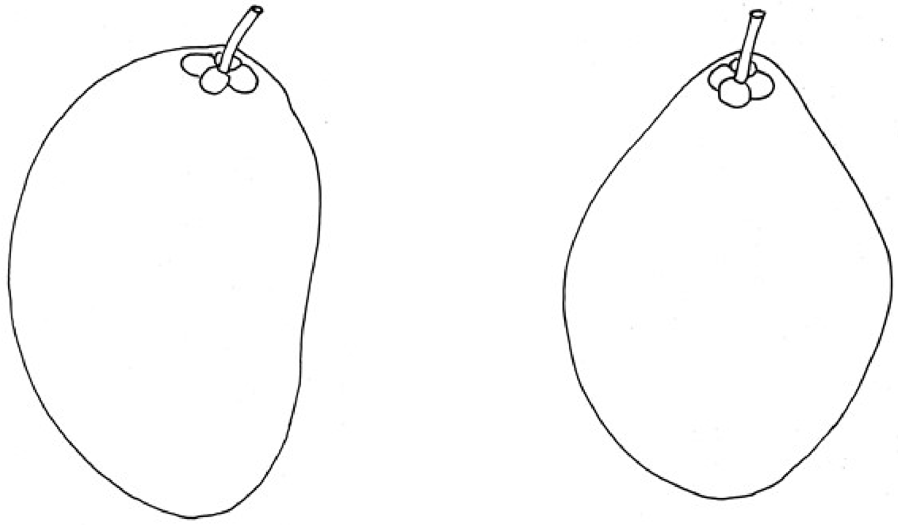
Spherical – 35 %



Oblate – 2 %



Ellipsoid – 17 %



Kidney-shaped – 4 % Rhomboidal – 14 %

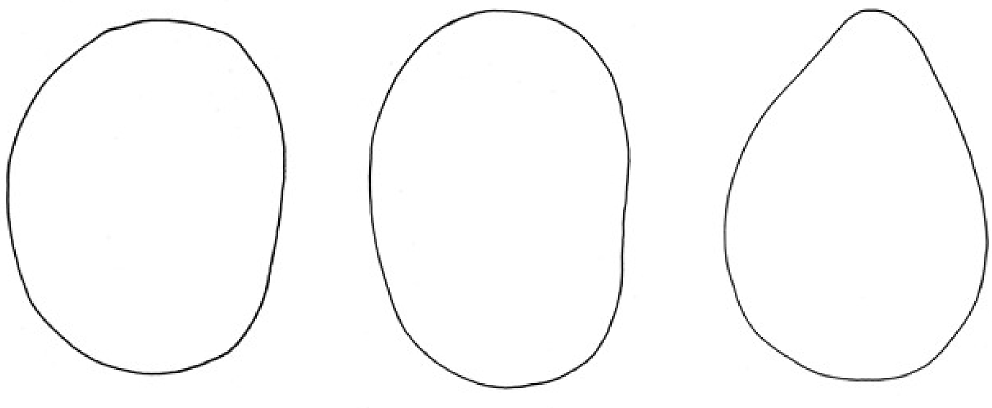
Figure 14. Different shapes of *G. kola* fruits and their frequencies. Source: author's drawings.

Table 10. Fruit morphological quantitative data among different study sites in Southwest region of Cameroon; mean ± standard deviation and median.

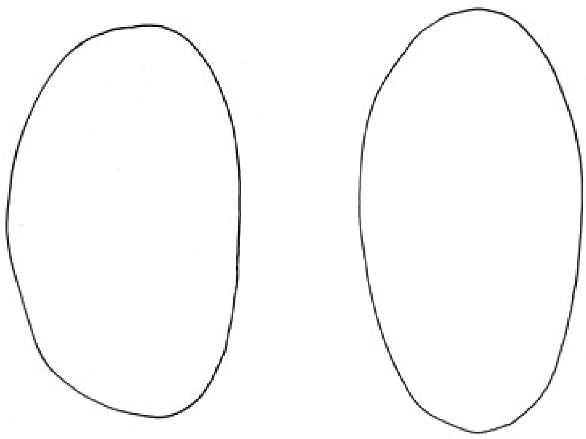
Study site	Weight (g)		Diameter (cm)		Length (cm)	
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median
Kumba	133.68 ± 33.31	136.68	6.80 ± 0.57	6.61	6.25 ± 0.90	6.18
Lebialem	144.51 ± 31.24	143.15	7.04 ± 0.49	7.17	6.16 ± 0.74	6.06
Mamfe	149.42 ± 42.29	139.72	7.02 ± 0.55	6.93	6.58 ± 1.01	6.33
Tombel	148.79 ± 62.56	138.49	6.85 ± 0.95	6.82	6.88 ± 1.14	6.89
Average	143.95 ± 43.91	139.91	6.93 ± 0.66	6.91	6.47 ± 0.99	6.36

From the fruits, 1,821 seed were extracted. Predominantly, the kernels were of ellipsoid shape, which was found in 939 cases (52 %). This type was the most common in all study sites. Furthermore, other prevailing types included oblong shape, which was detected in 465 (25 %) seeds, and elongated pattern, found in 301 (17 %) kernels (Figure 15). Few seeds were categorized as irregular (only 1 % from the total amount), mainly due to their immaturity or poor development. In Tombel, elongated shape of kernel was the second most frequent right after ellipsoid, on the other hand, in Kumba, Lebialem and Mamfe the second most common shape was oblong. Colour of seed testa was usually brown, sometimes having a slight orange/reddish tint. In general, 65.4 % of seeds were identified as light brown, 11.4 % had orange tint, 8.5 % were brown, 4.2 % dark brown and 1.5 % had reddish tint. Few of the kernels had no seed coat and therefore the colour was determined as white or beige, this occurred in 3.7 % of seeds.

