Original Research Article

PHYLOGENETIC ANALYSIS OF *ACACIA NILOTICA* AND *COFFEA ARABICA* USING PROTEIN SEQUENCE FROM CHLOROPLAST *rbcL* GENE

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Abstract

Phylogenetic analysis is the study of evolutionary relationships among living organisms. In the context of protein sequence data, phylogenetic analysis is one of the pillars of comparative sequence analysis in both plants and animals. Therefore, the objective of this study deals with the evolutionary analysis of *Acacia nilotica* in comparison with *Coffea arabica*. The genus Acacia is of economic importance to local communities in sub-Saharan Africa for its medicinal and beverage usage. The bark extract is used by the same locals to make a coffee-like concoction. The beverage like concoction is brown in color hence the locals refer to this plant as 'Wild coffee' and therefore the needs bioinformatics tool approaches to compare the evolutionary relationship of this plant with *Coffea arabica* to determine whether they are monophyletic, paraphyletic, or even polyphyletic and whether they are closely or distantly related using a phylogenetic tree. Hence, the utility of cpDNA regions *rbcL* was tested in this study because of its high discriminatory power sequencing and better alignment thus it is vital for evolutionary study in plants. Also, this region of cpDNA is conserved across all plants. The sequences generated from *rbcL* also indicated that *Acacia nilotica* and *coffea arabica* are monophyletic. The evolutionary analysis on the basis of *rbcL* proved that *Acacia nilotica ssp. Subalata* and *Acacia nilotica* ssp. *hemispherica* are closely related

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as they form the sister groups. Also the same was seen with *Coffea arabica* and *Coffea salvatrix and C. racemosa.*

Keywords: *rbcL, Acacia nilotica, Coffea arabica*, MEGA, bootstrap, phylogenetic tree

INTRODUCTION

In addition to the nuclear (nDNA) and mitochondrial (mtDNA) genomes, plants have an additional genome, the chloroplast genome (cpDNA) which is not the case in animals. Because of its complexity and repetitive properties, the nuclear genome is used in systematic botany less frequently, Zabta K. Shinwari *et al.*, 2014. The mitochondrial genome is used at the species level due to its rapid changes in its structure, size, configuration, and gene order. On the other hand, the chloroplast genome is well suited for evolutionary and phylogenetic studies above and at the species level, because cpDNA, is a relatively abundant component of plants total DNA, thus facilitating extraction and analysis. Secondly, contains primarily single-copy genes. Thirdly, it has a conservative rate of 2 nucleotide substitution; and fourthly extensive background for molecular information on the chloroplast genome is available, (Doebley *et al.*, 1990). Therefore, data from cpDNA genes are used in phylogenetic reconstructions in plant systematics. Plastid-encoded *rbc*L gene is the most common gene used to provide sequence data for plant phylogenetic analyses (Chase *et al.*, 1993; Donoghue *et al.*, 1993). This single-copy gene is approximately 1430 base pairs in length, is free from length mutations except at the far 3' end, and has a fairly conservative rate of evolution. The function of the *rbc*L gene is to code for the large subunit of ribulose 1, 5 bisphosphate carboxylase/oxygenase (RUBISCO or RuBPCase) Duvall and Morton, 1996.

The enzyme ribulose-1,5-bisphosphate carboxylase (Rubisco) is responsible for the fixation of carbon dioxide in the Calvin cycle, Michael (1993). The holoenzyme is formed by a 16-mer structure that includes eight identical chloroplast-encoded large subunit polypeptides and eight small subunit polypeptides, Michael (1993). In green algae and in land plants, the genetic information for the small subunit is encoded in the nuclear genome, typically in a small multigene family (Meagher *et al.*, 1989 Palmer, 1991). Owing to its central importance in photosynthetic carbon fixation and owing to the early technical advantages associated with the study of the chloroplast genome, the molecular characterization of the *rbcL* gene was a major goal of plant molecular biology in the 1970s, Michael (1993). Cloning and determining the

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sequence of the *rbcL* gene was first accomplished by (Coen *et al*., 1977) and by McIntosh *et al*. 1980 working with maize (Zea mays).