Average seed weight was found to be between 5-7 g, the seeds were about 2.8 cm long and had 1.3 cm in width (Table 11). In Mamfe. We found significantly heavier seeds compared to Lebialem, longer seeds compared to Lebialem and Kumba and wider seed compared to all other study sites. Generally, we calculated that one fruit contain 2.4 seeds on average. The highest portion of seeds per fruit was observed in Kumba (2.7), whereas the lowest number occurred in Mamfe (2.3).



Spheroid – 4 % Oblong – 25 % Drop – 1 %



Ellipsoid – 52 % Elongated – 17 %

Figure 15. Different shapes of *G. kola* seeds and their frequencies. Source: author's drawings.

Table 11. Seed quantitative morphological data among different study sites in Southwest region of Cameroon; mean ± standard deviation and median. Numbers with the same letters within a same column are not significantly different at 0.05 level.

Study site	Weight (g)		Length (cm)		Width (cm)	
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median
Kumba	6.02 ± 1.33[ab]	6.10	2.96 ± 0.35[ab]	2.80	1.19 ± 0.11[a]	1.21
Lebialem	5.10 ± 1.49[a]	5.33	2.59 ± 0.43[a]	2.56	1.29 ± 0.17[a]	1.31
Mamfe	6.96 ± 2.05[b]	6.65	3.11 ± 0.47[b]	3.05	1.50 ± 0.16[b]	1.53
Tombel	5.61 ± 1.68[ab]	5.65	2.96 ± 0.42[b]	2.87	1.18 ± 0.14[a]	1.19
Average	5.90 ± 1.76	5.80	2.90 ± 0.46	2.80	1.29 ± 0.19	1.27

Further on, we found significant ($p < 0.05$) but not very high ($r = 0.524$) correlation among whole fruit weight and total weight of the seeds inside the fruit. That means, in heavier fruits we could expect a higher weight of seeds.

5.3. Seed nutritional composition

In total, 80 samples (one of each sample tree) consisting of the mix of grounded seeds were subjected to various nutritional analyses. Average moisture content in fresh kernels was found to be around 42 %, with statistical significant differences among populations from Mamfe, where we found the lowest amount, and Lebialem with highest portion (Table 12). Ash content hovered about 0.3 %, crude fibre varied around 2.3 % and crude protein was determined at 6.5 %. The amount of crude fat was, compared to other study sites, significantly lower in samples coming from Mamfe, but the level was 1.5 % on average. It was calculated that the samples contained on average 47 % of nitrogen free extract (NFE). Principal Component Analysis (PCA) showed that populations from Kumba, Lebialem and Tombel were similar in the seed nutritional composition, while Mamfe was found to be the most distinct from the study sites (Figure 16).

Table 12. Mean nutritional composition of *G. kola* seeds from Southwest region of Cameroon. Numbers with the same letters within a same column are not significantly different at 0.05 level. Results are shown in % of dry weight basis.

Study site	Moisture (%) ± SD	Ash (%) ± SD	Crude fat (%) ± SD	Crude fibre (%) ± SD	Crude protein (%) ± SD	NFE (%)*
Kumba	42.33±8.25^{ab}	0.24±0.14	1.60±0.26^{b}	2.27±0.36	6.64±1.18	46.92
Lebialem	44.59±4.41^{b}	0.42±0.17	1.49±0.18^{b}	2.26±0.56	6.68±0.80	44.56
Mamfe	39.53±5.18^{a}	0.35±0.20	1.21±0.25^{a}	2.39±0.62	6.35±0.78	50.17
Tombel	42.63±6.06^{ab}	0.26±0.21	1.59±0.20^{b}	2.15±0.30	6.25±1.53	47.12
Average	42.30±6.33	0.33±0.19	1.48±0.27	2.27±0.47	6.48±1.12	47.19

*NFE (%) = 100 – (CP % + CF % + Crude fat % + Ash %); NFE – Nitrogen free extract; ± SD – Standard deviation

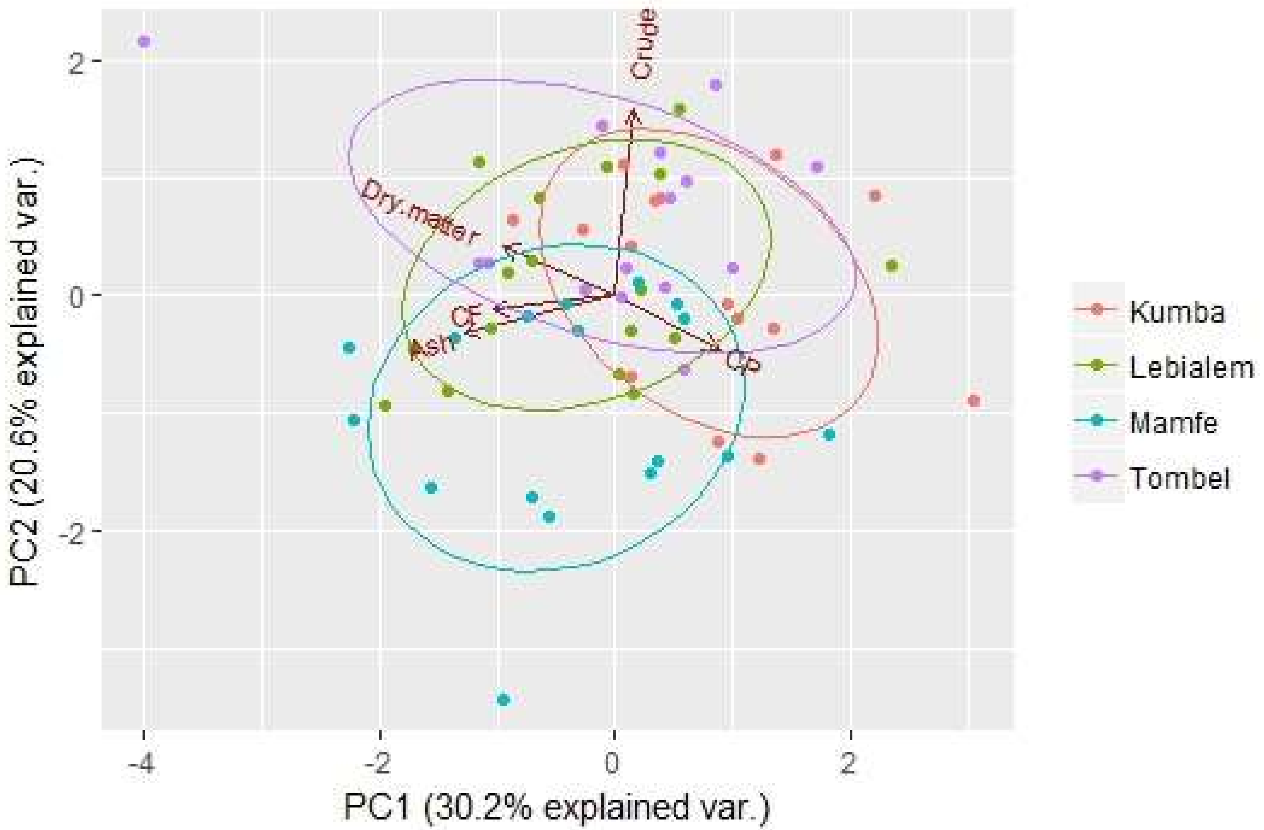


Figure 16. Principle component analysis of *G. kola* seed nutritional values: dry matter, ash, crude fibre (CF), crude fat (Crude) and crude protein (CP), among different study sites in Southwest region of Cameroon.

5.4. Linkages between morphological and nutritional characteristics

Quantitative morphological data of fruit weight, diameter and length together with seed weight, length and width were correlated to the seed nutritional values (Table 13). The analysis had not proved any strong statistical dependence among the parameters (the correlation coefficients were usually bellow 0.3), however it pointed out an interesting relation between amount of crude fat and seed width (r = -0.561). The result suggested that narrower seeds could have higher content of fat.

Table 13. Correlation coefficients between seeds and fruits morphological data and seeds nutritional values. Data marked with * have statistically significant dependence at $p < 0.05$.

Parameter	Ash (%)	Crude protein (%)	Crude fibre (%)	Crude fat (%)	Moisture (%)
Seed weight (g)	-0.185	0.128	-0.166	-0.415*	-0.368*
Seed length (cm)	-0.215	0.173	-0.251*	-0.283*	-0.424*
Seed width (cm)	0.029	-0.067	0.047	-0.561*	-0.056
Fruit weight (g)	0.003	-0.234	0.079	-0.088	0.043
Fruit length (cm)	-0.210	-0.046	-0.093	-0.124	-0.246*
Fruit diameter (cm)	-0.052	-0.193	0.013	-0.079	-0.078

PCA that included all morphological and nutritional data of fruits and seeds also showed that the samples from different study sites are quite similar in morphological as well as in nutritional traits (Figure 17). On the other side, the analysis revealed that particular trees coded as TEE1, from Tombel, and MNO1, from Mamfe, possessed distinct characteristics compared to the other samples.