The *rbcL* gene of chloroplast contains high substitution rates within the species and is emerging as a potential candidate for to study of plant systematics and evolution (Sathishkumar *et al.,* 2008). It has long been evident that molecular sequences contain useful information about evolutionary history, (Johnkress and Kenneth, 2005). The *rbcL* gene has ideal size, a high rate of substitution, a large proportion of variation at nucleic acid and protein level at first and second codon position, a low transition/transversion ratio, and the presence of mutationally conserved sectors. These features of *rbcL* gene are exploited to resolve genus and species-level relationships. Polymorphism of chloroplast DNA especially *rpoB*, rbcL, and intergenic *rpocL, rpoC* regions has been used to study the phylogeny of various plants, (Sathishkumar *et al.*, 2008). The sequence data of the *rbc*L gene are widely used in the reconstruction of phylogenies throughout the seed plants and flowering plants.

The objective of this study is to evaluate the generic, species variation, and phylogenetic relationships of Acacia and Coffea plants by using the chloroplast *rbcL* gene sequences available from the Genbank. Acacia is a flowering plant among the 751 genera and has about 1380 species. The genus *Acacia* contains many species that are traditionally medicinal to cure various disorders and in some local communities they use the bark extract to make a coffee-like concoction Kshipra Dhabhai and Amla Batra (2012). Therefore, this study tries to compare the evolutionary analysis between *Acacia nilotica* and *Coffea arabica* from a phylogenetic tree. Thus the sequences from the *rbcL* gene were used to compare their evolutionary relationships between *Acacia* and *Coffea* genera and their species to analyze whether they are monophyletic, paraphyletic, and polyphyletic.

Statement of the problem

Acacia nilotica (Subalata) is reported to treat ear, eye infection, skin rashes, painful urination and as a beverage by the Pokots (*Aloush et al.,* 2006). It is also used to treat cancer, diarrhea and diabetes (Kalaivani & Methew, 2010 &Del, 2009). It makes coffee like concoction for medicinal use. There is no scientific knowledge on comparison of inhibitory activities between *A. nilotica* (subalata) and *Coffea arabica* varieties extracts on the microorganism that cause the above mentioned ailments. Also there is no scientific facts on evolutionary relatedness between *A. nilotica* (subalata) and *Coffea arabica* varieties.

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Because of prolonged use of *A. nilotica* (subalata) extracts to make coffee like concoction and treat diseases by locals whereas no scientific work has been done to compare evolutionary relatedness of *A. nilotica* (subalata) and *Coffea arabica* varieties can cause long term health effects. Therefore due to the taxonomic relatedness of these two plants, it is important to study and evaluate their evolutionary relationship in order to provide scientific evidence of their closeness.

Specific objective

To compare their evolutionary relationship between *Acacia nilotica* and *Coffea arabica.*

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Literature review

Acacia nilotica (subalata)

Taxonomy and ecology of *Acacia nilotica*

The Brenan (1983) taxonomy of *Acacia nilotica* subspecies subalata is as follows; domain (*eukaryota*), kingdom (*plantae*), phylum/division (*spermatophyta*), subphylum (*angiospermae*), class (*dicotyledonae*), order (*fabace*), family (*fabaceae*), subfamily (*mimosoideae*). There are over 1380 acacia species worldwide. In Africa there are about 130 species (Rajvaidhya *et al*., 2012).

Acacia nilotica (subalata) grows well in riverine alluvial soil and black cotton soil. It grows on saline and alkaline soils. *Acacia nilotica* grows thrives well in sub-tropical to tropical climatic conditions (Godghate *et al*., 2014). This plant can withstand high temperature of up to 50 ^{o} C but adequate moisture is needed for full growth and development. The average annual rainfall ranges from 250-1500 mm (Bargali & Bargali, 2009).

Economic importance of *Acacia nilotica*

Acacia nilotica subspecies subalata is a source of fodder, timber, gum and fuel, and also have pharmacological effects (Bargali & Bargali, 2009). The major primary use of *Acacia nilotica* subsp. subalata is wood fuel (Ali *et al.*, 2012). Its charcoal burns well without too much smoke and sparks. *Acacia nilotica* subsp. subalata wood is used to make pulp in paper industry (Ali *et al*., 2012) while its timber makes good furniture. Leaves and pods are used as fodder for animals

and production of biogas (Ali *et al*., 2012). This plant has common names from various tribes and subtribes in Kenya, Uganda, and Sudan. For instance in Kenya: Kipsigis (chebitet), Masai (chebiwa),Tugen (chebiwo), Turkana (ekapelimen), Samburu (ikoloriti), Rendille (illgiti), Luhya (inyanya), Pokot (kopka), Swahili (mgunga), Kamba (muthi), Giriama (muhegakululu), Somali (twerr); Sudan: Lutoho (asit); Tanzania: Mbulu (bariomot), Sukuma (dubilo), Masai (elarai); Uganda: Suk (kapka). (http://www.Acacia local names/index.html).