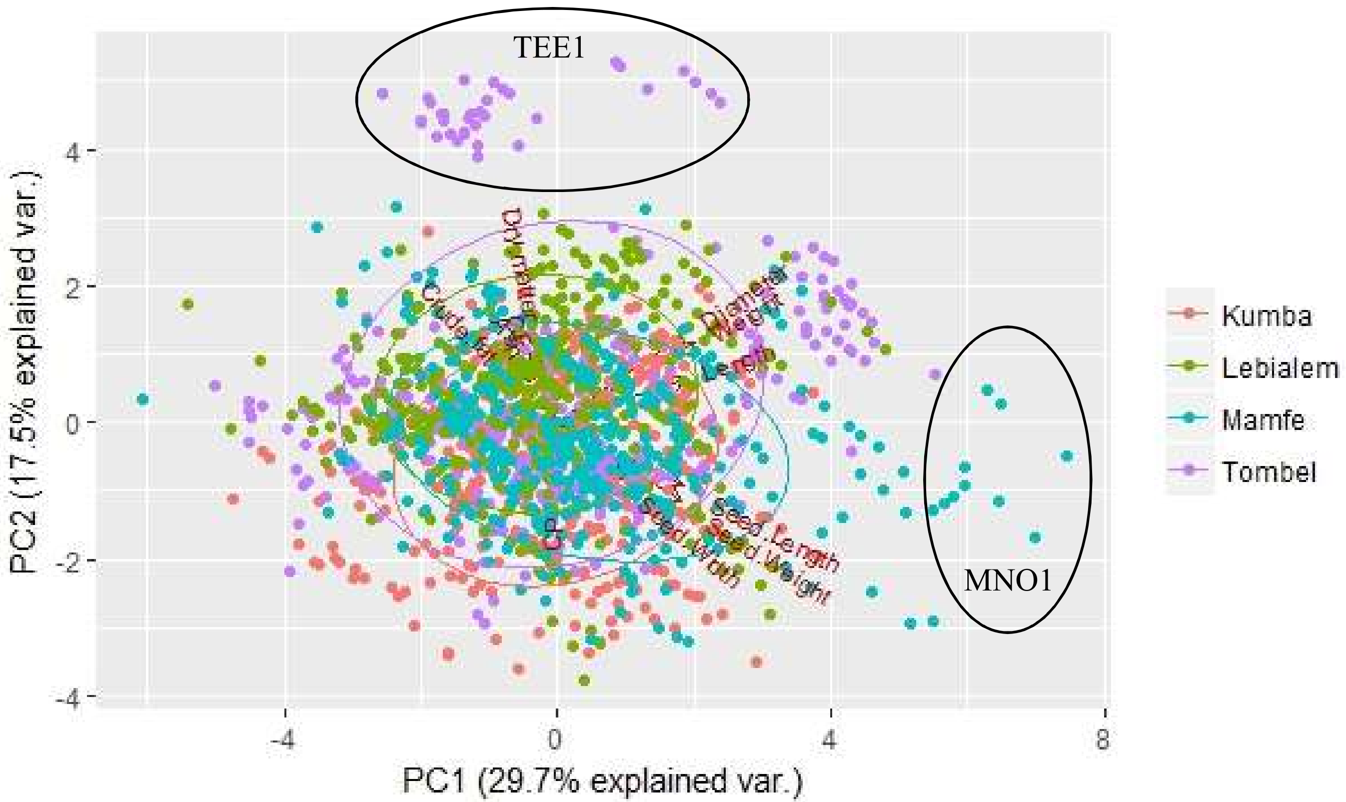


Figure 17. Principle component analysis (PCA) among: Fruit weight, length, diameter; seed weight, length, width; and nutritional values of seeds: dry matter, ash, crude fibre, crude fat and crude protein. Every dot represents one specific characteristic, black ellipse marks a particular tree.

DISCUSSION

Tree domestication is one of the processes that may lead towards sustainability and creation of functional agroecosystems in agriculture. Its benefits are in income diversification, improvement of local diets and health or simply in saving species, which are under threat of extinction from wild due to deforestation or over-harvesting (Leakey and Simmons 1998; Akinnifesi et al. 2007; Ofori et al. 2014). The process of tree domestication consists of many steps, e.g. species priority setting, selection of desired traits, superior trees selection, integration of trees into farmlands, vegetative propagation, targeted plant breeding and product commercialisation (Clement et al. 2010; Franzel and Kindt 2012; Leakey and Van Damme 2014). These steps cannot be taken unless solid information on species morphological, phytochemical and genetic characteristics are revealed. Therefore, this study has started the necessary steps in the domestication process of *G. kola*. We found a lack of data on the species morphology, e.g. no botanical descriptors and just few studies with sufficient number of samples. For this reason, we decided to describe phenotype variations in *G. kola* trees, leaves, fruits and seeds. Next, we wanted to focus on species phytochemistry, mainly secondary metabolites because the seeds are chewed as stimulant. However, during our study we revealed a confusion in the seeds nutritional values with different results pointing out rather contradictory information. We decided to arrange these disarrays first before targeting on the secondary metabolites. Further, our study was also complemented by farmers' survey which should have allowed us to understand more about the cultivation and utilization of *G. kola*.

6.1. Management and utilization of *G. kola*

More than half of the trees were grown on farmlands, in agroforestry systems, however large proportion (38 %) of *G. kola* were also cultivated in homegardens. This is much higher number than reported by Onyekwelu et al. (2015a), who described that in Ondo state, Nigeria, 96 % of the trees are cultivated on farmland and just 4 % in homegardens. This may indicate that the domestication process is a bit more advanced in Cameroon, because the farmers are purposely planting *G. kola* in their gardens as valuable fruit tree species. Most of the trees in our study were planted from seed or transplanted from natural regeneration as wildling, even two farmers tried vegetative propagation. Methods of vegetative propagation are considered as one of the major tools to fasten the domestication process. Their advantages are certainly in shortening the tree maturation period and thus earlier fruit production or in exact duplication of the mother tree genotype (Sciana et al. 1998; Tchoundjeu et al. 2006). On the other hand, this type of propagation may also make the plant weaker and some species do not respond well to it, which is for example the case of mangosteen, *G. mangostana,* close relative to *G. kola* (Mad 1990). Bin Osman and Milan (2006) reported that vegetative propagation even lowered the fruit production of *G. mangostana* in South-East Asia. Therefore, not only suitable methods of vegetative propagation for *G. kola* but also long termed studies monitoring the development of propagules, their growth and production, should be developed.

In our study, more than 80 % of farmers used the seeds for treatment of gastric disorders, however this does not agree with results of Fondoun and Tiki Manga (2000) from humid forest zone of Cameroon. They found out that the farmers use *G. kola* mainly as aphrodisiac or as ingredients for palm wine production. This variance might be a matter of interpretation. From our experiences, local people know about the potential use of the seeds as aphrodisiac but usually do not utilize them in that way. From the practical point of view, curing of gastric problems should be the main use. According to Awono et al. (2016), 50 tons of *G. kola* seeds are sold annually in Cameroon. The authors proposed an average price for 1 kg of kernels as 2,500 CFA (4 USD). Compared to our study, their suggested mean price is a bit higher, reaching 37,500 CFA (61 USD) per 15 l bag/bucket. Most of our respondents sold their products in range of 10,000-30,000 CFA (16-49 USD) per bucket/bag. The price was significantly higher just in Lebialem. The reason might be in its topography rugged by steep hills and valleys, where altitude varies greatly between 200 – 2,400 m a.s.l. This is combined with harsh conditions characterized by strong winds and low sunshine as well as with the fact, that Lebialem is considered as the rainiest division in the whole Cameroon (Focho et al. 2009; Etiendem et al. 2011). These facts not only influence morphology of the tree but also trading opportunities, and, consequently, might lead towards a higher price of *G. kola* seeds in this division.

If stored properly, the seeds can last for a year or even longer and be sold in off-season for a greater fare. We have identified seven basic methods that are practised by farmers to store the kernels, four more then described in studies of Dosunmu and Johnson (1995), Adebisi (2004a) and Adebisi (2004b). Those studies reported storing the seeds in cool, dry place; wrapping them in leaves; and putting them between layers of soil or in dust piles. Apart from that, storing the seeds in plastic bag or air-tight container were considered as useful methods by our respondents. More research should be conducted to define the specific ways of *G. kola* kernels storage along with their durations to optimize the storage procedure.

6.2. Morphological characteristics

If we look at the median of the tree morphological measurements, the most vigorous trees grew in Mamfe, the least in Lebialem. Again, this result was, most probably, caused mainly by the different climatic conditions and topography. The unambiguously smallest leaf blades were spotted in Tombel, which might be a matter of the fact that the site had the highest average elevation from our study sites. In terms of fruit measurements, there were no significant differences among the populations, but comparing the median values, the biggest and heaviest fruits were collected in Lebialem. In contrast, Lebialem also possessed the smallest seeds, which is an interesting trend pointing out that the pulp percentage was the highest there. Once again, this might be attributed to the specific climatic and geographical conditions in the division as described above. The biggest and heaviest seeds were sampled in Mamfe, which is the place of lowest average altitude and highest temperature. This may show that the seeds did not need so much protection and therefore the fruits contain lower amount of pulp. It seems that the best conditions for seed development are lowlands, with high average temperatures and rainfall.

Due to lack of studies focusing solely on *G. kola* morphology, there is not much information to which we can compare our results. However, Onyekwelu et al. (2015a) had measured 50 *G. kola* trees in Ondo state, Nigeria. Our sampled trees were generally of smaller size in terms of tree height, DBH and crown diameter but had bigger leaves. Onyekwelu et al. (2015a) described mean fruit weight as 127.5 ± 47.4 g and seed weight as 3.2 ± 0.5 g. Our results were much higher with 143.9 ± 43.9 g in case of fruit weight and 5.9 ± 1.8 g for seed weight. Ondo state should have mostly similar climatic conditions compared to our study sites, so the variation in the results might be caused by diverse approach from farmers. Because the authors did not specify the origin of the sampled trees, we can guess that they could have remained after forest clearing. That would have led to higher age of the trees and, consequently, to the differences in the morphological parameters compared to our trees, which were mostly planted by their owners. We can also hypothesize that the trees in our study had already undergone some kind of primary selection, which, as a consequence, led to slightly bigger fruits and seeds.

6.3. Nutritional values

During the nutritional analysis of *G. kola*, we did not identified much differences among the study sites. On the other hand, we came up with robust results (80 samples), which may help to define reliable nutritional values for the *G. kola* kernels. Data from Table 2 (in 'Literature review') showed very discrepant results in the seeds nutritional composition. We put these variations into comparison with our findings (Table 14).

Table 14. Comparison of published data and author's results on nutritional composition of *G. kola* seeds.