Plate 1.: (a) A mature whole plant and (b) stemof Acacia nilotica (subalata) in its natural habitat; source (Photo courtesy of George Kisoi at Kolowa in Baringo County, November; 2014).

Health benefits of *Acacia nilotica*

Banso A (2009) studied the antimicrobial activity of ethanolic extracts of the stem bark against *Streptococcus viridans*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Shigella sonnei* using the agar diffusion method and found the minimum inhibitory concentration of the stem bark extract of the plant ranged between 35 and 50 mg/ml. *Acacia nilotica* is also known to contain active ingredient sused for treatment of human diseases including cancer, diabetes, diarrhea, and headache,(Kalaivani & Methew, 2010). Its bark is used extensively as anti-diarrhoea, dysentery and bleeding piles (Del, 2009).The stem bark powder have been used against tumors of ear, eye and testicles, (Kalaivani & Methew, 2010).Methanol extract from the bark has been reported to be anti-oxidant, (Agrawal *et al.*,2010).

Acacia nilotica has been used for the treatment of infectious bacteria including *Escherichia coli* (Rajvaidhya *et al*., 2012). It has also showed antifungal activity of methanolic extracts and aqueous extract of *A. nilotica* with percentage inhibition ranging from 34.27± 1.45 to 93.35±1.99. Methanol extracts of *Acacia nilotica*, showed highest antibacterial activity *against B. subtilis.* and *Staphylococcus aureus* with inhibition zone 15±0.66mm, (Mahesh B. and Satish S. (2008))

Coffea arabica

Taxonomy and Ecology of *Coffea arabica*

Coffee belongs to kingdom *plantae*, division *tracheophyta*, class *dicotyledonae*, order *gentianales*, family of *Rubicae*, genus *Coffea* and species *arabica* (Farah, 2012). It is grown in more than 50 countries in three continents (Asia, Australia and Africa).In Africa it is grown in countries such as Kenya, Ethiopia, Rwanda and Uganda. There are seventy types of coffee varieties but only two are of economic importance namely Arabica and Robusta. In Kenya, coffee arabica is grown in the highlands such as Kiambu, Nyeri, Limuru, Muranga, Meru whereas Robusta coffee grows in low lying regions such as Baringo (Mureithi, 2008).*Coffea arabica*has many varieties which include Ruiri 11, SL34, 38, Batian 27 and 28 (Mureithi,

2008).The pictures of *Coffea arabica* varieties; Ruiru 11 (a) and Batian 27 (b) are shown in plate 2.

Plate .2: (a) Ruiru 11 and (b) Batian 27 with mature berries respectively. Source (Photo courtesy of George Kisoi at CRF in Kiambu County, November; 2014).

Coffee is a plant which grows in areas with temperatures ranging of between 15-24°C and 24-30°C for Arabica and Robusta respectively. The plant needs an annual rainfall of between 1500-3000 mm and thrives well in volcanic red earth soil (Mureithi, 2008). The pattern for rainy and dry periods is very important for growth, budding and flowering of coffee plants. The chemical compounds found in coffee are used by the plant as a natural defense against herbivores because it produces toxic substance that protects the plant berries (Mureithi, 2008).

Economic importance of *C. arabica*

Coffee is an important beverage with over 2.25 billion cups consumed daily (Farah, 2012). Unfortunately, consumption takes place in developed countries whereas its production (90%) takes place in developing countries (Farah, 2012). It is the second most valuable commodity in the world after petroleum with 25 million farmers in 50 countries cultivating coffee (Dimins *et al.*, 2011). There are over 70 varieties of coffee but only two are of major economic value. These are Arabica and Robusta where the former accounts for almost 75% and Robusta accounts for the rest of the world's consumption (Dimins *et al.*, 2011). Coffee earns Kenya foreign exchange,

creates employment, improved standard of living and it has led to development of roads and schools (Mureithi, 2008). .