Parameters	Range of published results** (mean)	Results of this study (mean ± SD)
Moisture %	7.2 – 92.7	42.30 ± 6.33
Ash %	0.33 – 5.9	0.33 ± 0.19
Crude protein %	0.58 – 7.8	6.48 ± 1.12
Crude fat %	0.19 – 14.5	1.48 ± 0.27
Crude fibre %	1.23 – 20.51	2.27 ± 0.47
NFE %	10.85 – 91.35	47.19

* NFE – Nitrogen-free extracts, SD – Standard deviation

** Dosunmu and Johnson (1995), Arogba (2000), Asaolu (2003)[a], Eleyinmi et al. (2006), Ibekwe et al. (2007)[a], Esiegwu and Udedibie (2009)[a], Odebunmi et al. (2009), Adesuyi et al. (2012), Onyekwelu et al. (2015b); a – published in Esiegwu et al. (2014)

Our results on moisture content were closest to Odebunmi et al. (2009), ash content came out similarly to Onyekwelu et al. (2015b), crude protein content was almost the same as published by Arogba (2000), in terms of crude fat and crude fibre our results corresponded mostly again to Onyekwelu (2015b) and amount of NFE was closest to Ibekwe et al. (2007) in Esiegwu et al. (2014). One of the reasons for the differences might be because some of the authors were analysing the kernels without hulls, whether the others included them in the determinations. Moreover, different studies used distinct measurement units and without clearly stated basis of the data, the results could not be fully re-counted. Next, the inequalities may reflect the fact that the content of nutrients depends on external factors such as season, climate, soil condition or time of evaluation (Eleyinmi et al. 2006; Odebunmi et al. 2009, Simbo et al. 2013). Also, a period for which the seeds were stored before selling should be considered as an important factor.

6.4. Diversity and domestication

Our results revealed that the morphological diversity within the whole population is much higher than the diversity between populations. Even on one tree we often identified a variety of fruit shapes, sizes and colours. That might also be the reason why there were no statistical differences in fruit types among the study sites. We can guess that the morphological diversity is still influenced mainly by external conditions rather than by genetics. Due to the high tree-to-tree variation it is evident that the species has a good adaptability to diverse conditions; even though *G. kola* is classified as lowland species, we detected it from 139 - 755 m a.s.l.

The tree can be, according to Clement et al. (2010), categorized as incipiently domesticated. The process of domestication is at its very beginning, yet there is an obvious effort for the species meaningful cultivation and, maybe, also selection of the best individuals.

CONCLUSION

Our study was targeted on a summary and extension of knowledge on diversity, management and utilization of *G. kola* in Cameroon. We interviewed 48 farmers from 50 farms and examined altogether 759 fruits along with 1,821 seeds and 402 leaves coming from 81 trees. Sampling was done in four different divisions of Southwest region. After the morphological evaluations, seeds from each tree were further analysed for their nutritional values. From the farmers survey we found out that *G. kola* is grown mostly in cocoa agroforestry systems or homegardens. The trees were usually planted from seed or transplanted as wildling by their owners, only two respondents tried to propagate the trees vegetatively. The most valued product were clearly the seeds, which served mostly for treatment of gastric disorders. Their price fluctuates throughout the year and was found higher in Lebialem region compared to other study sites, probably because of its inaccessibility and difficult conditions for tree cultivation. Bark of the tree was also used, mainly to relieve malaria or for palm wine fermentation. In terms of *G. kola* morphology, we developed specific botanical descriptor by identification and creation of its morpho-types. Significant differences were revealed only in species DBH, leaf blade length and width, and in seed weight, length and width. Interestingly, Lebialem possessed the biggest fruits but also the smallest seeds, which is most likely a matter of its specific climatic and topographical conditions. The biggest seeds occurred in Mamfe, which may have the best conditions for *G. kola* cultivation – lowland areas along with hot, moist climate and high rainfall. Seed nutritional composition showed significant statistical differences in moisture and crude fat content. However, the main benefit of our work was in overall determination of seeds nutritional content, which was differently interpreted by various authors. Overall, we found higher intrapopulation diversity, in terms of species morphological and nutritional characteristics, than diversity between populations/study sites. There was also a high variability of morphological traits within single trees. This diversity was probably influenced more by ecological conditions than by tree genetics and may refer to high species adaptability.

For the future, more samples from other parts of Cameroon (other countries from West and Central Africa, respectively) should be sampled and compared to our results to see, whether there are significant differences among more distant places/study sites. The research should not only focus on seeds, long-term study might be conducted to reveal fluctuations among secondary metabolites in the both species' seeds and bark. Besides, field trials focusing on methods of vegetative propagation and the propagules regeneration as well as fruit bearing have to be established to prove, whether the vegetative way would not cause lower yields, as in the case of *G. mangostana*. Our data should be further supported by studies analysing species genetic variation and market/value chains to make a real progress in terms of *G. kola* domestication. To fasten the process, priority should be given to superior trees selection and their further multiplication.

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APPENDICES

Appendix A – Questionnaire

Name: Gender: Age:

Village: Estimated size of farm:

Number of bitter kola trees on farm:

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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?
8) How do you harvest the fruits?
9) What is the best time for harvesting?
10) Do you sell the seeds? For how much CFA?
11) Is it possible to store the seeds, how, for how long?

Appendix B – *G. kola* habitus

(Author's personal photo documentation)

Erect tree trunk

Bark exudating sticky yellow latex

Dense crown

Slightly drooping branches

Fruit set from Lebialem

Leaves set from Lebialem

Fresh fruit with seeds embedded in fibrous pulp

Rotten fruit for easier seeds removal

Seeds divided according to mother tree

Method of fruits softening

Seeds during morphological analyses

Wildlings

Appendix C – Modified descriptor for *G. kola*

<u>Site characterization</u>

Owner of farm:

GPS: Elevation:

Location on farm (homegarden, forest, agroforestry):

Topography: 1) Flat 0-0.5% 2) Almost flat 0.6-2.9% 3) Gently undulating 3-5.9% 4) Undulating 6.0-10.9% 5) Rolling 11.0-15.9% 6) Hilly 16.0-30.0% 7) Steeply dissected >30%, moderate elevation range 8) Mountainous >30%, great elevation range

Soil texture class:

Vegetation surrounding the collecting site: Farm, homegarden, forest

<u>Tree characteristics</u>

Age: Tree type (seedling, veg. prop.);

Condition: 1) Dying 2) Old – declining 3) Mature – diseased 4) Mature - non-vigorous 5) Mature – vigorous 6) Young - not yet bearing 7) Healthy - cropping poorly 8) Healthy - cropping well

Tree height: Trunk height: Crown diameter: DBH:

Crown shape (fig. 3): pyramidal, spherical, oblong, elliptical

Branching pattern (fig. 4): erect, semi-erect, horizontal, irregular

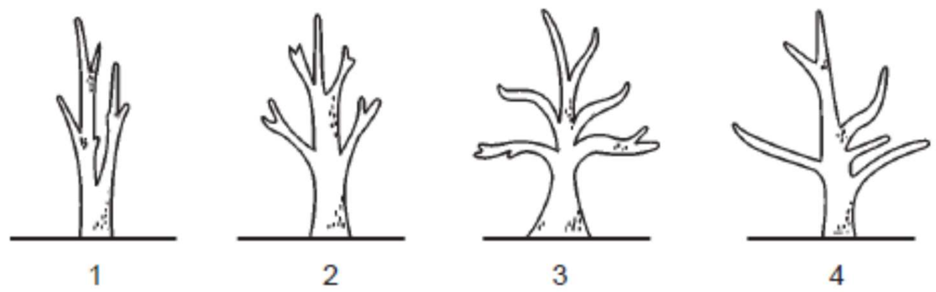


Fig. 4. Branching pattern

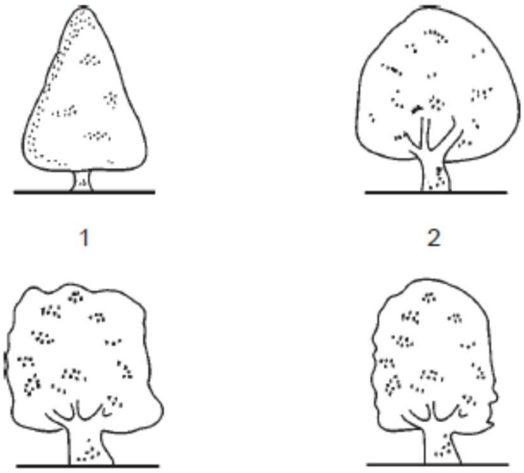
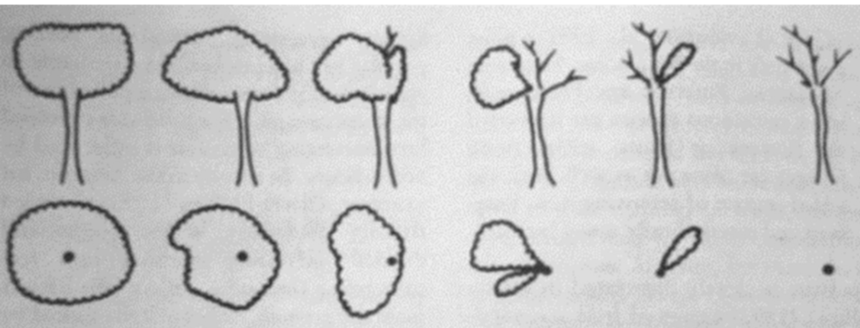
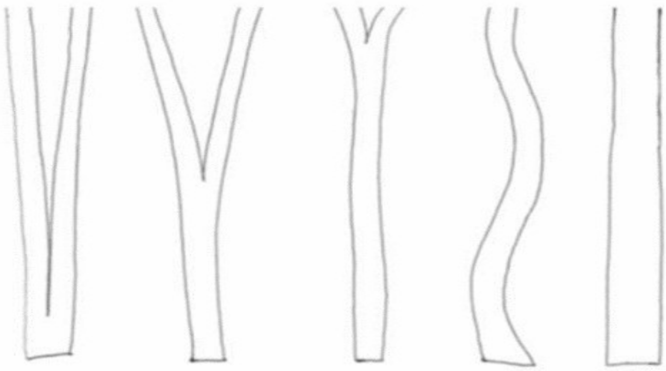


Fig 3. Crown shape



Definition of crown ratings
0 = Perfect: excellent size and development, wide, symmetrical and generally circular in plan.
1 = Good: slightly asymmetrical with some dead branch tips ('silviculturally satisfactory' to foresters).
2 = Tolerable: markedly asymmetrical, some dieback.
3 = Poor: extensive die-back, leaves form less than half original crown size.
4 = Very poor: badly damaged, unlikely to survive.
5 = Dead.