Health benefits of *C. arabica*

Caffeine is among the phytochemicals found in coffee which has been shown to possess health benefits (Durak & Dziki, 2014). Coffee consumption lowers the risk of Type Two diabetes, liver disease and cancer (Kang *et al*., 2011). *Coffea arabica* is rich in alkaloids, flavonoids and amino acids (Daruk & Dziki, 2014). The content of phytochemicals in coffee varies depending on species, climate, soil composition, season, age, zone, agricultural practice and maturity (Wanyika *et al.*, 2010).

Studies suggest that low to moderate intake of caffeine leads to increase in alertness, heartbeat, learning capacity and decrease in fatigue leading to clearer flow of thoughts, whether in tablet form or not (Wanyika *et al.*, 2010). High to acute caffeine consumption is associated with high blood cholesterol and coronary, urinary excretion of calcium (Wanyika *et al.*, 2010). During lactation, it may cause irritability and wakefulness in a breastfed baby. Other studies suggests that long term consumption of caffeine leads to most of these acute effects to disappear. This is because of metabolic adaptations in the body and other compounds that can counteract these acute effects of high consumption of caffeine. It stimulates the central nervous system as an adenosine receptor antagonist (Wanyika *et al.*, 2010)

According to Jansen (2006), Arabica coffee produce 100% world's best coffee thus it is more expensive than Robusta coffee beans. To achieve specific and desirable aroma the two types of coffee are blended in different proportions to produce a wide variety of taste and aroma of coffee beverage (Jansen, 2006).

Ribulose-1, 5-Bisphosphate Carboxylase (*rbcL*) gene for phylogenetic analysis

Ribulose-1, 5-Bisphosphate Carboxylase (*rbcL*) gene is among other conserved genes such as maturase kinase (matK) found in chloroplast genome used in phylogenetic tree construction and determination of phylogenetic relatedness. Because of its complexity and repetitive properties, nuclear genome is used in systematic botany less frequently. The mitochondrial genome is used at the species level due to its rapid changes in its structure, size, configuration, and gene order.

On the other hand, chloroplast genome is well suited for evolutionary and phylogenetic studies. It is a relatively abundant component of plant total DNA.
Secondly, contains primarily single copy genes. Thirdly, extensive background for molecular information on the chloroplast genome is available (Doebley *et al.,* 1990).

Ribulose-1, 5-bisphosphate carboxylase *(rbcL)* was the first gene that was sequenced from plants (Zabta *et al.,*2014).It is a single copy gene approximately 1430 base pairs in length, free from length mutations except at the far 3' end, and has a fairly conservative rate of evolution. It encodes the large subunit of rubilose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO). This enzyme Ribulose-1, 5-Bisphosphate Carboxylase (Rubisco) is responsible for fixation of carbon dioxide in the Calvin Cycle, Michael (1993). In green algae and land plants, the genetic information for the small subunit is encoded in the nuclear genome, typically in a small multigene family (Meagher *et al.,* 1989 & Palmer, 1991).

The ribulose-1, 5-bisphosphate carboxylase *(rbcL)* contains high substitution rates within the species and is emerging as potential candidate to study plant systematics and evolution (Sathishkumar *et al.,* 2008). It has long been evident that molecular sequences contain useful information about evolutionary history. The ribulose-1, 5-bisphosphate carboxylase *(rbcL)* gene has ideal size, high rate of substitution, large proportion of variation at nucleic acid and protein level at first and second codon position, low transition/transversion ratio and the presence of mutationaly conserved sectors. These features of *rbcL* gene are exploited to resolve genus and species level relationships. Polymorphism of chloroplast DNA especially ribulose-1, 5-bisphosphate carboxylase*rbcL* has been used to study the phylogeny of various plants (Sathishkumar *et al.,* 2008). The sequence data of the ribulose-1, 5-bisphosphate carboxylase *(rbcL)* gene are widely used in the reconstruction of phylogenies throughout the seed plants and flowering plants and more than 10,000 ribulose-1, 5-bisphosphate carboxylase *(rbcL)* gene sequences are already available in GenBank (Newmaster *et al.,* 2006 and Chase *et al.,* 2007).

Comment [R10]: Literature and reviews is not needed here

Methodology

Comment [R11]: Materials and methods, sequence after introduction

Data collection

Comment [R12]: Write some details of region where study conducted

The entire coding region of *rbcL* sequences of 13 different species belonging to both generic Acacia and Coffee and outgroup information were obtained from the taxonomy database of the National Centre for Biotechnology Information (NCBI), www.ncbi.nlm.nih.gov/GenBank.

Sequence analysis

The data analysis was done for the plant species *Acacia nilotica* and *Coffea arabica* for which their sequences are available in Genbank to find the interspecies variation. Multiple sequence alignment was performed by using MUSCLE, which is offline software that performs optimum alignment for sequence. Alignments were not complicated due to the occurrence of indels and were not included in data analysis, Thompson (1994). Aligned sequences were edited by using the software JALVIEW.

Comment [R13]: See comment 3 and 4

Phylogenetic analysis using (Maximum Likelihood estimation and Neighbor-Joining)

The basic sequence statistics including amino acid frequencies, transition/transversion (ns/nv) ratio, and variability in different regions of sequences were computed by Molecular Evolutionary Genetics Analysis (MEGA), (Kumar S and Tamura, 2004). The sequence data were analyzed by Maximum Likelihood Estimation (MLE) (Felsenstein, 1985) by using MEGA. Distances were calculated using the Neighbour-join method. Bootstrap analysis was performed by NJ plot. Various clades were determined by MEGA.

Results

Comment [R14]: Write the results in details with proper tables and diagrammatic representation

A.lebbeck	MFTSIVGNVFGFKALRALRLEDLRIPPSYSKTFQGPPHGIQVERD
A. n. ssp. Hemispherica	MFTSIVGNVFGFKALRALRLEDLRIPPSYSKTFQGPPHGIQVERD
A. karoo	MFTSIVGNVFGFKALRALRLEDLRIPPSYSKTFQGPPHGIQVERD
A. nilotica	MFTSIVGNVFGFKALRALRLEDLRIPPSYSKTFQGPPHGIQVERD
M. araujei	MFTSIVGNVFGFKALRALRLEDLRIPPAYSKTFQGPPHGIQVERD

C. arabica
MFTSIVGNVFGFKALRALRLEDLRVPPAYIKTFQGPPHGIQVERD
T. cacao
MFTSIVGNVFGFKALRALRLEDLRIPPAYSKTFQGPPHGIQVERD
P. sinensis
MFTSIVGNVFGFKALRALRLEDLRIPPAYSKTFQGPPHGIQVERD
F. indica
MFTSIVGNVFGFKALRALRLEDLRIPPAYSKTFQGPPHGIQVERD
C. salvatrix MFTSIVGNVFGFKALRALRLEDLRVPPAYIKTFQGPPHGIQVERD
C. racemosa MFTSIVGNVFGFKALRALRLEDLRVPPAYIKTFQGPPHGIQVERD
L. longifolia MFTSIVGNVFGFKALRALRLEDLRIPPAYSKTFQGPPHGIQVERD
T. bacterium VFTSLVGNVFGFKAVRSLRLEDVRFPIAYVMTCNGPPHGIQVERD

Figure 1. *An example of an alignment of part of the chloroplast -encoded Rubisco, large subunit of ribulose-1,5-bisphosphate carboxylase (rbcL) alignment in the MEGA format.*

Comment [R15]: Give the detailsls in systematic tabular form

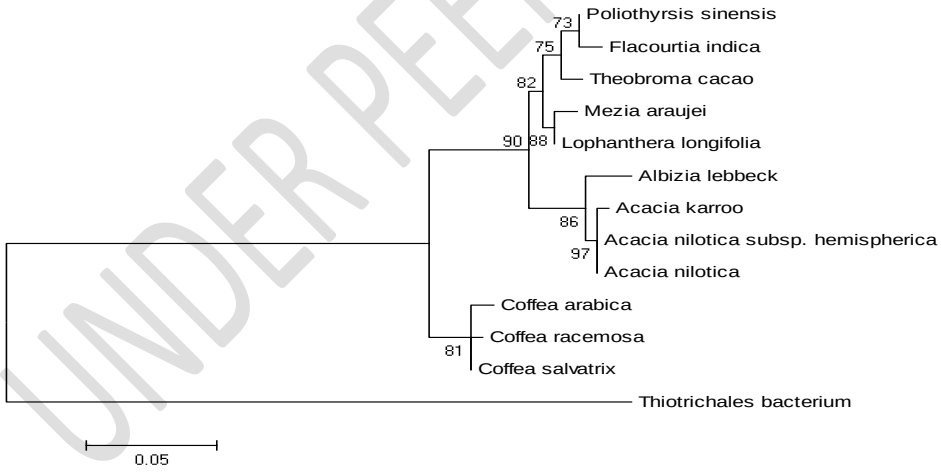

Figure 2. *NJ tree of genetic distance for Acacia nilotica and Coffea arabica based on rbcL. The numbers above branches correspond to bootstrap support. Thiotrichales bacterium taken as an outgroup is the sister taxa of Coffea*

Comment [R16]: Give proper descriptions on given figure

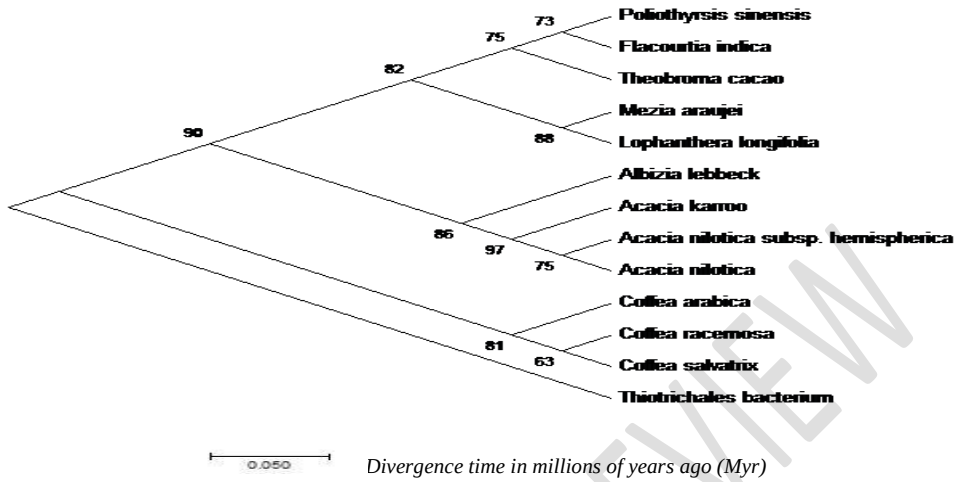

Figure 3. *ML tree rooted on* Thiotrichales bacterium. *Bootstrap support values depicted on the maximum likelihood tree*

Comment [R17]: No description given in results

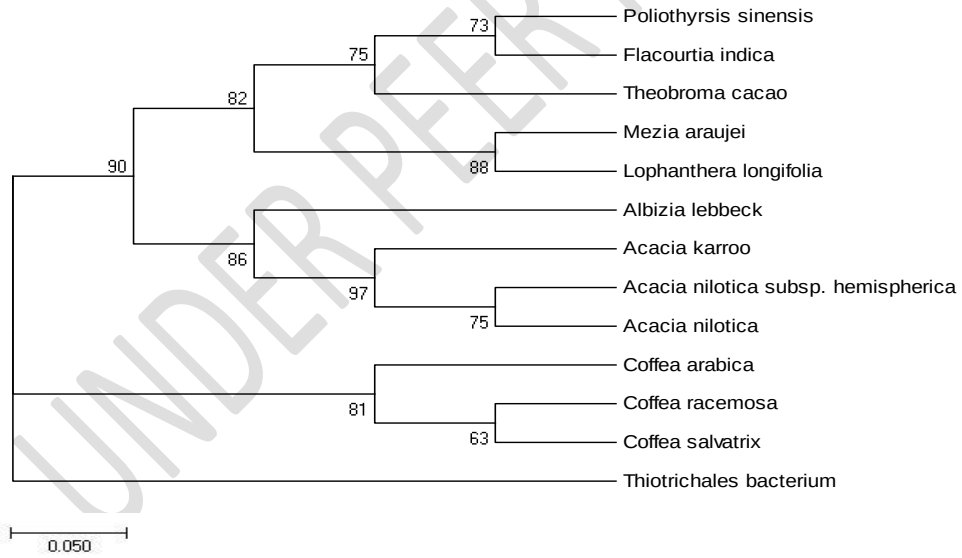

Figure 4*. The Maximum Likelihood tree shows the relationship between Acacia nilotica and Coffea arabica with the related taxa and Thiotrichales bacterium as an outgroup. The percentage of replicate trees, in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown (>50%) next to the branches. The scale bar represents the branch length measurement in the number of substitutions per site that is genetic change.*

Comment [R18]: No description

Discussion

Taxonomy

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The morphological description and geographic distribution of the genus *Acacia* is well documented by (Rajvaidhya *et al*., 2012). There are over 1380 acacia species worldwide that have bark as the outermost layer on the stem. In Africa, there are about 130 species (Rajvaidhya *et al*., 2012). The Brenan taxonomy of *Acacia nilotica* subspecies subalata is as follows; domain (Eukaryota), kingdom (Plantae), phylum (Spermatophyta), subphylum (Angiospermae), class (Dicotyledonae), order (fabace), family (Fabaceae), subfamily (Mimosoideae).

Comment [R20]: Discussion start with proper description of findings and then quote supportive literatures and reasonings

According to Brenan (1983), *Acacia nilotica* is categorized into 9 subspecies where 3 are in India and the rest are spread across Africa. They are distinguished by their size, shape, and degree of pubescence on pods and branchlets. It is a single-stemmed tree that grows between 2.5- 15 meters high with grey to black and brownish bark. It has a very deep and extensive root system.

This *Acacia nilotica* subspecies subalata flowers in April and November and are yellow-like in color. They have very long pods which are green but when it ripens, it turns grey with seeds inside. The barks are smooth when the plant grows but gets scaly as it matures. The leaves are densely hairy and are compound pinnate with leaflets that are narrow with parallel margins. The tips of these leaflets are rounded (Godghate *et al.*, 2014).

Phylogeny (Maximum Likelihood, Maximum Parsimony, and Neighbor-Joining)

In this study, the protein sequence of the chloroplast gene *rbcL* of *Acacia nilotica* and *Coffea arabica* was assessed to know the generic and interspecific differences. The entire coding region of *rbcL* sequences of *Acacia nilotica* and *Coffea arabica* were retrieved from GenBank and the BLAST search showed 95% sequence similarities with multiple plant species. In this process, the sequence is assigned on the basis of its similarity to a set of reference (identified) sequences (Ross *et al.*, 2008). The related sequences were retrieved from the GenBank database to determine the phylogenetic analysis of the studied specimen. Multiple sequence alignment done using Clustal W which is in MEGA software shows that there are variable numbers of Indels in the gene *rbcL*. Tree analyses were conducted using maximum likelihood and neighbor-joining methods. The studied specimens were phylogenetically related with *Coffea racemosa*,

Coffea salvatrix, Albizia lebbeck, and Acacia nilotica subsp. Hemispherica and *Acacia karroo* (Fig. 2; supplementary Figs. 3 and 4).

All the trees that were inferred from the partial rbcL gene sequence of both *Acacia nilotica* and *Coffea arabica* and related taxa demonstrated a distinct lineage of the studied specimen; thus, could distinguish the species of *A. nilotica* and *C. arabica* and show their relatedness as they share a common ancestor. The sequences generated from *rbcL* also indicated that *Acacia nilotica* and *coffea arabica* are monophyletic. The evolutionary analysis on the basis of *rbcL* proved that *Acacia nilotica ssp. Subalata* and *Acacia nilotica* ssp. *hemispherica* are closely related as they form the sister groups. Figure 3. Figure 1. Shows a section of *rbcL* sequences alignment of part of the chloroplast -encoded Rubisco, the large subunit of ribulose-1, 5-bisphosphate carboxylase (*rbcL*) of 13 different species where the majority of the sequences were conserved.

Conclusion

The presence of *rbcL* sequences in the databases for different plants has aided in determining the phylogenetic relationship of plants. Nowadays phylogenetic analysis not only does it complements and often outperforms similarity searches and transition/transversion rate in protein sequence when dealing with sequence identity. Molecular Evolutionary Genetics Analysis (MEGA) software provided a framework for qualified identification of protein sequences of *Acacia nilotica* and *Coffea arabica* is provided with the interspecies relationship. The tree analysis shows that *Acacia nilotica* and *Coffea arabica* are monophyletic as they share a common ancestor through distantly related. *Acacia nilotica* has higher bootstrap values than *Coffea arabica* making the evolutionary sense between the two genuses. Thus, from this study, it can be suggested that the *rbcL gene* is good for phylogenetic analysis of the protein sequence of *Acacia nilotica* and *Coffea Arabica* members and other species.

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Comment [R21]: Arrange the references as per the journal format

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Appendix I. Accession numbers of sequences retrieved from GenBank.

rbcL	
KC417042.1	AJ402991.1
KC417043.1	AM235003.1
KC417041.1	X81095.5
GQ981898,1	HQ247539.1
KR529342.1	JX572420
AF344502.1	JX572421
KP793075.1	