Figure 4.14 *Although used by Dawkins (1958) to rate tree-crown condition, due to various reasons, including competition, crown ratings also reflect tree health generally, for instance as a secondary response to bark or root use*



Trunk shape: 1 – forked from bottom; 2 – fork at less than 6 m height; 3 – forking starts above 6 m height; 4 – stem twisted; 5 – stem straight

Leaves

Petiole length and width: Leaf blade length and width:

Leaf blade shape (fig. 5): ovate, obovate, elliptic, oblong, lanceolate

Leaf apex shape (fig. 6): acute, acuminate, retuse, obtuse

Leaf base shape (fig. 7): oblique, rounded, cuneate, shortly attenuate, truncate

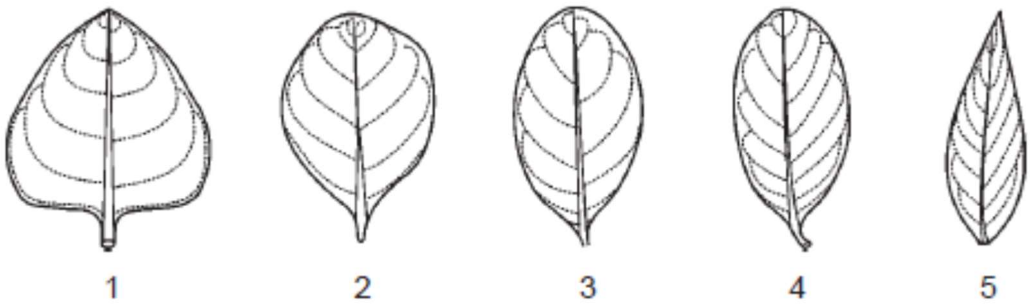


Fig. 5. Leaf blade shape

Fig. 6. Leaf apex shape

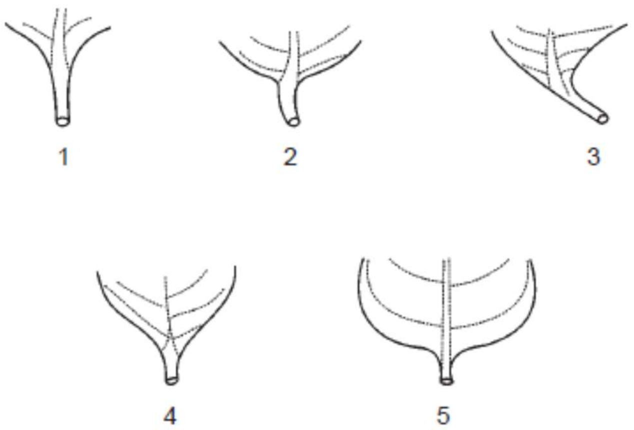


Fig. 7. Leaf base shape

<u>Fruit</u>

Fruit weight: Diameter: Length:

Fruit shape (fig. 9): spherical/round, flattened, ovoid, oblong

Colour:

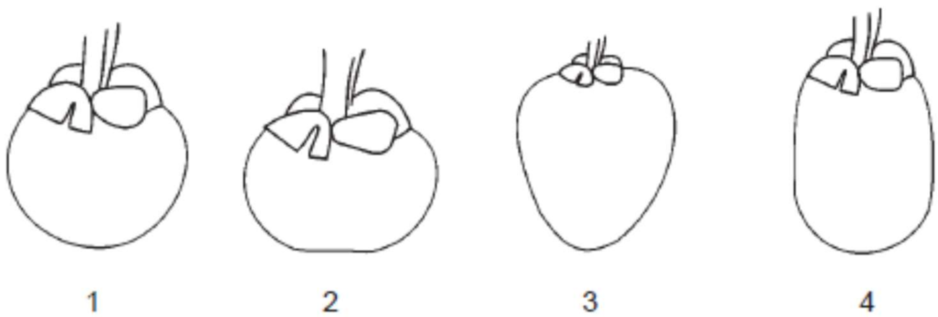


Fig. 9. Fruit shape

<u>Seed</u>

Seed weight: Width: Length:

Number of seeds per fruit:

Seed shape (fig. 12): spheroid, ellipsoid, elongate, oblong, reniform and irregular

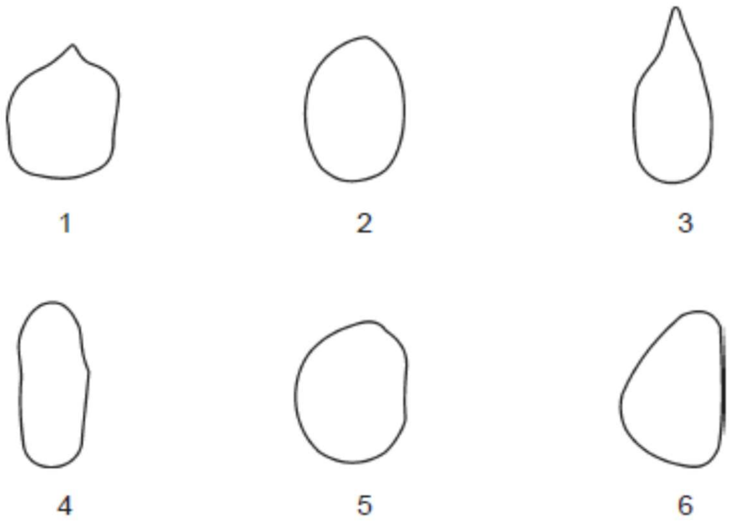


Fig 12. Seed shape

Seed coat colour: