MORPHOLOGIC AND MOLECULAR CHARACTERISATION OF *Ricinodendron heudelotii* (Baill.) Pierre ex Pax IN SOUTHERN NIGERIA

BY

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CERTIFICATION

I certify that this work was carried out by Alfred O. ONEFELI under my supervision in the Department of Forest Production and Products, Faculty of Renewable Natural Resources, University of Ibadan.

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DEDICATION

This work is dedicated to the Yahweh Nissi, who protected me throughout this study as well as my wife Mrs Oluchukwu Joy ONEFELI and my daughter Esther Ifoma ONEFELI for their love, care, patience and understanding.

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ABSTRACT

Ricinodendron heudelotii is an ethnomedicinally important indigenous tree species found in wild populations in Nigeria, but its utilisation is limited by insufficient taxonomic information. Morphologic and genetic characterisations provide detailed taxonomic description for effective identification of indigenous tree species. However, such information is scarce with respect to *Ricinodendron heudelotii* in Southern Nigeria. Therefore, morphology and molecular characteristics of leaf and fruit of *Ricinodendron heudelotii* trees in Southern Nigeria were investigated.

Wild *Ricinodendron heudelotii* trees were purposively selected from Oyo [Ibadan (n=1) and Onigambari (n=5)], Ondo [Oloruntele (n=12) and Akure (n=4)], Osun [Osu (n=8), Ikoyi (n=4) and Ile-Ife (n=10)], Edo [Benin (n=4)] and Cross River [Boki (n=2)] States, based on availability. Fifty random samples of uniformly sized leaves, from each location, were assessed for Leaf Length (LL, cm), Petiole Length (PL, cm), epidermal cell shape, Guard Cell Area (GCA, µm2), Pore Size (PS, µm2), Stomata Length (SL, µm) and epidermal cell length (ECL, µm) following standard methods. For fruit and seed morphology, 50 matured fruits were randomly collected from each location and used to determine Fruit Length (FL, mm), Pulp Weight (PW, g), Fruit Largest Width (FLW, mm), Fruit Roundness Ratio (FRR), seed length (mm) and Seed Diameter (SD, mm2) using standard procedures. Six leaves from each location were subjected to molecular characterisation using 19 Inter Simple Sequence Repeat (ISSR) markers following standard methods. Polymorphic Information Content (PIC), genetic diversity, similarity index, and unique allele were determined. Data were analysed using descriptive statistics, Principal Component Analysis (PCA), Cluster Analysis and ANOVA at α0.05.

Leaf length significantly increased from 22.3±5.7 (Osu) to 53.0±5.8 (Onigambari), while PL varied from 8.9±0.1 (Boki) to 30.9±5.0 (Onigambari). Epidermal cells were polygonal in all sites, except Akure with irregular shape. Highest GCA (243.1±30.5), PS (322.8±78.5), SL (29.4±2.4) and ECL (43.7±8.8) were in Akure, while the least were in Ikoyi (72.7±7.0), Onigambari (40.3±8.0), Ikoyi (20.4±3.6) and Ibadan (19.2±8.7), respectively. Boki had highest (45.4±2.6) FL, while Ile-Ife had least (30.2±11.5). The PW and FLW were highest at Oloruntele (34.3±7.2; 44.2±4.0) and least at Akure (18.4±3.3; 31.2±1.3). The FRR and seed length varied from Oloruntele (14.4±1.7; 0.77±0.3) to Akure (17.1±0.7; 1.31±0.11), while SD ranged from 12.9±0.9 (Akure) to 16.3±0.6 (Ile-Ife). The GCA (0.48), PS (0.57) and SL (0.39) had highest contribution to the 76.8% total variance in leaf and fruit morphometrics. The ISSR marker-840 had highest PIC (0.42), while ISSR marker-848 had the least (0.21). Genetic diversity increased from Akure (0.09) to Oloruntele (0.24). Highest genetic similarity (60.3%) was between Ibadan and Osu, while the least (1.0%) was between Akure and Benin. Three unique allele (1600-2000bp) were identified in Oloruntele. The population of *Ricinodendron heudelotii* clustered into four groups; Akure, Benin and Oloruntele were distinct, while others formed a group.

Leaf-based characters showed distinct taxonomic differences across populations of *Ricinodendron heudelotii* in Southern Nigeria. The most genetically diverse population was found in Oloruntele, which indicates potential germplasm for domestication of the species. The unique alleles identified could be used for marker assisted identification of the population.

Keywords: *Ricinodendron heudelotii*, Morphological taxonomy, Epidermal characterisation, Molecular delimitation, Inter Simple Sequence Repeat markers

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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
FHI	Forest Herbarium Ibadan
ANOVA	Analysis of Variance
CA	Cluster Analysis
CRD	Completely Randomized Design
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic Acid
GPS	Global Positioning System
ISSR	Inter simple sequence repeat
OTU	Operational Taxonomic Unit
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PIC	Polymorphic Information Content
RAPD	Random amplified polymorphic DNA
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
STE	Sodium Chloride-Tris-EDTA
TAE	Tris base, acetic acid and EDTA
TETFund	Tertiary Education Trust Fund
THSD	Tukey Honestly Significance Difference
USDA	United State Department of Agriculture

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

The highly diverse Nigerian forest ecosystem is diminishing steadily over time in complexity as well as germplasm population due to uncontrolled exploitation. Nigeria has been ranked number one among the ten countries of the world with the highest deforestation rate (Ladipo, 2010; Olagunju, 2015). According to Ladipo (2010), the deforestation rate in the country is about 3.5% per year, which translates to a loss of forest land of about 350,000–400,000 ha per year. As a result of this loss, only 2.5% of the forest land is left (Ladipo, 2010) and this is far below 25% of the total land area originally recommended in the nations forest policy (Lowe, 1984). This remnant forest area is dominated by coppices resulting from human forces such as agricultural development, urbanisation, fuelwood generation and indiscriminate forest harvesting. These problems have undoubtedly aggravated the current food insecurity experienced in the country. Regrettably, most of the germplasm of edible seed-producing and medicinal indigenous tree species such as *Ricinodendron heudelotii* (Baill.) Pierre ex Pax is a victim of these human forces without any conservation consideration. According to Lawal *et al.* (2016), one of the ways to enhance the conservation of the indigenous fruit trees in Nigeria is through taxonomic study. However, a detailed taxonomic description that would enhance the effective identification of any plant species could be achieved with characterisation at both the morphologic and molecular level.

Ricinodendron heudelotii is an indigenous tree species, which belongs to the family Euphorbiaceae. In southern Nigeria, the species has been used widely most especially by rural dwellers for livelihood. Almost every section of the tree is used for some reason or another. Food, medicine, and culture are the three most important uses of *R. heudelotii*. The tree produces seeds of high nutritional values (Boko-Haya *et al.,* 2021). Due to the importance attached to the value of its seed in various part of Africa, the tree received the

common names groundnut tree and African nut tree. In Cote d'Ivoire for instance, the kernel extracted from the seed is usually consumed after cooking by boiling it in water (Diomande *et al.,* 2019). In some cases, the kernel is prepared into a sauce in tandem with vegetables. According to Olasehinde *et al.* (2016), the species' kernel is one of the main flavouring agents that rural dwellers cannot live without in their traditional dishes. This is because it contributes to a well-balanced diet.

Some agroforestry qualities have been established for this tree species, in addition to the three major benefits listed. In southwestern Nigeria, for example, it has been observed that the trees are retained deliberately in agricultural lands such as *Theobroma cacao* farms. During the dry season in Nigeria, the leaves have been found as good quality fodder for sheep and goats. In its rhizosphere, arbuscular mycorrhiza has also been found (Musoko *et al*., 1994). Based on the fact that the wood from the species dries very fast and burns very well, it is popularly used as fuelwood in the southern part of Nigeria (Odinga and Nwaokezi, 2020). The wood of the species is also a very good source of material in the production of drums in southern Nigeria.

Its ethnomedicinal significance cannot be understated. A decoction made from various parts of the plant has been used to treat a variety of diseases. Fever, malaria, anaemia, stomach pain, headache, toothache, and quick child delivery are all treated with extracts from the leaves (Tchoundjeu and Atangana, 2006). In terms of socio-culture, Ibos in Nigeria use the seeds for the "Okwe" game, while the wood is historically used to make drums (Fondoun *et al*., 1999; Tchoundjeu and Atangana, 2006).

Given the benefits derived from *R. heudelotii*, it is important to characterise the species to provide knowledge that could help the species' future survival and food security.

1.2 Statement of the problem

Over the years, some technical issues such as proper identification of tree species, seed viability and availability have been discovered to hinder the domestication and ex situ conservation of multipurpose indigenous tree species particularly *R. heudelotii* from their wild type (Lawal *et al*., 2016). As a result of inadequate information on distinctive morphological tree characters, germplasm collection has been very difficult, consequently, domestication becomes unattainable. Species morphological diversity is very significant to

the survival of trees and the sustainability of the forest ecosystem. Without a rich pool of genes, most species would become endangered due to the lack of adaptability to changing environments (Loewe and Hill, 2010). There is scarce recent information on the morphological variation in *R. heudelotii*. However, morphological variation brings about difficulty encountered in the identification of most indigenous trees both in space and in time. Hence, this variability could be a good character to delimit the genus to a smaller taxon.

Molecular delimitation of the population of *Ricinodendron heudelotii* is very significant to its genetic distinction for conservation purposes. Achieving a natural and more reliable classification of any tree species, however, requires an integrative application of molecular data to complement morphological taxonomic characters. Unfortunately, there is presently no recent published information on the genetic differentiation of the population of the study species.

There is limited information on the effective germination of *Ricinodendron heudelotii* in Nigeria. However, the germination potential of this species has been well established in some other African Countries such as Cameroon (Anjah *et al.,* 2013), the Republic of Benin (Boko-Haya *et al.,* 2021), etc. According to Boko-Haya *et al.* (2021), the geographical location of the seed sources plays a significant role in determining the germinability of the seeds of *R. heudelotii*. There is, therefore, a need for urgent information on germination potentials of Nigerian *Ricinodendron* for domestication purposes. On many occasions, seed germination tends to vary due to differences in dormancy level as influenced by the pre-germination treatments applied. A viable germination analysis is needed to identify the pre-germination treatment with a high germination percentage. In terms of efficient domestication and conservation of agroforestry tree species, the growth rate is also very important. In forestry, however, identifying tree species with a significant quick growth rate remains a challenge. This is due to the fact that most indigenous tree growth experiments end up in the nursery, so the field's early growth potential is unknown. This has unquestionably hampered the use of indigenous plants for afforestation, reforestation, and agroforestry.

Tropical soils, such as those in Nigeria, are nutrient-deficient (Oludoye and Ogunyebi, 2017). This has resulted in habitat degradation and loss of biological diversity. Poor-nutrient soils have exacerbated the low yields of many arable crops, such as maize, which are grown solely in an open field without the benefit of tree cover. As a result, the country continues to struggle with food insecurity.

1.3 Objectives of the study

The main objective of this study is to investigate the taxonomy and alley cropping potential of *Ricinodendron heudelotii* with a view to contributing some information to the taxonomic revision and optimise the utilisation of the species.

The specific objectives are to;

i. assess the pattern of leaf epidermal and macro morphological variation of the species;

ii. examine the levels of genetic differentiation among the populations of *R. heudelotii*

iii. determine appropriate seed germination protocol for the species;

iv. assess the early growth of the species and its effect on soil nutrients, growth and yield of maize.

1.4 Justification of the study

The problem of tree identification is a challenge in Nigerian forest resources management. As a result, various forestry activities on indigenous trees such as nursery operation, plantation establishment, forest inventory, etc. are difficult to execute. However, previous studies have related this identification issue to the unsteady morphological differences possessed by the indigenous species (Tchoundjeu and Atangana, 2006). This has directly or indirectly hindered the right utilisation and conservation of some of the tree species. This is because tree species are not fixed or unalterable. They evolve and adapt in response to the environment in which they live. Since environmental conditions are also dynamic and constantly changing both spatially and temporally, the processes of evolution and adaptation for tree species are never complete. Morphometric analysis is commonly used in resolving contentions of taxonomic rank in plant species. Specifically, morphometric studies are useful tools to understand which morphological characters vary between related taxa and to describe the characters used in the delimitation of threatened

tree species. Due to the nomenclatural contentions of *Ricinodendron* occasioned by its morphological diversity, different specific names such as *Ricinodendron heudelotii*, *R. africanum*, R. *gracilis* subsp. *Heudelotii*, *R. schliebenii*, *R. tomentellum*, *Jatropha heudelotii* and *J. Mahafalensis* have been ascribed by Taxonomists (Tchoundjeu and Atangana, 2006). However, a detailed taxonomic study has not been done on Nigerian *Ricinodendron* to determine its present specific diversity. Collecting morphological and other taxonomic information about this species will not only contribute to the amelioration of the problem in its identification and classification but will serve as a guide for other indigenous tree species taxonomy. Therefore, it is pertinent to carry out the characterisation of *Ricinodendron* Mull. Arg.

One of the factors contributing to the difficulties of maintaining remnant forests and restoring deforested areas is the lack of knowledge on the propagation and growth rate of the indigenous tree species (Onefeli and Adesoye, 2014). Scientific information on the artificial regeneration of tropical trees is very limited. Such knowledge would enhance conservation of the threatened species through their inclusion in enrichment planting, agroforestry and other planting programmes thus, facilitating both in-situ and ex-situ conservation of threatened species.

In light of Nigeria's decreased agricultural production potential due to land degradation, introducing *R. heudelotii* into agricultural cropland such as maize would be one way to improve food security for local farmers as well as the entire population. If well planned and implemented by local farmers, it has the potential to diversify production output, which would otherwise be limited to a single crop under current agricultural management practices. Furthermore, it will likely lead to poverty reduction on both a local and national level.

1.5 Scope of the study

This study covered nine populations (Osu, Onigambari, Oloruntele, Ile-Ife, Ibadan, Akure, Benin, Ikoyi and Boki) of *Ricinodendron heudelotii* in Southern Nigeria. The taxonomic study focused on epidermal morphology, macro-morphometric and molecular characteristics of the species. The agroforestry aspect utilised alley cropping treatments using two alley widths with the incorporation of maize into tree component.

CHAPTER TWO

LITERATURE REVIEW

2.1 The family Euphorbiaceae and its taxonomic documentation

Euphorbiaceae, otherwise known as the spurge family is a very large family of angiosperms. It is commonly known as euphorbias, which is equally the name of the type genus of the group. The family is well represented basically in all life forms; herb, climber, shrub and tree (Mwine and Van-Damme, 2011).

A good example of the herbaceous member of the taxon is *Euphorbia hirta*, which is popularly used in the treatment of Asthma in Nigeria and some other countries in Africa. *Alchornea cordifolia* is one of the shrub members of the family while *Plukenetia conophora*, which is commonly known as Nigerian Walnut belongs to the climber category. Economically important species such as *Ricinodendron heudelotii, Hevea brasiliensis* and *Jatropha curcas* are some of the representatives of trees in the family, which many African countries cannot do without.

The family Euphorbiaceae is taxonomically unstable due to the inconsistency in the description and classification of the members of the taxon. Many times, various subgroups are being moved from one hierarchical level to another. There are also cases of synonymy, which have undoubtedly brought about taxonomic variableness. Hence, there is rarely agreement in the numbers of the entity in each hierarchical level reported by different authors.

According to Mwine and Van-Damme (2011), Euphorbiaceae is a genetically diverse family of the plant which comprises eight thousand, nine hundred and ten (8,910) species distributed into three hundred and twenty-two (322) genera. Some years later, it was documented that the family comprised of seven thousand, five hundred (7,500) species arranged into three hundred (300) genera (Gillespie and Armbruster, 2018; The Plant List, 2017). On the contrary, Gupta (2021) reported that there are seven thousand three hundred

(7,300) species and two hundred and eighty-three (783) genera in the family. Also, it was recently discovered by Kew (2021) that the family consists of two hundred and twenty-seven (227) genera. All the highlighted fluctuations in the taxonomy of the family is a function of the resultant effect of the cumulative diversity existing in the various populations, species and genera.

Members of the family Euphorbiaceae popularly occur in three tropical regions; Africa, tropical America and Indo-Malayan region. However, species in the group are more concentrated and less diverse in the last two tropical regions than in Africa. In other nontropical areas such as the Southern United States, Mediterranean Basin, South Africa and the Middle East, species of Euphorbiaceae are also represented in a good number. Many members of the family survive in hot desert conditions while others are rainforest herbs and trees (Mwine and Van-Damme, 2011). This implies that Euphorbiaceae is a complex taxon with much research potential. According to Sharmin (2017)**,** the variability in genetics and morphology, as well as the complexity in the range of habitat, has made the classification of Euphorbiaceae difficult and therefore requires much attention for delimitation to the populational level. Many angiosperm families have peculiar characteristics that allow the members to be grouped as a taxon. Such characteristics are cut across every member of each family. For instance, possession of a pod is a unique character for members of Leguminosae. Members of Poaceae are also uniquely characterised by parallel venation but in the case of Euphorbiaceae, it is very difficult to find observable trait can be used for the classification of the members.

Despite all the existing taxonomic studies on Euphorbiaceae, Webster (1994) lamented that there is no single feature that can be used to delimit all members of the family. Rather, he stated the anatomical features such as the stomata, trichomes and some wood structures are significant for the characterisation of the family. He further noted that lower taxonomic groups like subfamily, tribe and genus in the family can be delimited using the combination of inflorescence types, nuclear number of pollen, type of pollination and exine of pollen.

The taxonomic work on Euphorbiaceae is a long-standing history. The first taxonomic study on the family Euphorbiaceae was undertaken by Adrien Jussieu in 1824 (Webster,

1994). He identified the genera in the family while Jean Mueller was the second taxonomist to study the family. The work of Jean Mueller was the first detailed classification that delimited Euphorbiaceae into different sub-tribes, tribes, and sub-families (Webster, 1994). He opined that the classification provided for Euphorbiaceae by Mueller was an excellent breakthrough. He then accorded the achievement of Mueller as the first significant phylogenetic study that withstood the test of time for a long period. Consequently, Mueller's finding was employed as a bedrock for Webster's classification with the use of taxonomic characters like anatomy and pollen morphology.

Webster separated the family into 5 different subfamilies, which included Oldfieldoiideae, Euphorbioideae, Crotonoideae, Phyllanthoideae and Acalyphoideae. Based on the classification, Acalyphoideae, Crotonoideae and Euphorbioideae were characterised by uni-ovulate ovary (Webster, 1994). This implies that the taxa contain one ovule per locule while Phyllanthoideae and Oldfieldoiideae are bi-ovulate. This traditional system of classification was the accepted taxonomic grouping. As this form of classification is common to such a diverse Euphorbiaceae family, there has been much pressure for a detailed reconstruction of the family boundaries (Tokuoka and Tobe, 1995; Wurdack *et al*., 2005). This is necessary to exclude from the group those that are not fitted to the family and as well include the yet to be discovered well-fitted species. Therefore, starting from the population as an operational taxonomic unit is an explicit option to achieve a robust and natural classification for the family.

2.1.1 Description, distribution and Ethnomedicinal importance of the *Ricinodendron heudelotii*

Existing pieces of literature show that *Ricinodendron heudelotii* can grow as tall as 50 m within a few years and as such referred to as a fast-growing species (Tchoundjeu and Atangana, 2006; Onefeli *et al.,* 2019). According to Assanvo *et al.* (2015), the average diameter of its trunk varied from 1.5 m to 2.7 m. The tree is characterised by a straight trunk (Appendix 1), which is usually thicker with short buttresses at the base. At the young stage, the branches are arranged in a whorl, which usually arches upwards (Onefeli *et al.,* 2019). The wood is very soft and is pale yellow or white but becomes darken on exposure (Sut *et al.,* 2019).

The leaves are compound (Appendix 2), which are alternately arranged on the branches while the leaflets are digitate in the arrangement. The apex of each leaflet is usually acuminate or acute. The leaflets are in most cases united at the base in such a way that the lamina is lobed digitately, with lobes ranges from 3 to 6 (Assanvo *et al.,* 2015).

Ricinodendron heudelotii is dioecious and flowers start appearing from April to May every year while fruiting is from September to October (Tchoundjeu and Atangana, 2006). The tree produces indehiscent fruits (Appendix 3), which are green in colour but turn yellow after dropping. According to Fondoun *et al.* (1999), the fruit is mostly spherical with lobes varying from one to three. The colour of the seeds is usually black to reddish-brown (Appendix 4), which consist of a testa with a white or yellow kernel (Appendix 5). Literature (Fondoun *et al.,* 1999; Onefeli *et al.*, 2019) have shown that there are mostly four different types of seed numbers. These include; three seeded fruit having three lobes, two-seeded fruit with two lobes, two-seeded fruit with an aborted lobe and single-seeded fruit with an aborted lobe (Appendix 6).

Ricinodendron heudelotii is endemic to Africa (Sut *et al.,* 2019). It is an indigenous tree in Angola, Benin, Cabinda, Cameroon, Central African Repu, Congo, Equatorial Guinea, Gabon, Ghana, Guinea, Guinea-Bissau, Gulf of Guinea Is., Ivory Coast, Kenya, Liberia, Mozambique, Nigeria, Senegal, Sierra Leone, Sudan, Tanzania, Uganda, Zaire (Figure 2.1). Apart from the distribution in the native countries, the tree has also been successfully introduced as an exotic plant to Zambia and Madagascar (Figure 2.1).

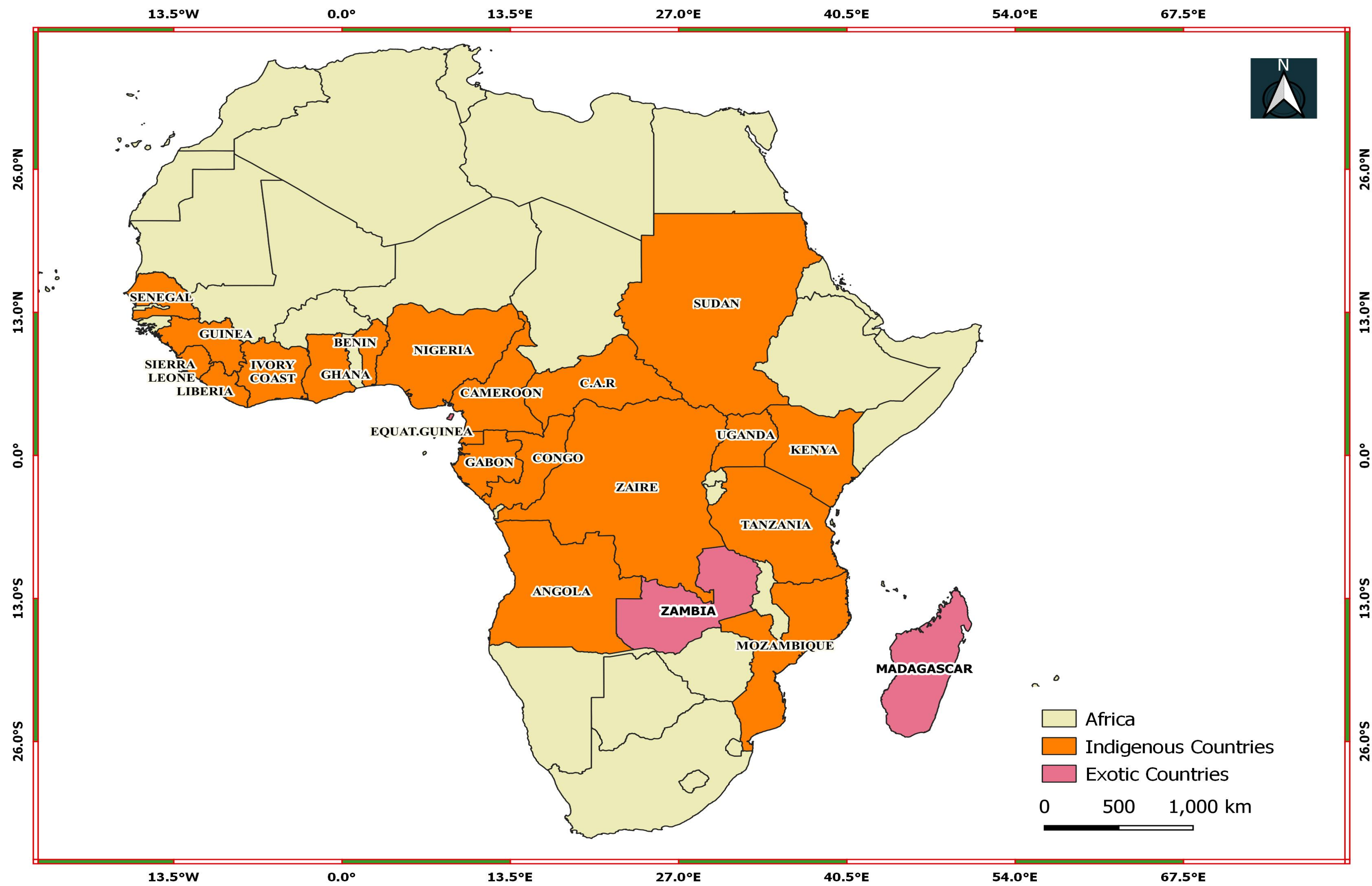


Figure 2.1. Map of Africa showing the Countries of the distribution of *Ricinodendron heudelotii*

Ricinodendron heudelotii is an ethnomedicinally important indigenous tree species found in wild populations in Nigeria. The seed has a unique taste and as such used as a condiment for soup making (Assanvo and Baruah, 2015). Different parts of the plant are being prepared for the treatment of some diseases. According to Tchankou *et al.* (2009), oil prepared from the seed is efficacious for the reduction of cholesterol in the system. Paste prepared from the seed is highly effective as a toothache reliever (Assanvo and Baruah, 2015). Studies have also shown that the bark of the tree is very useful for the treatment of cough, infertility, sexual problems, ulcer, and menstrual pain as well as a booster of breast milk in lactating mothers.

2.2 Plant Taxonomy, Systematics and Biodiversity

According to Biocyclopedia (2021), taxonomy can generally be referred to as the method through which immense diversity of living things existing on the planet are being classified and organized by various scientists and conservationists to discover and understand the affinities between them. Another scientific classificatory approach in which the relationships among organisms are studied, especially with special consideration to higher levels is known as plant systematics. It is closely allied to taxonomy. A special distinction from plant taxonomy, however, is the inclusion of evolutionary relationships. In other words, plant taxonomy plus evolutionary study gives plant systematics. Ayodele (2017), opined that Systematics emphasises more on the knowledge of the wide complexity and diversity of plants, causes of the variability as well as the understanding of the patterns of variation to utilise them for classification, identity determination and nomenclatural activities. This may have been accounted for the reason why Keogh (1995) simply defined taxonomy as the science of biodiversity's documentation.

As much as taxonomy is viewed as the oldest, basic and most embracing of all the biological sciences, it is equally the most controversial and misunderstood science. This is based on the fact that no two individuals are the same, therefore the reason for grouping the diversity of plants in a systematic order for better identification. Biodiversity can be described as the variety and richness of life existing on earth and has been identified to be the most important and complex component of the planet. It will therefore be practically

impossible to have sustained life without biodiversity. Because, biodiversity includes various microorganisms, plants and animals which maintains the sustainability of life.

According to Heywood and Baste (1995), it may equally be said to be the variety, number and variability of living organisms in species within a specific ecosystem. Hence, biodiversity is assessed at three different levels, which are intricately interrelated; genetic, species and ecosystem.

In the real sense, genetic diversity elucidates the intricate genetic information that all individual organisms possessed (i.e. variations among genes) (Prasad, 2014). It usually occurs between and within populations of a species. Species diversity explains the variability that exists between and among different species of organisms, whereas, ecosystem diversity is the diversity between different ecosystems (Siew *et al.,* 2018). Having explained the conglomeration of biodiversity, it is pertinent to note that plants play major roles in the sustenance of biodiversity. This is based on the fact that they are the food producers for other components. There is a wide diversity of habitats that house an array of plant species. This varies from various forest types and ecosystems to swamps and arid areas. Most importantly, there is a link between taxonomy and plant diversity. This is because plant taxonomist attempts to create order from the myriads of the diversity of plants of the world. By so doing, different species of plants are being discovered daily by these expert taxonomists.

According to Ayodele (2017), as gathered from the report of the Royal Botanic Gardens, Kew in its global assessment of flora of the world, there are about 390400 flowering plants minus Hornworts, Mosses, Algae and Liverworts, which are reported to account for about 40,000. Meanwhile, the recent report by Kew (2021) indicated that one million, one hundred and ninety-two thousand (1,192,000) plants species have been discovered and identified up to the species level on a global basis. This number may still be little compared to the total global existing plant species. Hence, with the intensification of taxonomic exploration, many more plants will still be identified. Unfortunately, this effort has been variously jeopardized by the drastic decrease in the available number of existing taxonomic experts globally vis-à-vis the imminent deforestation occurring in many tropical ecosystems of the world.

Scaling this down to the case of Nigeria in terms of plant diversity and taxonomy, it is very evident that there may be a geometric reduction in the number of the described and identified plant species soon. Hence, many plants will never be discovered considering the alarming rate of loss of habitats, species' invasiveness, as well as climate change. The reason is not far fetched; aside from the unavailable taxonomists, few young researchers are not keenly interested in specialising in plant taxonomy due to the tediousness and robustness of the field study (Keogh, 1995). It is therefore discouraging to note that Africa, particularly Nigeria is lagging while very explicit and aggressive taxonomic cum general floristic studies are currently going on in the world especially in America, Europe and Asia.

As far back as 1996, approximately four thousand, six hundred (4,600) plant species were identified by taxonomic experts (Lumpur, 1996). With this report, Nigeria was ranked number eleven in the whole of Africa for diversity. According to the report, about two hundred and five (205) of the total identified species were estimated to be endemic in which Nigeria was ranked the ninth highest with plant endemism among the African countries. The taxonomic works in Nigeria are however poor to the extent that records on the flora today were obtained from the collections undertaken by early collectors and willed to us by colonial masters (Borokini, 2014).

2.3 Significance of Morphological variation in Plant Taxonomy

Morphology is defined as the study of the form and structure of an organism. The measurement of this form and structure is referred to as morphometry. Morphology can be either macro or micro in form. It is macro when it can be viewed and measured without the aid of any structure magnifier such as lens and Microscope. Examples of macro-morphological variables in plants include leaf length, leaf width, fruit length, petiole length, diameter at breast height, crown length, etc. Conversely, micro-morphological variables in plants are those that can be measured with the aid of a structure magnifier such as a microscope. Variables such as those assessed through epidermal characterisation is a true representation of macro-morphology (Petronela and Nevena, 2010; Khan *et al*., 2011a; Khan *et al*., 2011b).

2.4 Macro-morphology and Monotypic Taxonomy

Identification of a population of species with outstanding characteristics is a precursor to efficient conservation efforts. Conservation strategies in many cases utilise the morphological qualities that are essential for rural people who use the species daily (Assogbadjo *et al*., 2006). The conservation strategies based on intraspecific variation observable among populations should, therefore, involve these morphological features. Besides, numerical classification and identification of lots of tree species fly on the wing of the morphological variation and affinities at specific taxon.

Macro-morphology has been reported to be one of the important aspects of numerical taxonomy used significantly in classifying many plants as well as interpreting the results of taxonomic studies. According to Sonibare *et al.* (2004), cluster analysis and principal component analysis (PCA) are the most commonly used techniques for macro-morphological data to develop taxonomic relationships among a group of plants.

Morphometric diversity in baobab (*Adansonia digitata*) populations across different climatic zones of Benin was examined by Assogbadjo *et al.* (2006), significant differences were discovered among the different populations.

Dorji and Yapwattanaphun (2011) studied the morphological diversity of mandarin (*Citrus reticulata*) in the major growing areas of Bhutan and established the fact that leaf and fruit quantitative characters differed significantly among the locations. The finding was however ascribed to the influence of genetic diversity and environmental factors. Conversely, it was further noted that flower characters might not be useful for identifying and delimiting one mandarin from another due to the insignificant difference observed in their qualitative and quantitative characteristics. The traits could, therefore, be applied at a higher taxonomic rank. Sanjeewa *et al.* (2013) morphologically characterised populations of *Terminalia chebula* in Sri Lanka and observed no significant variation in the flower characters of the species while leaf and fruit variables of the species were reported to be significantly variable. This finding corroborates Dorji and Yapwattanaphun (2011). However, the classification resulted in four (4) clusters based on leaf and fruit characters with leaf characters having the highest contribution to the first principal component of the

principal component analysis result. Sanjeewa *et al.* (2013) concluded that it is possible to classify the population of *T. chebula* trees according to their fruit shapes.

Some ethno-varieties of *Vitellaria paradoxa* from Uganda were assessed to determine the patterns of their morphological variation and establish the congruence between morphological variation and folk classification by Gwali *et al.* (2012). Hierarchical cluster analysis using quantitative morphometric data produced three groups without clear aggregation based on ethnographic or geographic separation. It was reported that quantitative morphological data alone does not resolve any discrete forms of *V. paradoxa* that are related to folk classification. However, augmenting the quantitative with qualitative traits according to farmers' perception provided good congruence with folk classification. A similar study was also carried out by Djekota *et al.* (2014) on populations of *V. paradoxa* in India. High variations were pointed out among populations of the species. The dendrograms analyses revealed the existence of four groups instead of the six ethno-varieties described by folk classification with the utilisation of leaf, fruit and nut traits. It is however evident from these facts that morphological attributes are prime to proper identification of a population of trees for conservation.

2.5 Contribution of Plant Epidermal Characteristics to the taxonomic delimitation of plants species, genera and families

The epidermis can be described as a system of cells that varies in structure and function, which constitute the covering of the plant body in the primary state (Evert and Eichhorn, 2006). Variables obtained from leaf epidermal studies include but are not limited to the nature of the epidermal cell membrane on periclinal walls, abaxial and adaxial surface indumentums, adaxial and abaxial trichomes, stomata type, density, length, area and apparatus level (Wang *et al.,* 2015).

Epidermal characters essentially contribute relevant and significant information to various levels of plant taxonomic revision (Ibrahim and Ayodele, 2013; Shokefun *et al.,* 2017). Based on the importance of these characters, several plant species have been successfully moved from one position to another. In other cases, different groups of plants are being merged to form bigger and more natural groups. Hence, the literature contains some documentation, where epidermal data has been widely utilised to settle contentious issues

in plant taxonomy at various taxa (Olowokudejo, 1990; Olowokudejo, 1993; Ugbabe and Ayodele, 2008; Ibrahim and Ayodele, 2013; Talebi *et al.,* 2017; Arogundade and Adedeji, 2019).

Annonaceae being the largest family in Magnoliales was studied through the comparison of the West African taxa of *Annona* using foliar epidermal characteristics (Olowokudejo, 1990). The epidermal characteristics of the species revealed that wax distribution and the appearance of the cell wall (e.g. the prominent ridges of the anticlinal walls in *A. glauca* var. minor), varies among the taxa after a critical examination under the light microscope. For instance, the anticlinal walls in *Annona squamosa* were reported to be prominently sinuated whereas in *A. muricata* and *A. glabra*, they were curved and in *A. glauca*, *A. senegalensis* var. *senegalensis* and *A. glauca*, the anticlinal walls were in a straight position. In terms of the numerical differences in the epidermal variables among the *Annona*, Olowokudejo (1990) stated that stomatal size was largest (33.45 × 21.22 µm) in *A. glabra* and smallest in *A. chrysophylla* (10.66 × 4.63 µm). The average size of abaxial cells according to this author was largest (37.07 µm) in *A. muricata* and smallest (13.53 µm) in *A. squamosa*. On the adaxial surface, the stomatal size of *Annona* varied from 17.7 (*A. squamosa*) to 45.21 µm (*A. glauca* var. minor) (Olowokudejo, 1990). Despite this wide gap in the stomatal sizes of the genus *Annona*, Paracytic was the only type of stomata that visibly identified in all the assessed epidermal peels. According to Folorunso and Olaniyan (2009), such a taxonomic character may be regarded as typical for the genus or even cut across the whole family of Annonaceae. It was emphasised that the most targeted morphological and anatomical traits in the works of systematics and taxonomic identification of plants are those that separate taxa (Folorunso and Olorode, 2006; Ugbabe *et al*., 2014). Such traits are mostly discovered by subjecting groups of characters understudy into some statistical analysis like principal component analysis to identify their corresponding factor loadings. This, therefore, explains one of the significances of epidermal characteristics in plant taxonomy and made the works of taxonomists in revising the traits regularly to catch up with the current and existing taxonomic distances among the various taxa (Ayodele, 2003; Folorunso and Abayomi, 2014).

Aside from the usefulness of epidermal characters in setting boundaries among taxa, they have also been very useful to the survival and spatial distribution of plants. According to

literature (Olowokudejo, 1990; Folorunso and Abayomi, 2014), the existence of a high stomatal index, reasonably sized abaxial and adaxial cells, pitted anticlinal walls as well hydropoten cells are responsible for the survival of tree species like *Annona senegalensis* in the savannah areas. Metcalfe and Chalk (1979) explained that hydropoten cells are the most significant epidermal cells that facilitate water and mineral salts absorption in plants, which confers the ability to survive in the arid regions. On the other hand, undulating and large size irregularly shaped epidermal cells have been reported to be characteristics of *Persicaria lapathifolia* at both abaxial and adaxial surfaces (Yasmin *et al.,* 2010). *Persicaria lapathifolia* on basis of these traits grow and survive better in humid conditions (Ayodele and Olwokudejo, 2006).

Foliar epidermal features of eleven tree species of the family Bignoniaceae in Nigeria was carried out macroscopically and with a light microscope by Ugbabe and Ayodele (2008). The alignment of the anticlinal walls was greatly varied among Bignoniaceae members in a pattern that some of the species possessed wavy or curved walls while other species walls were straight (Ugbabe and Ayodele, 2008; Ugbabe *et al.,* 2014). As reported by Ugbabe and Ayodele (2008) and recently confirmed by other authors (Ugbabe *et al.,* 2014; Mahmoud *et al.,* 2016), that have studied the family Bignoniaceae, Anomocytic is the most common stomatal type except in *Kigelia africana* that is characterised with diacytic stomata. The positioning of striae in abaxial and adaxial surfaces is a major trait that can be employed by the taxonomists to bring about the striking difference between and among taxa of Bignoniaceae. For example, it was reported (Mahmoud *et al.,* 2016) that striae are present on the abaxial surface of *Spathodea campanulata*, while in *Oroxylum indicum*, it is located on the adaxial surface. Other unique epidermal taxonomic characters in this family that are of taxonomic importance include knobs, epidermal cell shape, and stomatal index. However, the size and number of epidermal sizes were presumed to be of little diagnostic value by Ugbabe and Ayodele (2008).

A preliminary leaf epidermal study of some West African species of *Desplatsia* by Shokefun *et al.* (2017) revealed that the epidermal cells in the genus are polygonal and irregular with variation in anticlinals such as curvy, wavy and straight walls. The two types of stomata discovered from the genus are staurocytic and anisocytic and are said to be distributed on the abaxial layer. The hypostomatic nature of the plant was discussed by

Goldschmidst (1996) as an adaptive feature that is ecologically advantageous for perennial survival in the prevailing environmental condition. *D. dewevrei* from *D. subericarpa* were delimited using trichomes, stomatal size and density. Shokefun *et al.* (2017) highlighted that *D. subericarpa* is distinguished by having the largest cells as well as the least density and size of stomata.

Khan *et al.* (2011a) reported that medicinally important arboreal flora could be easily identified if leaf epidermal markers such as size, shape and distribution of epidermal cells, stomata and trichomes are utilised. Petronela and Nevena (2010) characterised the leaf epidermis of two *Sesleria* species (*Sesleria heufleriana* and *S. uliginosa*) and discovered that both species differ adaxially and abaxially in costal and intercostals zones of the epidermis and as such helpful in distinguishing them. However, both species were reported to have long cells, prickles hairs, stomata and bulliform cells by these authors. This, therefore, established the fact that two different species exhibit some similarities and differences in leaf epidermal characteristics.

Ibrahim and Ayodele (2013) investigated the taxonomic application of leaf epidermal characters in the family Loranthaceae with light microscopy and explained that the characters were of more taxonomic importance in showing the affinity among groups at the family level than at the lower taxa. The authors equally reported that Trichome and trichome bases were restricted to the genus *Phragmanthera* while striations were absent in *Agelanthus brunneus*, *Englerina gabonensis* and *Tapinanthus cordifolius*. They emphasised that features such as Amiphstomatic leaf type, polygonal cell shape, straight to curve anticlinal wall and pericytic stomatal types were common to all the species and therefore concluded that the generated anatomical characters will help in the identification of the leaves of some of the species even when in fragments.

Leaf epidermal morphology of selected four species of Meliaceae; *Khaya grandifolia, Azadirachta indica, Khaya senegalensis* and *Cedrela odorata* were comparatively analysed by Akinyele *et al.* (2020). It was stated that the taxonomic distances mong the taxa exhibited by the epidermal characterisation technique was an unbiased indicator. The authors however linked the phylogenetic relationship derived from the epidermal characters in the family to be the basis for the existing molecular delimitation. On this

basis, it was highlighted that *Azadirachta*, which is in the subfamily melioideae formed a paraphyletic taxon with *Khaya* and *Cedrela*, whose subfamily is Swietenioideae. It was equally indicated that leaf epidermal characters were gene-independent and could provide cost-effective and stable taxonomic characterisation. In a bid to discover stable and useful taxonomic characters to mark striking differences among the genera *Daniellia*, *Caesalpinia*, *Senna* and *Bauhinia*, an intensive morphological analysis of leaf epidermis was carried out by Aworinde and Fawibe (2014).

Epidermal characters such as stomatal types, epidermal cell types and the existence of trichomes were greatly significant in determining the affinities among the species. This significant contribution and informativeness of the markers were since they were variable in some species and constant in others. The authors noted the characters as a novel because *Senna siamea, Senna alata*, and *Senna siberiana* were epistomatic whereas other species investigated were amphistomatic. Regardless of the stomatal positioning on the abaxial and adaxial layers of the leaf, macromorphologic variables like stomatal density, stomatal index, stomatal length and width showed reasonable variability based on their reflection of taxonomic delimitations. Therefore, molecular and phytochemical studies of the taxa were suggested by the authors based on the striking differences and similarities identified among the group to prove the effectiveness of the leaf epidermis.

According to Gul *et al.* (2019), Lamiaceae is one of the largest angiosperm families having about seven thousand (7000) species distributed into two hundred and forty (240) genera. Members of this family are characteristically aromatic, which have uncomparable medicinal with economic values and are distributed throughout the world (Gul *et al.,* 2019). These impeccable benefits, therefore, necessitated the explicit taxonomic evaluation of the taxon using epidermal markers to optimise the utilisation potential derived from the species. On this note, it was emphasised by Gul *et al.* (2019) that micromorphological features of the leaves are an undoubtedly useful tool to elucidate the systematics and taxonomy of the members of the family. In the study, a light microscope and scanning electron microscope were used to investigate the micromorphological features of 22 Lamiaceae belonging to 15 genera to solve the problem of identification of the species. Consequently, glandular and non-glandular trichomes were recorded as informative epidermal features for describing the group while morphometric

measurements, such as trichome index, stomatal complex, width and length of trichomes as well as the epidermal cells were taken for the full classification of the species. The characterisation generated some taxonomic keys that would aid the correct identification. In line with Gul *et al.* (2019), phylogenetic and molecular studies were then suggested to strengthen the systematics of Lamiaceae.

Consequent to the notable medicinal applications in tandem with the threatened nature of some members of Apocynaceae in Nigeria as well as the negative effects resulting from various medicinal adulteration and substitution, a taxonomic revision of selected species was carried out by Onefeli and Kehinde (2020). The idea was to provide an additional marker that end users can afford, that will equally contribute effectively to the correct use and identification of the selected species. The authors emphasised the fact that even though the floral characterisation was the order of the day as far as informative discrimination is concerned, however, due to the fluctuation of the flowering of the indigenous species in Nigeria, epidermal markers was tried using a biological microscope with a camera attachment. Epidermal morphometric findings obtained from the study indicated that stomatal length increased from *Rauvolfia vomitoria* (20.88 µm) to *Thevetia neriifolia* (25.92 µm). All the epidermal cells on the adaxial layer were therefore reported to be significantly different among the studied species while the epidermal cells were hexagonal or pentagonal in *Vocanga africana* and *Alstonia boonei.* The stomatal crypts were recorded to be very useful in the species identification coupled with the fact that it was the first account of the family in Nigeria.

In a similar study on the family Apocynaceae, Bashir *et al.* (2020) obtained some representative specimens from different locations in the University of Peshawar's main campus, Parkistan during different seasons. Epidermal characteristics were studied under light and Scanning electron microscopy for accurate classification and identification. In the assessment, both quantitative and qualitative features such as the width of guard cells, stomatal pore, length of guard cells, subsidiary cells, stomatal crypts and trichomes were observed on the two sides of the leaves. According to the report of the authors, *Alstonia scholaris, Plumeria rubra, Trachelospermum lucidum*, *Raulfia serpentine, Catharanthus roseus* and *Thevetia peruviana* demonstrated hypostomatic leaves. It was indicated that *Nerium oleander* does not demonstrate any type of stomata, but rather exhibited stomatal

crypts, in which stomata were enveloped in clusters of trichomes. This trait is said to be a remarkable marker for separating the species from other members of Apocynaceae in Pakistan. Other taxonomic keys noted for the family include the fact that *A. scholaris* is delimited other taxa with an anisocytic stomata on the abaxial, complete absence of stomata on the adaxial layer and thick cell wall. *C. roseus* is distantly differentiated from others with an average adaxial stomatal index of 19.9% while *Carissa carandas* was the only species having an irregular epidermal cell shape. In *P. rubra*, the epidermal cells were rectangular with a straight margin. *T. peruviana* was taxonomically unique for having a Paracytic stomatal.

According to Cheng (2006), Schisandraceae is an important and economic plant family with some taxonomic issues. Both Schisandraceae and Illiciaceae were classified under Illiciales, which was considered primitive angiosperms (Yang and Lin, 2005). The family having sixty (60) species was delimited into two genera; *Schisandra* and *Kadsura* regarding the fruit and shape of the torus in the pistillate flowers and is primarily distributed to the tropical and temperate regions of Southeast Asia (Cheng, 2006). To produce a natural classification of the taxa in the family, the morphological characteristics of epidermal markers and their systematic significance was assessed by Cheng (2006). Out of the 60 previously described species, twenty-three (23) were analysed by the author using electron microscopy. The finding showed that the shape of the epidermal cells, cuticular ornamentation as well as types of stomata were very helpful for determining the taxonomic relationship within and between the species of the family because they are usually constant within species. Hence, the family was reported to have either irregular or polygonal shapes of the epidermis. Thus, such a trait may help sort out the various sections within the family as well as separate the family from other related families of Angiosperms. Besides, Anticlinal walls in the family were identified to vary from sinuate or sinuous to straight while the stomatal apparatus belong to the Paracytic.

The family Annonaceae is a woody family which has been used effectively for the treatment of diseases such as fever, menstrual pains, diarrhoea, fatigue and wounds (Yao *et al.*, 2019). It is considered as one of the most primitive and diverse families consisting of 2500 and 200 species and general respectively, which are distributed to various locations around the world. The family is said to be the most successful family because a

large number of its species are scattered within several genera. The species in the family mainly occur in the rainforest of neotropics and paleotropics (Nielsen, 1993). The Histomorphological studies of five medicinal species of the family; *Xylopia aethiopica*, *Cleistopholis patens*, *Xylopia quintasii*, *Enantia polycarpa* and *Monodora myristica* collected from the Pra - Suhien forest in the central region of Ghana were assessed for a clearer distinction of them (Ameyaw and Akotoye, 2007). The fives species have been observed over the years to be very useful to the human communities living around the forest as a result of their vast utilisation for the treatment of ailments. Also, various parts of the plants are sold as spices and crude drugs in the markets. It, therefore, necessitated the taxonomic identification from the epidermal characters. According to Ameyaw and Akotoye (2007), leaves of the species are mainly paracytic which are hypostomatically distributed. The value of the stomatal index was highest in *E. polycarpa* (0.199) and least in *X. quintasii* (0.086). However, cluster type of epidermal crystals was observed only on all the species while *E. polycarpa* was distinct by having a trihydric crystal. Another striking epidermal marker that the author used to distinguish *E. polycarpa* from other species was epidermal hairs, which varied from stellate to non-glandular. The epidermal cells of the family varied from wavy to straight.

Trifolieae is one of the major groups assigned to the subfamily of Papilionoideae. The tribe suffered from different taxonomic opinions due to the appreciation in the use of contemporary tools like foliar epidermis and molecular characters (Debelle *et al.,* 2001). According to Mabberley (1997), the tribe consists of six genera; *Ononis*, *Melilotus*, *Medicago*, *Parochetus*, *Trifolium* and *Trigonella* worldwide. However, *Melilotus*, *Medicago*, *Trigonella* and *Trifolium* are the only genera found in Pakistan represented by thirty-six (36) species. Rashid *et al.* (2019) studied the taxonomic significance of leaf epidermis in the Papilinioideae and Leguminosae families represented in the tribe to assess the characters that could be utilised for the systematic positioning of the taxa in the families and tribe. In the study, the positioning of *Trigonella* and *Melilotus* in the *Grammocarpus* section was reexamined based on leaf epidermal features. Hence, the presence of anomocytic type of stomata which was greatly variable in size, non-glandular trichomes with a characteristic acute apex were the significant diagnostic features for delimiting the species. The highest trichome density was reported for the genus *Medicago.*

According to the finding, *Trigonella foenograceum* had the highest stomatal index (83%) while *Trifolium dubium* had the least value (24.6%). Generally, the authors concluded that the delimitation result of the epidermal characters on the members of the two families are mostly intrinsic and sustained to the species as well as promising taxonomic value. The authors indicated that the combination of other traits in correlation with epidermal variables has a good potential for taxonomic resolution at the specific level in the families. Further study on phylogenetic studies was also recommended to explain more about the intrageneric relationships in the taxa.

With regards to mono-specific taxonomy of plants, Kadiri and Ajayi (2009) studied *Musanga cercropioides* taxonomically and recorded bulbous trichome bases, flaky strand-like waxes, hypo-stomatic leaf, anomocytic stomatal type, abaxially restricted simple unicellular and non-glandular trichomes as well as crescentiform and hairy petiole as the most useful anatomical features which can be used for its proper identification. This tree intraspecific taxonomic relationship could also be linked to the findings of Oyegoke *et al*. (2014) on the population of *Vitellaria paradoxa* in different locations in Nigeria. They discovered significant similarities and differences in leaf anatomical characters concerning location influence and concluded that epidermal variations in *V. paradoxa* are influenced by environmental conditions. The type of epidermal cells reported in the leaves of the tree was polygonal epidermal cells, which had wavy to slightly straight anticlinal wall patterns while the stomata in the leaves from the three locations were hypo-stomatic with paracytic stomatal complexes. The authors reported that variations in epidermal cell shapes and anticlinal wall patterns observed in the leaves were probable adaptations to environmental factors and therefore suggestive of the possible occurrence of different varieties of *V. paradoxa* in Nigeria.

2.6 Molecular Taxonomic Studies of Plants

Molecular taxonomy is the organisation or classification of organisms based on the genetic diversity within and among taxa. Molecular methods have been successfully applied on many occasions to determine the genetic affinity among plants of different taxonomic groups (Onefeli, 2021). It is especially useful in taxonomic studies, where the morphological techniques have failed to accurately elucidate the correct relatedness and

differences among taxa of plants. One of the ways by which the relationship between different or closely related taxa can be determined is to identify the amount of DNA polymorphism. DNA polymorphism can be referred to as the differences that exist in the sequence of nucleotides between individuals. It, therefore, implies that the more the polymorphic DNA of the evaluated taxa, the higher their genetic or taxonomic variation. The differences can occur by insertions, deletions, changes in single base pairs or changes in the number of copies of a given DNA sequence. The data obtained are converted to binary form and used to construct phylogenetic trees, which epitomize the taxonomic relationship.

There are presently different molecular makers utilised for molecular taxonomy. The first group of molecular markers used for characterisation was isozymes. Isozymes have been used for the genetic characterisation of fruit trees (Uzun *et al.,* 2003). They are however disadvantaged as a result of the limited number of isozyme systems available coupled with the fact they are greatly affected by environmental factors and produce very low polymorphism. Hence, the existence of Polymerase Chain Reaction (PCR) based molecular methods has brought about many more efficient markers. Molecular markers like Inter simple sequence repeat (ISSR), Random amplified polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP), has made it possible for an improved molecular characterisation. Such molecular markers are less influenced by environmental factors and as well as highly polymorphic (Gopale and Zunjarrao, 2013; Prasad, 2014; Escobar-Saucedo *et al.,* 2018; Siew *et al.,* 2018).

Gopale and Zunjarrao (2013) characterised 20 accessions of *Jatropha curcas* using RAPD markers and discovered that 10 primers were able to produce some reproducible bands. Further analysis of the bands indicated 125 scored out of which 75.2% (94) was polymorphic while the rest 24.8% were monomorphic among the genotypes. Wide variations discovered by the authors in the number of bands produced by different primers were however reported to be a good indication for the used primers being able to resolve the taxonomic variation in the populations studied.

Genetic relationship among varieties of *Hibiscus rosasinensis* was examined by Prasad (2014) through random amplified polymorphic (RAPD) marker with the use of two

arbitrary decamer primers OPA9 and OPD10; a total number of 59 bands were obtained. About 71.19% of the bands were found to be polymorphic while 20.34% and 8.4% were reported to be monomorphic and unique respectively. The author further explained that there is a high genetic relationship among the studied varieties of *H. rosasinensis* due to the same DNA profile that was obtained for all the varieties. However, some bands for the different varieties were discovered to be different from each other while the phylogenetic tree generated segregated the population Bangalore from India.

Recently, in another study by Escobar-Saucedo *et al*. (2018), the genetic diversity of *Malus domestica* (Apple tree) was analysed using nine ISSR primers and identified 124 visible DNA bands but with a lesser polymorphism of 63%. The relationship among the evaluated cultivars of apple was recorded to be variable with genetic diversity of about 0.24 and with an average number of 1.637 alleles even though only two groups were formed by the resulted dendrogram. Using the same ISSR marker, Siew *et al.* (2018) assessed the molecular taxonomy of Malaysian Durian varieties and observed 91.73% (122) polymorphic bands out of the clear and reproducible 133 total DNA fragments. These 133 bands were reported to be amplified with just 12 primers, giving an average of 11 fragments per primer. In the same vein, Yegenoglu and Sesli (2017) carried out a similar study on *Olea europaea* (Olives) with ISSR and obtained 92 visible bands having 100% polymorphism.

2.7 Molecular taxonomy and Indigenous tree species in Nigeria

Considering some indigenous trees to Nigeria and Africa at large, the genus *Milicia* is one of such African taxa that have gained so much attention from scientists due to the quality of timber produced from this genus. However, much emphasis is laid on the populations in Cameroon and Ghana (Dainou *et al.,* 2012a). Phylogenetic studies by some authors (Dainou *et al.,* 2012b; Dainou *et al.,* 2014) revealed a significant difference between *M. regia* and *M. excelsa.* It was established that *M. excelsa* is distributed from South Africa to West and East Africa while *M. regia* is found in West Africa, which is distributed from Ghana to Senegal. Despite the existing phylogeographical knowledge and molecular taxonomic delimitation findings among the genus *Milicia*, Dainou *et al.* (2016)

highlighted that the current phylogenetic knowledge on the genus is still incomplete. This was based on the following two facts:

1. East African operational taxonomic unit for *M. regia* was limited and poorly represented as claimed by Dainou *et al.* (2016) and this unit could serve as the origin of the genus as stated for *Tamarindus indica* (Diallo *et al.,* 2007) and *Prunus africana* (Kadu *et al.,* 2011) that are widely distributed;

2. The insufficiency of the sampling intensity in the previous studies might have a high influence on the detection power of the distinct closely related taxa (Fogelqvist *et al.,* 2010). Therefore, an improved sampling intensity may bring about additional patterns of clusters and delimitation among the taxa.

Based on the highlighted limitations on the molecular taxonomic status of *Milicia*, 435 samples of *Milicia* species were obtained from West, East and Central Africa and subjected to SSRs and SNPs genotyping to shield more light on the existing clusters in the genus (Dainou *et al.,* 2016). To improve the naturalness of the produced clusters and remove any element of doubt, the authors augmented the genotyping data with the analysis of two plastid regions (psbA-trnH, trnC-ycf6). It was discovered that there are three distinct clusters of *Milicia* in Africa constituting *Milicia excelsa* in East and West Africa and *Milicia regia* in West Africa. In other words, three different species of *Milicia* were recognised by the research outcome of Dainou *et al.* (2016) as against the two species previously identified for this genus (Dainou *et al.,* 2012a; Dainou *et al.,* 2012b; Dainou *et al.,* 2014). It, therefore, implies that there are presently one species of *M. regia*, which belongs to West Africa and Two different species from *Milicia excelsa*, in which one is domiciled in West Africa, and one in the East African region.

The molecular taxonomic study of important indigenous tree species is not only limited to *Milicia*. *Ceiba pentandra* with the trading name Ceiba was long ago ranked as one of the most important multipurpose tree species in Ghana (Irvine, 1961; Burkill, 1985). As part of the efforts to improve the proportion of indigenous tree species plantation in Ghana, this species was therefore incorporated into the National Forest Plantation Development programme. Brondani *et al.* (2003) emphasised that the poor molecular evidence from *C. pentandra* could be a threat to its sustainable management and in the long run, lead to

complete erosion from the natural range of the species. However, thirty-six genotypes of *C. pentandra* were sampled from five ecological populations in its natural range and phylogenetically analysed with ten RAPDs and ISSRs markers by Abengmeneng *et al.* (2016). It was identified from the principal component analysis (PCA) that the first two principal components determined approximately 67% variation existing among the genotypes. Abengmeneng *et al.* (2016) reported that the ten polymerase chain reaction markers utilised were informative for the separation of the genotypes revealing nine accessions with a high dissimilarity. It was therefore recommended that the accessions should be used as seed trees candidates for breeding and conservation in Ghana. Darwish *et al.* (2015) conducted a similar study by fingerprinting *C. pentandra* in Egypt. In their study, the DNA fingerprint of the different varieties of the species was developed for future taxonomic identification at the genomic level.

Adansonia digitata commonly known as the Baobab tree is also one of the widely utilised multipurpose indigenous species in sub-Saharan Africa basically because of its high-quality oil and fruit pulp utilised in the cosmetic and juice industries (Edkins *et al.,* 2007). The genetic differentiation and diversity of five operational taxonomic units sampled from Malawi were studied using microsatellite marker by Munthali *et al.* (2013). Low genetic diversity was identified among the units. This was therefore attributed to anthropogenic factors as well as the taxonomic units that are growing in the genetic centre of diversity of the species. It was equally indicated that ecological differentiation influenced the clustering patterns observed in such a way that samples obtained from Likoma Island were genetically distanced from those in the mainland. Kamau (2016) investigated the molecular characteristics of eight populations of *Adansonia digitata* sampled from Kenya/Tanzania transect using sixteen microsatellite markers and discovered a low genetic variation among the populations with only three clusters formed. It was also confirmed by the author that the clusters obtained correlated with the geographic origin of the species. The low genetic difference was attributed to gene flow among the populations.

2.8 Classification methods in plant Taxonomic study

Despite the advances that have been made in forestry and botany, there exist lots of plants yet to be discovered, classified and identified. However, the discovery and classification

of the unknown plant are tantamount to having a new treasure. It is a known fact that no one can study all the plants that exist on the planet earth. Even if only the angiosperm is considered, it is still an impossible situation because this is the dominant plant group on the earth (Okonek, 2019). It is on this note that plants are categorised based on similarities and differences at different levels. According to Okonek (2019), plant classification is the placement of plants into groups according to the differences and similarities in their characteristics for orderliness and proper identification.

In some decades back, only a few arbitrary taxonomic characters were utilised for the delimitation of taxa regardless of any relationship amongst them (Cesaroni and Allegrucci, 1991). This type of classification typically known as artificial classification is utilised only for the sake of convenience. Most times, plants that are related in the real sense are placed under different groups while unrelated plants appeared in the same taxon, but the unknown plants are easily identified.

Artificial classification brought about the taxa such as edible plants, poisonous plants, medicinal plants, trees etc. Due to the major disadvantage of artificial classification of not being able to elucidate a true relationship among taxa, a more encompassing plant classificatory system known as the formal or natural system was developed. In this system of classification, morphological correlated characters that can be examined with the naked eye are used (Bhattacharyya, 2009). Because as many as possible characters are used, closely related plants form a group.

Natural classification utilises the data that are available at a particular time. Therefore, the relationship obtained among the taxa is a function of the existing overall similarities and dissimilarities. This classification system that is based on the overall similarity is also technically known as phenetic classification. Plants are grouped based on their morphological character or other observable traits without any reference to their phylogenetic relationship or ancestral groups.

All living plants are related in one way or the other. The fact that all present-day plants are the product of the evolution of the ancestral plants that were sharpened through periodic modification by the environmental changes was proposed by Darwin (1859). This was the beginning of phylogenetic classification. After the publication of Darwinian evolution

theory, more interest in the phylogenetic classification of plants with the incorporation of the evolutionary history was developed by taxonomists (Gnoli, 2006). Therefore, the grouping of plants based on their evolutionary relationship is known as phylogenetic classification. In this type of classification, species that are genetically related are represented in a tree known as the phylogenetic tree. The most common method for presenting plants that are related in a phylogenetic tree is called cladistics, which was derived from the word clade. Whereas, a clade is a group of plant that consists of an ancestor and all its descendants. A phylogenetic tree that represents an ancestor with all its descendants is referred to as a cladogram.

Plant classification today has now incorporated characters from different properties such as morphometric, molecular, chromosomal, behavioural, etc. to arrive at a robust natural vis-à-vis phylogenetic relationship among the groups under consideration. Managing this nature of robust data, therefore, requires multivariate approaches to classify and discriminate the species.

Out of the available multivariate approaches, Principal Component Analysis (PCA) and Cluster analysis have been widely utilised by plant taxonomists for the classification of Operational Taxonomic Units (OTU).

2.9 Principal Component Analysis and Classification

Principal Component Analysis (PCA) is a mathematical procedure in which linearly uncorrelated variables are produced through a linear transformation of possibly correlated multi variables. The transformed linear variables are referred to as the principal components. The resultant number of components, which explains the percentage variations in the original data is equal to or lower than the number of variables included in the analysis (Cesaroni and Allegrucci, 1991).

Principal Component Analysis was originally not well accepted for data dimensionality reduction until the advent of electronic computers because it is not feasible to use PCA without a computer, but it has now become one of the sophisticated multivariate analysis tools for taxonomic studies (Mishra *et al.*, 2017). It has also been well entrenched in many statistical packages.

The major goal of the principal component analysis is to reduce dimensionality (i.e. analyse large sets of variables to few ones) and retaining as much as possible variation available in the data set.

Principal component analysis transformation is designed in such a way that the first component usually accounts for the highest variation and the succeeding component has a higher contribution than the following components. Few of the produced components accounted for a significant variation based on some selection criteria. These criteria include eigen value one criterion, percentage variance explained and scree plot (Mishra *et al.*, 2017). According to the eigen value one criterion, components having an eigen value higher than 1 are selected for the explanation of the variations in the data. It stands that the higher the eigen value of a component, the stronger the explanatory power of such a component. It, therefore, implies that the first principal component will always have the best taxonomic discrimination efficiency. The percentage variance criterion requires that selected components account for at least 65 percent of the total variance, whereas the scree plot criterion requires that components be chosen until the graph line breaks or flattens out (Tharwat, 2009).

Aside from the mentioned three selection criteria, another important Principal Component Analysis parameter is the eigenvector. It is also known as component values or loadings. Component values could range from zero to one. The closer the component values to one, the better the contribution of the corresponding variable to the variation within the considered taxa. Component values can also be negative or positive. It means that loadings with the same sign contribute the same way within the component while those with the opposite sign will contribute in an opposed way. The eigenvectors equally identify important descriptors required for the delimitation of groups of organisms (Coccia, 2007).

2.10 Cluster Analysis

The bulkiness of the data employed in taxonomic studies necessitated the use of cluster analysis. Cluster analysis is a significant multivariate analysis used for the classification of organisms such as plants. This classification technique is achieved with the use of the characteristics possessed by the plants to be grouped.

Plants successfully grouped are then represented in a graphical form known as the dendrogram or phylogenetic tree. The dendrogram, which indicates all the samples or accession included in the analysis consists of branches and nodes that resembles a tree physiognomy. Similarities among the clusters are determined by the branch length not the order of the arrangement of the branches on the tree (Holland, 2006). According to Restrepo *et al.* (2007), a dendrogram could be viewed as a graph that is capable of bifurcating into subgroups of the entities involved.

The major aim of a cluster is the ability to depicts similarity among the groups of plant species. This necessitated its wide application to plant molecular diversity and drug discovery (Restrepo *et al.,* 2007). The type of dendrogram produced is determined by the nature of data utilised in the analysis. Therefore, the following types of dendrogram have been recognised for plant classification studies:

i. Cladogram;

ii. Phenogram;

iii. Phylogram.

A cladogram is a form of dendrogram used to illustrate phylogenetic or evolutionary association among Organism (Holland, 2006). Every node on a cladogram contains two branches that indicate evolutionary divergence from the common ancestor. A cladogram is the least informative way of presenting a phylogenetic tree (Alex, 2016). The main information depicted by this form of a dendrogram is how the terminals are assumed to be related. One major peculiar characteristic of cladogram is the absence of scale bar and all the terminals (species) ending in flush (i.e. the same edge length). A phylogram is also a kind of dendrogram that represents a phylogenetic relationship among plants. However, phylogram presents a more informative relationship among groups as compared to the cladogram. The branch lengths in a phylogram correspond to the number of character changes that have been inferred along the branches (Alex, 2016). A scale bar always accompanies the phylogram and the edge lengths do not end in flush. Phenogram, on the other hand, is a dendrogram that shows the overall taxonomic relationships among the plant groups but without reference to their evolutionary relationships.

2.11 Dormancy of Forest Tree Seeds

In certain situations, uninjured and perfect seeds fail to germinate despite the prevailing necessary and favourable environmental conditions. Such seeds are said to be at a low level of physiological activity (Yildiz *et al.,* 2017). This is termed seed dormancy. However, dormancy has variously been viewed as a major hindrance and obstacle to a physiologist who is willing to achieve a reasonable germination success of tree seeds. Germination success is particularly needed in modern nurseries, where mass-production and mechanised techniques are being utilised.

Dormancy in seeds of forest trees can occur as a result of physical or physiological factors. When the germination process is prevented as a result of the presence of inhibitors, it is known as physiological dormancy. Seed germination inhibitors can be found in seed endosperm, seed coat or fruit pulp (Lawan *et al.,* 2011). The most common inhibitor is abscisic acid (Yildiz *et al.,* 2017). One major way in which physiological dormancy operates is by deactivating the germination process. Seeds can also be physiologically dormant as a result of the incomplete maturation of the embryo.

It has been reported that the embryo of the seeds of some tree species may consist of few undifferentiated cells at the time of fruit ripening (Lawan *et al.,* 2011). To induce properly the germination process in such seeds, they must be stored for some time. This is to enable a complete differentiation process that must precede germination.

Physical dormancy could represent hard impervious seed coats that inhibit water from entering the embryo without which the enlargement that causes breaking of seed testa cannot occur. This type of dormancy can be found in tree species such as *Ricinodendron heudelotii, Albizia lebbeck, Elaeis guineensis* etc.

Naturally, seeds with physical dormancy require the activity of the microorganisms in the soil to weaken the hard seed coat before water could get penetrated the seed. Alternatively, physically dormant seeds can be exposed to periodic watering, which over time performing the weakening of the seed coat. Similarly, physiologically dormant seeds require leaching by rain in addition to the microorganism activity (Sezik, 1997). This mechanism of seed dormancy is naturally employed to prevent germination in unfavourable conditions that seedlings could experience during the dry season. As a result

of the germination inhibition immediately after fruit ripening, seed dormancy is equally beneficial by facilitating the dissemination of seeds either naturally or by man. This, therefore, makes possible the exchange of seeds as well as the introduction of exotic species into a country.

2.12 Germination of Tree Seeds

The development of any seedling commences with the germination of the seed. This process starts with the absorption of water by the seeds and culminates with the appearance of the first true leaves. This, therefore, indicates the significance of water in the germination process. According to Chachalis and Reddy (2000), water is one of the most important environmental factors affecting the germination of seeds. Other environmental factors include pH, Light and Temperature. Aside from the environmental factors, the natural properties of seeds have been reported (Trivedi *et al.,* 2016) to inhibit the rate at which seeds germinate. Naturally, some trees produce seeds that possess a soft seed coat while some other seeds are characterised by a hard seed coat.

Any seed with a tough or hard seed coat requires scarification to remove the impediment hindering quick and easy germination of such seed. There are various methods of scarification, which could be used as pre-germination treatment. These include filing, abrasion, fire, hot water and sulphuric acid. Although, the optimum levels of these methods are species-specific, therefore, each species best level and method must be determined through experiment. Wakawa and Akinyele (2016) applied some treatments; control, soaking in hot water, lime juice extract, concentrated H_2SO_4 and cold water soaking overnight to the seeds of *Tetrapleura tetraptera*. Assessment of germination indices such as mean germination time, percentage germination and germination speed was carried out daily for one week and it was found out that all the germination indices were significantly affected by the various treatment techniques applied. It was added that concentrated acid used resulted in the highest percentage of germination while seeds soaked in hot water could not germinate at all. The authors, therefore, concluded that H_2SO_4 was effective in breaking seed dormancy and as well suggested the pre-germination treatment for *Tetrapleura tetraptera* seeds.

Reports (Mng'omba *et al.,* 2007) had shown that fire can be used to rupture the tough coat of seed but results in high seed mortality. However, fire is very supportive in the regeneration of many savannah tree species. Apart from the cracking of the seed that resulted from burning, some chemical components such as nitrogen oxides, ethylene, ammonia and ash which are released during this process induce germination potential of the seed. Acid can also be very effective for hard coat seeds scarification but if care is not taken, failure would be recorded. According to Mng'omba *et al.* (2007), 98% of seed embryos of *P. curatelifolia* was damaged due to the perforation of the seed testa by beetles. The use of a nutcracker for scarification had also proved ineffective and not efficacious enough to completely remove the tough coat of *P. curatellifolia* seeds. Filing and abrasion are however very difficult to utilise but positive results have been recorded. For instance, Mawahib (2004) observed that mechanical scarification of *Delonix regia* seeds at micropylar end improved its germination having about 7.4 days of germination rate and germination of 90%.

Seed characteristics such as seed weight and seed have been discovered to influence the germination capability of forest tree seeds. Ngulube *et al.* (1997) assessed the importance of seed weight on the germination of *Uapaca kirkiana* and observed significant variation in the seed germination after a few weeks of monitoring. In a related study, Upadhaya *et al*., (2007) investigated the influence of seed mass variability on germination of *Prunus jenkinsii*, it was found out that there were germination differences based on the seed character.

Aside from the germination of seeds by determining the influence of treatment methods, in vitro, experimental trials with different treatment combinations have also been efficiently used to germinate difficult-to-germinate indigenous trees like *Pycnanthus angolensis*. As claimed by Bello and Akinyele (2016), effects of inoculation media, priming and the interaction of the two in vitro germination factors were significant on percentage germination and the number of days to germination of *P. angolensis* seeds. This successful germination of *P. angolensis* seeds was recorded as one of the breakthroughs of indigenous tree domestication in Nigeria. This achievement was made through efficient seeds sterilisation together with inoculation by the combination of Murashige and Skoog with Kinetin, Naphthalene Acetic Acid, Benzyl Amino Purine and

Indole Butyric Acid. The best germination rate was however discovered from primed seeds, which were inoculated using Naphthalene Acetic Acid and low Benzyl Amino Purine on Murashige and Skoog.

2.13 Agroforestry Practices in Nigeria

It is a well-known fact that the population of Nigeria is increasing at a geometric rate, whereas, the size of the land since the creation remains unchanged. This hike in population has undoubtedly contributed to the problem of land hunger experienced in many developing countries like Nigeria. In some cases, however, there is a fragmentation of the land occasioned by the land tenure system through which land is being inherited by the children from their parents.

The rate of the utilisation of the usual agricultural practice of shifting cultivation which involves the alternation of fallowing and cropping period has drastically reduced in the country. This has culminated from the fact that there is a high demand for land. Despite the restoration of the soil fertility that occurs during the fallow period induced by the regrowth of perennial shrubs and trees, which in turn brings about the accumulation of organic matter (Place *et al.* 2005), the fallow period, therefore, amounts to a period of zero gain on such land. In order to ameliorate the problem of land hunger in Nigeria and many other parts of the world, agroforestry practices in which trees are grown concomitantly with the food crops could be a veritable option. Hence, the study (Amonum *et al.* 2009) has categorised Nigeria as one of the tropical countries having a good record of successful agroforestry experiments.

The adoption of agroforestry practices in Nigeria has been reported to be influenced by the differences in the ecological pattern that the country is composed of (Amonum *et al.* 2009). The country is generally segmented into savannah and high forest vegetation zones. The savannah zone is located in the northern region while the high forest is in the southern region of the country. The high forest region is composed of coastal vegetation, mangrove, freshwater swamps and the lowland rainforests while the savannah region is bifurcated into Sahel, Sudan, Northern Guinea, and Southern Guinea. In addition to the mentioned variant of ecological variation, there is a derived savannah that serves as a transition

between the savannah and rainforest types of vegetation. This usually occurs within the southern part of the country.

The agroforestry practices reported in Nigeria according to the vegetation zones described, therefore, vary from the cultivation of crops in tandem with shelterbelt trees in the Sahel and Sudan zones of the north part of the country to the shifting cultivation, which involves slash and burn in the derived savannah and high forest of the southern part of the country (Adegbehin and Igboanugo, 1990).

Shifting cultivation

Among all the agroforestry practised in Nigeria, shifting cultivation is the most traditional type. This agroforestry practice can only be sustainable where there is minimal population pressure with a kilometre square of land available for about twenty-five to thirty people on average. This agroforestry practice is commonly used for food production in the savannah and rainforest areas in Nigeria (Adegbehin and Igboanugo, 1990).

Shifting cultivation in Nigeria usually involves the farmers clearing a designated forested area, after which the cleared area is burnt to allow the fertilisation of the soil through the resulted ash. The land is cultivated for some period years until the depletion of the soil nutrients. Farmers then move to another forested area to allow the depleted soil to regain its nutrient by allowing the natural vegetation to cover the piece of land.

Due to the increase in population, approximately five hundred people, therefore, struggle for the same size land that was usually available for only about twenty-five people in the Southeastern part of Nigeria (Aweto, 2001). Hence, the practice of shifting cultivation, which requires a minimum of fifteen years fallow period to allow the soil to regain its fertility has drastically reduced in many parts of southeastern Nigeria due to the limited land resources. In those locations where the shifting cultivation is still ongoing, there is a problem of ecological disturbance resulted from overexploitation of the vegetation, poor soil fertility, low yields, which have cumulatively contributed to the poor socioeconomic status of the farmers. Also, this type of agroforestry is no more in existence and is being replaced by homestead gardens in the areas there is very high population pressure, especially in the southeastern part of the country. Where the population pressure is at a minimal level, the system is modified into an improved fallow through which the plots are

enriched with the introduction of perennials such as *Leucaena leucocephala*, *Acioa barteri*, *Gliricidia sepium*, *Anthonotha macrophylla* and *Alchornea cordifolia* (Adegbehin and Igboanugo, 1990).

Homestead gardens

Homestead garden is similar to the shifting cultivation agroforestry practice in that the woody and herbaceous plants are combined in a multi-storeyed pattern so that the environmental conditions such as the water, soil nutrients, space and sunlight are utilised maximally. For instance, due to the variety of the components, nutrients are taken at different layers of the soil with little or no competition.

In this agroforestry, horticultural crops such as *Citrus sinensis, Citrus aurantifolia, Anona muricata* are incorporated together with *Musa* spp., which usually serves as food sources and supplements to the family of the owner (Alahira, 2021). In many instances, livestock like poultry, piggery and ruminants are included. In this system, maintenance of the plot is usually achieved easily as a result of its proximity to the farmer's residence *cordifolia* (Adegbehin and Igboanugo, 1990). Fertilisation of the soil is equally maintained at little or no cost because refuse obtained from pens of poultry, piggery and those from households serve the purpose. Therefore, the returns from home gardens in some parts of Nigeria usually outweighed what was obtainable from the shifting cultivation method in a hundred folds.

However, to sustain a homestead garden and record a fairly good yield in the northern part of the country, there requires an extra irrigation system and fertilisation, most especially during the dry season.

This agroforestry is advantageous to the fact it produces a diversified output. The cost of production and risk is equally low because farmers make use of the idle nutrients and labour. In addition, farmers need only to spend a meagre amount of money on food items and other livelihoods requirements.

Taungya

Sapoba in Edo State was the first place of introduction of Taungya to Nigeria and it was widely used in both savannah and rainforest regions of the country (Egwunatum and

Ezealisiji, 2020). It was a modification of the shifting cultivation in the sense that farmers open up a forest being a part of the reserve and cultivate crops within a period of two to three years. As farmers shift their agricultural activities to a new forested land, the successive areas are occupied by tree plantations (Egwunatum and Ezealisiji, 2020).

In this agroforestry practice, there are two parties involved; the forestry department and the farmers. The Government own and allocate the land to the farmers while the farmers ensure the management of the agroforestry components. Therefore, the system benefits both parties. The most commonly used tree components include but are not limited to *Tectona grandis*, *Gmelina arborea*, while indigenous species such as *Triplochiton scleroxylon*, *Eucalyptus* spp., *Terminalia* spp. are seldomly used (Azeez *et al.,* 2017). The forestry department supplies the tree seedlings for the plantation why the farmers incorporate crops such as cassava, groundnut, okra, maize, tomatoes, etc. into the established plantation for their consumption.

There are two major types of taungya practice in Nigeria, the own your crop type and farming-for-pay type (Adegbehin and Igboanugo, 1990). The "own your crop type" is also referred to as the traditional taungya. Under this type, a forested area of about half a hectare is allocated to each farmer. Farmers take charge of all the pre-planting operations such as clearing, linning-out, pegging and planting of the tree seedlings supplied by the forestry department. In return for the farmers' labour, they are allowed to cultivate food crops, which continues for about three years before the tree canopy begins to close up. This taungya type is most commonly practised in Oyo, Ogun and Ondo States (Adegbehin and Igboanugo, 1990).

The second type of taungya is otherwise known as the departmental taungya and it is usually peculiar to Cross River State. The major distinction between the two types is that the local farmers are in the possession of only the food crops while both the crops and trees are owned by the government under the departmental type.

Alley cropping

Alley cropping is a farming method in which food crops are grown in between the rows of trees. The most common tree/shrub species used for this farming method are those with nitrogen-fixing properties (Onefeli *et al.,* 2019). Examples include *Leucaena leucocephala*

and *Gliricidia sepium.* Such tree species are used with the hope of replenishing the depleted soil nutrients. However, recently, multipurpose indigenous tree species, which do not necessarily fix nitrogen into the soil are being incorporated (Adegbehin and Igboanugo, 1990). In alley cropping, trees are pruned occasionally to avoid unusual competition between the woody perennials and crops. In the southern guinea and derived savannah, recommended hedgerow species include *Spondias mombin, Nauclea latifolia, Afzelia africana, Detarium microcarpum, Ceiba pentandra, Moringa oleifera* and *Prosopsis africana* (Adegbehin and Igboanugo, 1990).

As suggested by He *et al.* (2015), one of the most important qualities that should be looked out for while choosing the woody perennial for alley cropping should include regeneration after repeated pruning, rapid growth, deep rooting system, heavy foliage and multipurpose benefits such as medicinal values, fuelwood, fodder and food.

Examples of food crops commonly grown in the alleys are maize, cowpeas, cassava, rice, yam, etc. After the food crops have been harvested, the alleys are left on the field to serve as cover on the land as well as provide more wood products.

2.14 Potentials of *Ricinodendron heudelotii* for agroforestry

According to the United States Department of Agriculture (USDA, 2012), Agroforestry can be defined as the deliberate combination of shrubs and trees into crop and/or animal production systems in order to create benefits that are environmentally, economically and socially acceptable. Various agroforestry practices have been identified and used over the years. These include riparian forest buffers along watercourses; windbreaks used to protect livestock, farmstead and field; alley cropping in which alleyways are created using high-value shrubs or trees with the integration of crops in between the created alleys; Silvopasture, where trees, livestock and forages grow together; forest farming, in which food, medicinal as well as decorative products are grown together under the protection of a managed canopy (USDA, 2012).

The sustaining of soil productivity and provision of multiple products are the main attributes of multipurpose trees in agroforestry. The soil productivity sustenance is achieved through soil nutrient enrichment and erosion control. Nutrient enrichment is made possible through litter decomposition as well as root activities like nodulation and

nitrogen fixation, while deep taproot and good anchorage prevent erosion. Multiple products, both major and minor, are obtained from various parts of the trees ranging from the leaves, fruits, flowers and seeds to branches, stems, bark, root and so on.

Due to the presumed slow growth rate of indigenous tree species, agroforestry is rarely practised with deliberate planting of these trees from the early stage to maturity before the incorporation of crops (Onefeli and Adesoye, 2014). Traditionally, most farmers open a forest cover for cropping, during which some selected multipurpose trees are spared based on the benefits derived from them and for shading (Leakey, 1999). According to Duguma *et al.* (2001), small scale farmers popularly establish their farms having thinned the forest canopy and removing the understorey to enable the growth of their agricultural tree crops into a productive stage. For instance, “indigenous multipurpose tree species such as *Pycnanthus angolensis, Treculia africana, Monodora myristica, Dennettia tripetala* and *Ricinodendron heudelotii* have been reported to be retained as shade trees for the banana, and cash crop plantation in some western and eastern African countries” (Leakey, 1999; Onefeli *et al.*, 2019) (Appendix 7). In rapidly degrading landscapes, this practice was reported to have biodiversity conservation potential as a result of the related natural forest environment it creates (Leakey, 1999). However, as soon as the trees grow to the extent of producing heavy crowns, which could impede the growth of the crops of interest, farmers then kill them gradually by ring barking (Appendix 8). Similarly, some are destroyed by felling, which coppiced after a while (Appendix 9).

Ricinodendron heudelotii is a tree with great potentials for agroforestry since it has almost all the attributes required of multipurpose species. The root of this species has been found to accommodate mycorrhiza association (Tchoundjeu and Atangana, 2006) hence the attribute of soil enrichment through nitrogen fixation. It has a tap-root and lots of lateral roots for good anchorage. *R. heudelotii* produces leaves heavily, hence when it is in leaf, good shade is provided, and the temperature condition of the undercover environment is greatly ameliorated. Similarly, the leaf is palatable to livestock (Tchoundjeu and Atangana, 2006), therefore the fresh leaves could be lopped for fodder especially for small ruminants. However, all these highlighted potentials could only be achieved if demonstrated practically with an agroforestry practice on the field.

2.15 Early Growth of Tree Species and interaction with Arable Crops

The study of the early growth of trees is very imperative in determining their full potential and end uses applications. The developmental history (ontogeny) and Physiological parameters of the tree such as Net rate assimilation and relative growth rate cannot be possibly identified without the early growth assessment. However, most early growth studies of the indigenous species end up at the nursery stage (Onefeli and Adesoye, 2014; Akinyele and Wakawa, 2017; Bolanle-Ojo *et al.,* 2018) due to their assumed slow growth rate.

Some seedlings of *Buchholzia coriacea* experimented to determine the influence of six sources (Omo forest reserve, Ore Forest Reserve, Olukosi, Ogbere, Eleiyele, Ago-Owu and Erifun) on the early growth by Akinyele and Adegeye (2012). Data were collected on growth variables such as biomass production, leaf production, shoot height and collar diameter. Findings from their study showed that seedling sources significantly affected the height growth of the species in which Olukosi was recorded to have the highest height of 33cm. Despite the difficulty in the raising of this important indigenous tree, it was highlighted in the finding of the study that the plantation of *B. coriacea* could be established using the Olukosi or Ogbere source. In addition to the seedling sources effect on the growth of indigenous trees, Akinyele and Wakawa (2017) equally studied the response of *Tetrapleura tetraptera* to plant inevitable factors required for growth (soil, water and light intensity) to domesticate the species to different ecological regions. All the factors had a significant influence on growth; with loamy sand soil producing the tallest seedlings while daily watering and 100% light intensity positively affected the growth best. Jackfruit, scientifically known as *Artocarpus heterophyllus* is the largest fruit in the world with high nutritious value has hitherto been less cultivated. However, Bolanle-Ojo *et al*. (2018) recently carried out some domestication trials to determine its physiological and morphological responses to selected environmental factors. It was established that the chlorophyll content of the plant was influenced by varying the amount of light that was received. The author, therefore, concluded that *A. heterophyllus* can be raised under light intensity variation with minimal water stress.

There exist studies on on-site early growth studies of exotic trees (Calvo-Alvarado *et al.,* 2007; Akhabue *et al.,* 2020). According to Akhabue *et al.* (2020), the average height and diameter of *Gmelina arborea* after one year of on-site growth was about 1.5 m and 13 cm respectively. In an early growth study of both indigenous and exotic species carried out by Calvo-Alvarado *et al.* (2007) in Tropical moist forest, Costa Rica, the average height growth of *Gmelina arborea* was about 2.3 m while that of *Pinus caribaea, Vochysia guatemalensis, Vochysia ferruginea, Terminalia amazonia* and *Hieronyma alchorneoides* were less or equal to 1m after two years of assessment. According to Onefeli and Adesoye (2014), the average height and collar diameter of *Gmelina arborea* saplings were put at 38.11 and 4.23 cm respectively after six months.

Interaction between the tree and arable crop growth cannot be overemphasized. Before any tree species can be recommended for agroforestry purposes, the density of planting that favours crop yield must first be determined. This, however, may be varied among tree species and crops. According to Reynolds *et al.* (2007), maize that is grown under 6 m Poplar alley had significant better yield (4.61 t/ha), height (177.2 cm), whole plant leaf area (5026.5 cm^2) and whole plant leaf weight (26.0 gm) than those intercropped at 2 m alley width with the yield (2.89 t/ha), height (103.8 cm), whole plant leaf area (3769.2 cm^2) and whole plant leaf weight (17.3 gm).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Specimens collection and experimental sites

An initial reconnaissance survey was done to identify the mother tree locations of the species in the lowland rainforest area of Southern Nigeria. This was achieved with the assistance of forest guards, farmers, and villagers living in the localities of the existing collection records, that know the nooks and crannies of the localities as well as having knowledge about where *Ricinodendron heudelotii* could be found. The existing collection records were obtained from Flora of West Tropical Africa (Hutchinson and Dalziel, 2014), University of Lagos Herbarium and Forest Herbarium Ibadan (FHI). Based on the survey, Oyo, Osun, Ondo, Edo and Cross River States (Figure 3.1) were purposively selected for the sample's collection. Specifically, samples were obtained from Ibadan, Oloruntele, Onigambari, Osu, Akure, Ikoyi, Ile-Ife, Benin and Boki (Figure 3.2) based on availability.

The identified localities were traversed and the Geographical Positioning System (GPS) locations of the *R. heudelotii* sited were recorded (Table 3.1). Samples were collected from a total of 50 matured trees located in protected areas, free areas and farms.

Within each of the locations, leaves and fruits samples of the tree species were collected for taxonomic and agroforestry studies. Collections were achieved with the use of a pruning hook and sharp cutlass. Some of the collected leaf specimens were preserved in 50 % ethanol for epidermal study while the rest were preserved in sample collection bags containing Silica gel for molecular characterisation at the School of Biosecurity, Biotechnical and Laboratory Sciences (SBLS), Makerere University, Kampala, Uganda.

Figure 3.1. Map of Nigeria showing the states where *Ricinodendron heudelotii* were found

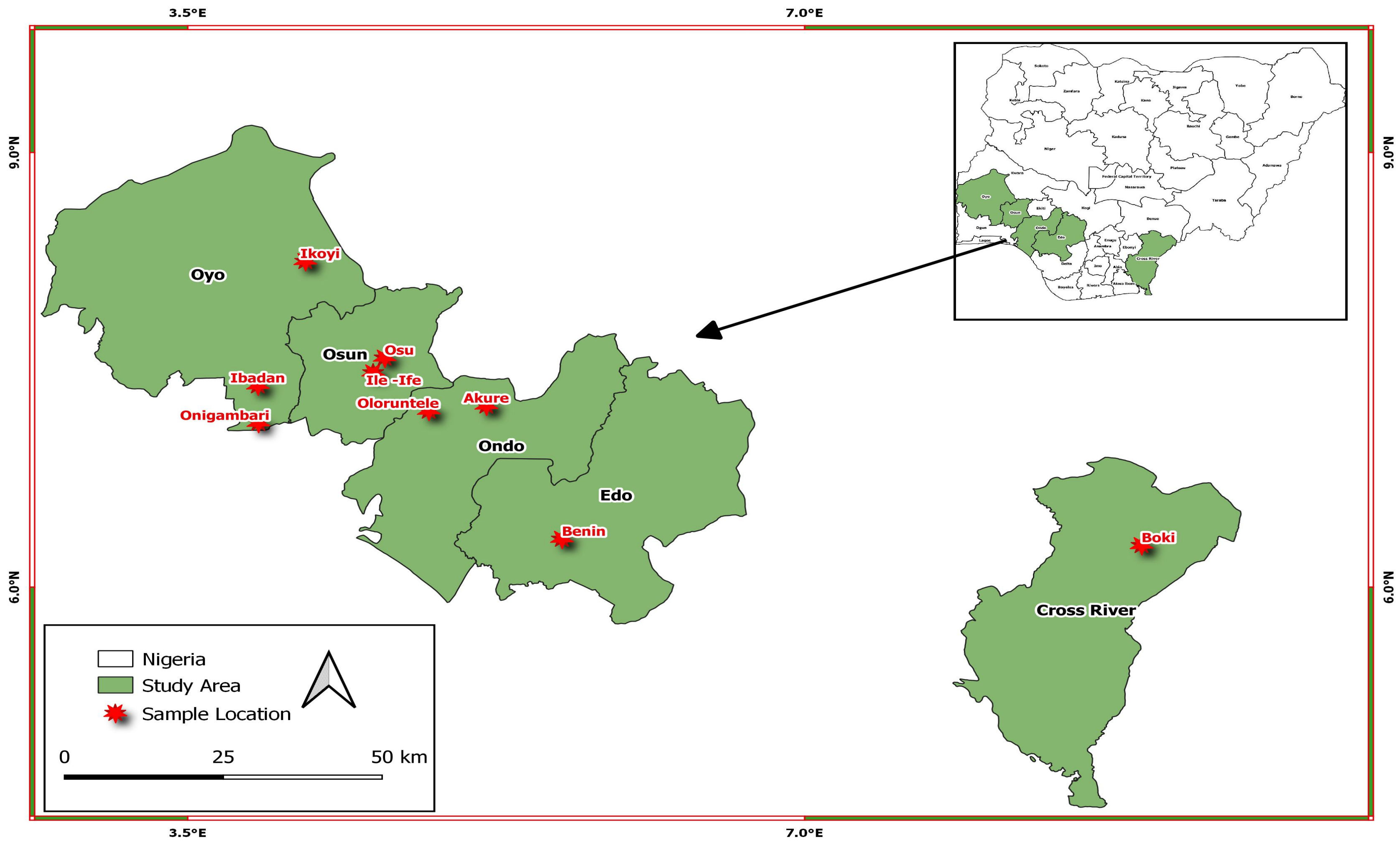


Figure 3.2. Localities for *Ricinodendron heudelotii* sample collection

Table 3.1. GPS locations of *Ricinodendron heudelotii*

S/N	OTUS	Latitude	Longitude	Elevation (m)
1	Ibadan	7° 26' 51.6912" N	3° 53' 46.4208" E	202
2	Osu	7° 34' 18.552" N	4° 37' 4.9224" E	330
3		7° 34' 12.7308" N	4° 36' 40.8204" E	341
4		7° 33' 56.4948" N	4° 36' 26.6076" E	307
5		7° 34' 18.0912" N	4° 36' 19.3464" E	324
6		7° 34' 11.5032" N	4° 36' 14.7096" E	315
7		7° 34' 7.5216" N	4° 35' 47.058" E	312
8		7° 34' 18.552" N	4° 37' 6.7764" E	330
9		7° 34' 7.5252" N	4° 37' 43.8564" E	347
10	ILE-IFE	7° 30' 11.9628" N	4° 31' 38.5536" E	250
11		7° 30' 11.772" N	4° 31' 35.346" E	257
12		7° 30' 10.7352" N	4° 31' 34.3812" E	260
13		7° 30' 7.7868" N	4° 31' 33.4524" E	256
14		7° 30' 10.6236" N	4° 31' 29.3232" E	258
15		7° 30' 15.9444" N	4° 31' 33.3372" E	262
16		7° 30' 21.8808" N	4° 31' 33.0276" E	262
17		7° 30' 22.5684" N	4° 31' 38.0892" E	256
18		7° 30' 30.8412" N	4° 31' 34.6116" E	255
19		7° 30' 31.7592" N	4° 31' 28.74" E	259
20	Ikoyi	7° 39' 8.0496" N	4° 17' 57.5628" E	293
21		7° 39' 4.6044" N	4° 18' 5.2884" E	289
22		7° 39' 1.4652" N	4° 17' 59.1864" E	289
23		7° 39' 9.0468" N	4° 17' 57.102" E	293
24	Oloruntele	7° 10' 38.5356" N	4° 44' 52.7352" E	227
25		7° 10' 25.0464" N	4° 44' 53.97" E	234
26		7° 10' 12.7848" N	4° 45' 8.1864" E	278
27		7° 10' 7.266" N	4° 45' 8.1864" E	289
28		7° 10' 7.266" N	4° 45' 21.1608" E	319
29		7° 9' 43.3512" N	4° 45' 37.8468" E	240
30		7° 9' 38.448" N	4° 45' 37.8468" E	240
31		7° 9' 38.448" N	4° 45' 55.152" E	240
32		7° 9' 29.25" N	4° 45' 55.152" E	253
33		7° 9' 29.25" N	4° 45' 48.9708" E	241
34		7° 9' 26.7984" N	4° 45' 45.882" E	250
35		7° 9' 23.1192" N	4° 45' 37.2312" E	243
36	Onigambari	7° 7' 59.5704" N	3° 54' 1.7604" E	122
37		7° 7' 58.9584" N	3° 54' 1.2996" E	123
38		7° 7' 58.0404" N	3° 54' 1.2996" E	121
39		7° 7' 57.5796" N	3° 54' 0.5256" E	121
40		7° 7' 56.046" N	3° 53' 59.91" E	121
41	Boki	5° 17' 29.3172" N	8° 1' 57.1836" E	51
42		5° 17' 37.3164" N	8° 1' 57.7992" E	65
43	Akure	7° 19' 5.5236" N	5° 5' 51.5616" E	391
44		7° 19' 5.5236" N	5° 6' 7.6284" E	367
45		7° 19' 21.4572" N	5° 6' 19.9908" E	354
46		7° 19' 54.5592" N	5° 6' 26.1684" E	366
47	Benin	6° 18' 26.2044" N	5° 36' 36.0036" E	68
48		6° 18' 25.668" N	5° 36' 35.6184" E	68
49		6° 18' 25.1316" N	5° 36' 33.3792" E	65
50		6° 18' 24.6708" N	5° 36' 33.5304" E	65

3.1.1 University of Ibadan

The University of Ibadan is the premier university in Nigeria, which is situated in Ibadan city within latitudes 7°25′55.200″N and 7°29′16.800″N and longitudes 3°52′19.200″E and 3°54′43.200″E. The climate of the University of Ibadan is mainly wet and dry seasons of which the wet period is usually longer than the dry season. Due to climate change consequences, the commencement of each season varies between different years. Many times, the dry period commences around November and culminates in March (Akinyele, 2010). The season is usually characterised by harmattan. The month of April is usually the beginning of the wet season, which in many cases runs through October (Akinyele, 2010). The wet season sometimes comes with both thunderstorms and winds.

3.1.2 Oloruntele

Oloruntele is a village in Ileoluji-Okeigbo local government, Ondo state. It lies between latitudes 7°7′N and 7°8′N and longitudes 4°46′E and 4°47′E. It lies in the dry lowland rainforest belt (Keay, 1989). It shares a boundary with Okeigbo town and Epe village. The climate is characterised by two seasons i.e. the wet and dry seasons. The dry season occurs between November and March while the wet season is normally between April and September.

3.1.3 Onigambari Forest reserve

Onigambari Forest Reserve is one of the important forests in Nigeria. Its geographical coordinates are latitudes 7°10′N and 7°48′N and longitudes 3°26′E and 4°57′E in Ibadan, Oluyole Local Government, Oyo State (Sanwo *et al.,* 2015). The reserve shares boundary with River Ona and Ibadan-Ijebu-Ode road on the west and east respectively. It was established by Sanwo *et al.* (2015) that the forest extends to an area of about 17,984 hectares. Both dry and wet seasons are experienced in the reserve. The dry season lasts for three months (December - February). The average annual rainfall is about 1140mm and the average annual temperature is about 26.4°C (Sanwo *et al.,* 2015).

The forest is of two types; plantation and natural forests. The plantation constituent was mainly established with two major exotic species, which are *Tectona grandis* and *Gmelina arborea.* Within the natural forest, tree species like *Triplochyton scleroxylon,*

Brachystegia enrycoma, Diospyros suaveoleus, Cordia millenii, Diospyros cauliflora and *Diospyros monbutensis.*

3.1.4 Osu

Osu is a Town in Osun State, which shares a boundary with the Western part of Ile-Ife. It is the headquarters of Atakunmosa West Local Government. It covers a landmass of about 577 km^2. Osu is characterised by tropical vegetation and lies within the dry lowland rainforest belt (Keay, 1989). The preponderance of the original forested areas has been destroyed and now replaced with tree crop plantations such as *Theobroma cacao, Elaeis guineensis* and *Cola nitida.* It experiences two seasons in a year; dry season, which persists for about 4 months (November to March), while the rainy season covers the rest of the months with an annual average rainfall of about 1413 mm (April to October) (Olatunde *et al*., 2013). The soil is a derivative of the basement complex, which is characterised by metamorphosed sedimentary rock.

3.1.5 Strict Nature Reserve (SNR 1), Akure

The Strict Nature reserve (SNR 1) is a tropical rainforest located within Akure Forest Reserve in Latitudes 5º 45’ and 8º 15’ North and Longitudes 4º 30’ and 6º East, Akure, Ondo State (Adekunle *et al.,* 2013a). SNR1 is characterised by bimodal rainfall patterns that last for about nine months (March-November). The dry season occurs between December and February. Iyagin and Adekunle (2017) highlighted that the mean annual rainfall in the reserve is about 1700 mm while the temperature ranges between 20.6 °C and 33.5 °C.

According to Adekunle *et al.* (2013b), it was demarcated from Akure forest reserve by the Forestry Research Institute of Nigeria (FRIN) in 1954. This was done to preserve the biodiversity for scientific studies, environmental monitoring and conservation of genetic resources. The reserve, which covers about 32 ha of the land area is bounded by the plantation of both exotic and indigenous species, which served as a buffer zone (Adeduntan and Olusola, 2013). Broadleaved hardwood tree species are the dominant vegetation in the reserve. According to some works of literature (Adekunle *et al.,* 2013a; Adekunle *et al.,* 2013b; Iyagin and Adekunle, 2017), the forest has some important indigenous tree species such as *Monodora myristica, Alstonia boonei, Trichilia*

heudelottii, Trichilia prieuriana, Chrysopyllum albidum, Mansonia altissima, Sterculia rhinopetala, Celtis zenkeri, Anogeissus leiocarpa, Tabernaemontana pachysiphon, Brachystegia nigerica, Cordia millenii, Brachystegia eurycoma, Bridelia micrantha, Enantia chlorantha, Lannea welwitschii, etc.

3.1.6 Abayomi Forest Estate, Ikoyi

Abayomi Forest Estate is a Secondary Private Natural Forest Reserve, located in Isokan Local Government, Ikoyi, Osun State. According to Alo and Adewole (2018), the forest covers a total land area of about 103.59 hectares. It is located between latitudes 7°17'47.85"N and 7°17'19.35"N and longitudes 4° 7'57.65"E and 4° 8'4.98"E (Alo and Adewole, 2018). The forest was established for the conservation of both flora and fauna diversity used for scientific studies as well as environmental maintenance. The topography of the forest is extremely undulating with some rock outcrops, especially at the riparian part. The forest is inhabited by some endangered tree species like *Lophira lanceolata, Monodora myristica* and *Brachystegia eurycoma.*

3.1.7 Obafemi Awolowo University, Ile-Ife

Obafemi Awolowo University is a Federal Government owned University in Nigeria. It was established in 1961 and is located in the ancient city of Ile-Ife, Osun State, Nigeria. It is geographically located on longitudes 4° 31.579' E and 4° 32.912' E and Latitudes 7° 30.458' 31.579' N and 7° 33.335'N, 256 m above the sea level (Akinola, 2009). The vegetation is characterised by some indigenous tall tree species, which create a serene and microclimatic environment on the campus. In some areas, there is thick undergrowth of shrubs and intertwining climbers among the existing trees.

3.1.8 Benson Idahosa University, Benin

Benson Idahosa University is a Private Christian University, which was founded in the year 2002 and named after Archbishop Benson Idahosa. It is located on longitude 5°38′E and latitude 6°20′N having 88m altitude in Benin City Nigeria (Oni *et al.*, 2016). Many indigenous tree species that are of ultimate benefits to humans, especially for amenity and avenue purposes are deliberately left and managed on the campus. The trees include but not limited to *Ricinodendron heudelotii, Alstonia boonei, Azadirachta indica, Newbouldia laevis*.

3.1.9 Afi River Forest Reserve, Boki

Afi River Forest Reserve is one of the tropical forest reserves located in Boki, Cross River State. The forest is characterised by a fast-moving river Afi, which has a high gradient that constitutes an important watershed. It is regarded as a biodiversity hotspot of global importance. The coordinates for its longitudinal location are 8° 50′ and 9° 05′E while that of latitudinal location is 6° 08′ and 6° 26′N (Aigbe *et al*., 2014).

The annual rainfall in the forest ranges between 3000 mm to 3800 mm while the mean annual temperature is about 22.2°C (Agbor, 2003; Aigbe *et al*., 2014). As maintained by Balogun (2003), the average relative humidity is 78 % at 7.00 hours annually.

According to Aigbe *et al.* (2014), the total land area on which the forest is covered is approximately 383 km^2. On average, its altitude is about 60m. The topography of the forest is very complex due to the presence of connected ridge systems, rock outcrops and isolated peaks. The existence of the Hills in the forest occurred as a result of the geological formation of the Cameroon Mountains that dovetailed into the forest.

3.2 Study 1: Leaf Epidermal and Macro-morphology of *Ricinodendron heudelotii*

3.2.1 Leaf epidermal characterisation

Samples collected from nine populations in Southern Nigeria; Ibadan, Oloruntele, Onigambari, Osu, Akure, Ikoyi, Ile-Ife, Benin and Boki were used for this study. These populations constitute the operational taxonomic units (OTUs). Leaf epidermal morphology was studied using both fresh and specimens preserved in 50% ethanol at the Department of Forest Production and Products, University of Ibadan. At least, ten (10) leaf samples from each OTU were used to prepare the epidermal peels. The specimens were prepared using the technique of Olowokudejo (1993) and Ayodele and Olowokudejo (2006).

Leaf sections of 5-8mm^2 were taken from a median portion of each leaf. The samples were then soaked in concentrated trioxonitrate (v) acid in Petri dishes for 2-4 hours. Thereafter, they were transferred into water in a Petri dish while abaxial and adaxial epidermises were carefully separated using forceps and dissecting needles. The epidermises were carefully cleaned with a camel hairbrush. The isolated epidermal layer was rinsed several times in

water before transferring into 50% alcohol for about 2 minutes to harden them. The tissue was then stained in safranine for about 3-5minutes and the excess stain was washed off in the water. The epidermises were subjected to 50%, 70%, 90% and absolute alcohol series to dehydrate them. They were mounted in glycerine on a slide.

Prepared epidermal peels were studied under CIWA XSP-35TV Biological microscope. Photomicrographs were taken using 200W Electronic Eyepiece. Qualitative and count epidermal variables such as stomatal type, leaf surface (LS), number of stomata (NS), number of epidermal cells (NEC), number of wall sides (NWS) were assessed by viewing through the microscope eyepiece. Epidermal cell length (ECL), epidermal cell width (ECW), stomatal length (SL), stomatal width (SW), Guard cell length and guard cell width (GCW) were measured using an ocular micrometre installed in the microscope eyepiece.

Guard cell area (GCA) was calculated according to Franco (1939) as:

$$GCA = Length \ x \ Width \ x \ k$$

k = 0.78524 (Franco's constant)

Stomal index (SI) was estimated using the formula of Salisbury (1927).

$$SI = {}^{S}/_{S + E}$$

Where S = Total number of Stomata per unit area

E = Number of epidermal cells in the same unit area as stomata

Pore size (PS) was calculated as:

$$PS = PL \ x \ PW$$

Where PL= pore length

PW = pore width

3.2.2 Morphometrics

Morphometrics is the measurement of multiple quantitative characters among a set of individuals (usually lengths, widths, and ratios) and using inferential statistics (e.g., ANOVA or multivariate statistical analyses) on the collected data to identify the relatedness among groups of individuals (Marhold, 2011). A study on Macro-morphology

was carried out at the Department of Forest Production and Products, University of Ibadan. The method of Adeyemi *et al.* (2013) was adopted for the macro-morphological characterisation. The macro-morphological features were obtained from the leaf, fruit and seed organs. All the leaves, fruits and seeds specimens collected from each Operational Taxonomic Unit (OTU) were bulked before the assessment.

The sampled leaves were obtained from projected branches on the tree crown that intercepted enough light. This was done to eliminate the shade effect on the leaves that were sampled and to ensure that the leaves sampled are almost of the same age. Eleven (11) characters were assessed on every fifty (50) randomly selected leaves from each OTU. The 11 characters assessed include (i) number of leaflets, (ii) petiole length, (iii) acumen length (apex), (iv) leaf total length, (v) leaflet length, (vi) petiole width, (vii) leaf width at base (0.25 length), (viii) leaf width at the middle (0.5 length), (ix) leaf width at the top (0.75 length), (x) number of secondary veins and (xi) space between secondary veins.

Fifty (50) fruits were also selected for the assessment of the following seven (7) variables in each OTU: (i) number of seeds per fruit (ii) number of aborted seeds, (iii) fruit largest width, (iv) fruit smallest width, (v) pulp weight, (vi) fruit length, (vii) fruit weight. Due to the limited number of seeds found in some OTUs, only twenty (20) seeds were assessed for (i) seed length, (ii) seed diameter, (iii) seed weight and (iv) roundness ratio.

Length and width of individual fruits were used to estimate the roundness ratio (RR) (Vihotogbé, 2012):

$$RR = {TL}/{\sqrt{Ld x Sd}}$$

TL = length of the fruit, *Ld* and *Sd* = its largest and smallest diameters, respectively.

Leaf total length, leaflet length, acumen length, leaf widths and petiole length were measured using a 30cm ruler. The fruit length, fruit diameter, seed length and seed diameter were measured using a digital calliper, while the fruit and seed weight were obtained using a weighing balance. The number of pairs of secondary veins, number of seeds per fruit and number of aborted seeds per fruit were obtained by visual counting.

3.2.3 Data analysis

Quantitative data were subjected to descriptive statistics, analysis of variance, and Tukey Honestly Significance Difference (THSD) while the qualitative data were analysed with Kruskal-Wallis to determine the variation among the different Operational Taxonomic Units (OTUs). Principal component analysis (PCA) was used to identify the most important traits that contributed best to the possible variations using the corresponding factor loadings within the species while Cluster analysis (CA) was used to produce a dendrogram based on morphological characters.

3.3 Study 2. Molecular Taxonomic Study of *R. heudelotii*

Five leaf samples were collected from each of the 48 stands of *Ricinodendron heudelotii* (Table 3.2) and used for this study. All the samples except for Benin were obtained from 3 months old seedlings raised at the Department of Forest Production and Products, University of Ibadan. Due to the lack of seeds on *R. heudelotii* in Benin, young leaf samples from this OTU were obtained on the mother trees. Each of the leaf sample specimens was packaged individually in a Ziploc bag containing 5g of silica gel for onward analysis at the School of Biosecurity, Biotechnical and Laboratory Sciences (SBLS), Makerere University, Kampala, Uganda.

Materials used for the molecular taxonomic study include Liquid nitrogen, STE (Sodium Chloride-Tris-EDTA) buffer, chloroform: isoamyl alcohol (24:1), 3M Sodium acetate, ice-cold 70% ethanol, ice-cold absolute ethanol, Elution buffer AE, Agarose powder, ISSR oligonucleotides, Nuclease-free water, 2x My Taq Red mix, Mg Cl_2, 1x TAE buffer, 50bp DNA ladder, Distilled water, Porcelain mortar and pestle, 1.5ml microfuge tubes, Vortex, Water bath, Thermometer, Laminar flow hood, Heating block, Refrigerators (-20^{0}C and 4^0 C), Thermocycler, Electrophoresis Tank and power source, Gel Documentary system, Analytical weighing balance, Micropipettes (P10, P100 and P1000), micropipette tips, Hand gloves, PCR tubes (200ul), Tissue papers and sample boxes.

Table 3.2. Number of Samples used for Molecular Taxonomic Study of *Ricinodendron heudelotii*

S/N	OTUs	Number of Samples
1	OSU	8
2	Ibadan	4
3	Oloruntele	3
4	Ife	6
5	Ikoyi	8
6	Onigambari	5
7	Akure	8
8	Benin	6
Total		48

3.3.1 DNA Extraction Procedures

DNA was extracted from young leaves using the Sodium Tris-Chloride EDTA (STE) method of Hosseinpour and Nematadeh (2013) with some modifications through the following steps:

3.3.2 Homogenisation

About 200mg of leaf sample was ground in liquid nitrogen to powder form using porcelain mortar and pestle. The ground **s**ample was transferred into a 1.5 ml microfuge tube and 500µl of STE lysis buffer [0.1M NaCl, 10mM Tris.Cl (pH 8.0), 1%SDS, 1mM NaEDTA (pH 8.0) and 0.6M β-mecaptoethanol] was added and mixed by vortexing for 15 seconds using a Biosan MSC-3000 Vortex (Appendix 10). The emulsion was incubated for one (1) hour at 65°C in a water bath with vortexing for every 15 minutes.

3.3.3 Phase separation

After incubation, the emulsion was centrifuged at 12000g for 5 minutes using an Eppendorf Refrigerated Centrifuge 5425R (Appendix 11) to separate the supernatant layer from the cell debris. The supernatant was transferred into a clean microfuge tube and 250µl of Chloroform: Iso Amyl Alcohol (24:1) was added to the supernatant, mixed by inversion and centrifuged at 13000 rpm for 1m at room temperature to properly separate the solution into two aqueous' phases.

3.3.4 DNA precipitation

The resulting upper aqueous phase, which contained the DNA was transferred into a clean microfuge tube, mixed with 3M sodium acetate (with the volume equivalent to 10% of the aqueous solution) and 3 volumes of ice-cold absolute ethanol. The microfuge tube was inverted severally for about 10s and incubated at -20°C for 24 hours for optimum precipitation of the DNA.

3.3.5 DNA wash

The centrifuge machine was first spun at 13000rpm for 15 minutes to adjust its temperature to 4°C before the precipitated DNA was centrifuged at 13000rpm for 30 minutes. The DNA pellet got stuck to the bottom of the tube and the supernatant was discarded, then 500µl of ice-cold 70% ethanol was added to the pellet while the

micropipette was used to disintegrate the pellet properly into the ethanol. The solution was centrifuged at 13000rpm for 15 minutes, after which the supernatant was discarded leaving only the washed DNA pellet in the microfuge tube.

3.3.6 Drying and Elusion of DNA Pellets

After wash, the microfuge tube containing the pellet was blotted and turned upside down on tissue paper spread under the laminar flow at room temperature for 30 minutes for proper drying of the pellet. DNA pellet was eluted in considerable volume (50-400µl depending on the quantity of the pellet) of elusion buffer AE and heated for about five (5) minutes on a heating block at 70°C.

3.3.7 Determination of the quality and quantity of extracted DNA

The presence of DNA was tested on a 1% agarose gel electrophoresis. The presence of a single band located close to the wells is an indication of a genomic DNA pellet. However, out of the forty-eight (48) samples subjected to extraction, nineteen (19) produced good quality genomic DNA. The extracted DNA was quantified using NanoPhotometer (Appendix 12), aliquoted and stored at -20°C for onward polymerase chain reaction (PCR).

3.3.8 Inter Simple Sequence Repeat (ISSR)

This study was carried out using a total of thirty-five (35) ISSR Oligonucleotides (Table 3.3), which were synthesized by Eurofins Genomics, Ebersberg Germany.

3.3.9 Reconstitution of Oligonucleotides

The stock solution of the primers was reconstituted to 100pmol/µl by adding a considerable volume of nuclease-free water into each vial containing the oligonucleotide as provided in the synthesis report of the primer manufacturer. The vials were centrifuged at 8000rpm for 30 minutes to get all the lyophilised DNA dissolved in the water. The working solution of the primers was further diluted to 10 pmols/µl by adding a 90µl of nuclease-free water to 10µl of the stock solution in a microfuge tube.

3.3.10 Polymerase Chain Reaction (PCR) Optimisation and Amplification of DNA

PCR optimisation was carried out for all the thirty-five (35) reconstituted primers. DNA template was taken from twelve (12) genomic DNA samples that appeared to be of good quality. PCR optimisation was achieved by varying the annealing temperature for different reactions using each primer to determine the optimum reaction condition (Appendices 14 to 28). PCR was carried out in a master mix reaction volume of 12.5µl consisting of 6.25µl 2x My Taq Red Mix, 0.3µl primer, 0.1µl $MgCl_2$, 4.85µl nuclease-free water, and 1 µl DNA template. The optimised PCR amplification was carried out using a 96-well S1000 BIO-RAD thermal cycler (Appendix 13) with an initial denaturation at 94°C for 4mins.

This was followed by 35 cycles of DNA denaturation at 98°C for 30 secs, annealing of primers (which varied from 42°C to 51°C depending on the primer) for 1min and elongation at 72°C for 3 mins. The final extension was performed at 72°C for 10mins. After completion of the amplification, the reaction was left at 10°C until electrophoresis.

After the optimisation process, the PCR was carried out for the 19 samples of the 48 having good genomic DNA.

3.3.11 Electrophoresis and Visualisation of the amplified DNA

Separation of the amplified DNA fragments was carried out on 2% agarose gel, which contained ethidium bromide in 1x TAE buffer through electrophoresis at a constant voltage of 125 for 30 minutes. The approximate molecular weight of the amplified DNA fragments was estimated using a 50bp bioline DNA ladder. Electrophoresed DNA profiles were visualised and photographed using a Gel Documentation system.

Table 3.3. List of Inter Simple Sequence Repeat Oligonucleotide used in this Study

S/N	Primer name	Primer sequences
1	Primer A	AGAGAGAGAGAGAGAGT
2	Primer B	ATGATGATGATGATG
3	UBC 859	TGTGTGTGTGTGTGTGAC
4	UBC 849	GTGTGTGTGTGTGTGTCG
5	UBC 848	CACACACACACACACAGG
6	UBC 812	GAGAGAGAGAGAGAGAA
7	UBC 816	CACACACACACACACAT
8	UBC 818	CACACACACACACACAG
9	UBC 825	ACACACACACACACACT
10	UBC 834	AGAGAGAGAGAGAGAGYT
11	UBC 836	AGAGAGAGAGAGAGYA
12	UBC 844	CTCTCTCTCTCTCTCTRC
13	UBC 857	ACACACACACACACACYG
14	UBC 864	ATGATGATGATGATGATG
15	UBC 885	HBHAGAGAGAGAGAGAG
16	UBC 822	TCTCTCTCTCTCTCTCA
17	A12	GAGAGAGAGAGACC
18	ISSR 3	GAG AGA GAG AGA GAG AT
19	ISSR 7	ACA CAC ACA CAC ACA CT
20	ISSR 17	GGCGGCGGCGGCGGC GGC
21	ISSR 21	CTT CAC TTC ACT TCA
22	ISSR 22	TAG ATC TGA TAT CTG AAT TCC C
23	ISSR 23	AGA GTT GGT AGC TCT TGA TC
24	ISSR 24	CAT GGT GTT GGT CAT TGT TCC A
25	ISSR 25	ACT TCC CCA CAG GTT AAC ACA
26	ISSR 808	AGAGAGAGAGAGAGAGC
27	ISSR 815	CTCTCTCTCTCTCTCTG
28	ISSR 816	CACACACACACACACAA
29	UBC 840	GAGAGAGAGAGAGAGAYT
30	UBC 873	GACAGACAGACAGACA
31	UBC 874	CCCTCCCTCCCTCCCT
32	UBC 823	TCTCTCTCTCTCTCTCC
33	UBC 842	GAGAGAGAGAGAGAGAYG/TG
34	UBC 845	CTCTCTCTCTCTCTCTRG
35	UBC 868	GAAGAAGAAGAAGAAGAA

Source: The current study

3.3.12 Scoring of DNA Bands and Data analysis

The presence of bands of different molecular weights was visually scored as one (1) while those bands that were absent were scored as zero (0). However, regardless of the intensity, bands having the same molecular weight and mobility level were taken as identical fragments. Scored bands were further coded into data matrices in a spreadsheet, which was transferred into the STATISTICA programme for statistical analysis.

Using molecular data, polymorphic information content (PIC), genetic diversity, similarity index, and unique allele were determined. Using unweighted pair group method with arithmetic average (UPGMA) (Nei and Li, 1979) distance, phylogenetic tree analysis was performed on Treecon version 1.3b (Peer and Wachter, 1994) at 1000 bootstrapping replicates to produce a phylogenetic tree.

3.4 Study 3: Germination study for *R. heudelotii*

3.4.1 Experimental procedure

This study was carried out at the Department of Forest Production and Products, University of Ibadan. Seeds were extracted from the collected fruits using a sharp knife to separate the fruit pulp from the seed. Based on the results of the existing studies on the germination of the tree species, a total of six hundred (600) seeds were used for the experiment.

The experimental design for this study was 3x2 factorial experiment in a completely randomized design (CRD). Two factors were involved in this study: pre-germination treatment and sowing media. The pre-germination treatment had two levels, which include scarified seeds soaked in cold water for 24 hours, scarified seeds without soaking and control.

The mechanical scarification method used was filing before subjecting them to further treatments. The mechanical scarification was achieved by removing the hard seed coat at the micropylar end of the seed using a file to allow penetration of moisture into the seed endosperm.

There were two levels of sowing media, sterilized river-sand and topsoil. The total number of treatment combinations obtained from the two factors was 6. Two (2) batches of seeds

with each batch containing fifty (50) uniform seeds were allocated to each of the treatment combinations that were used for this study. One seed batch is equivalent to one replication of the experiment.

Out of the total number, four hundred (400) seeds were scarified using mechanical scarification while two hundred (200) seeds used as control were un-scarified. Two hundred (200) out of the seeds scarified were subjected to soaking in water at room temperature for 24 hours; this was divided into two groups, in which each half was sown in topsoil (100 seeds) and sterilized river-sand (100 seeds) prepared in germination trays. The remaining two hundred (200) un-scarified seeds were sown directly into topsoil (100 seeds) and sterilized river-sand (100 seeds) without soaking. In the same vein, two hundred (200) seeds used as control were also sown in topsoil (100 seeds) and sterilized river-sand (100 seeds).

Seeds were watered once daily and monitored for germination daily. The experiment was terminated after twelve (12) weeks when no further germination was identified.

The Mathematical model for this design is:

$Yij = \mu + A_i + B_j + (AB)_{ij} + e_{ij}$

Yij = Individual observation

μ = Overall mean

Ai = Effect of pre-germination treatments

Bj = Effect of sowing media

$(AB)ij$ = Interaction effect

eij = Experimental error

3.4.2 Data analysis

Percentage of seeds germinated were transformed using Arcsin transformation and analysed with Descriptive Statistics, Analysis of variance (ANOVA) and t-test. ANOVA was used to analyse the seed germination potential of *R. heudelotii* while a t-test was used to compare the germination based on sowing media. Tukey HSD was used to compare the data based on the treatment combination.

3.5 Study 4: Alley Cropping Field Experiment of *Ricinodendron heudelotii*

3.5.1 Experimental procedure

Plot selection and demarcation

The purpose of this experiment was to determine the early growth of *Ricinodendron heudelotii* in tandem with the crop. The study lasted 15 months and was conducted at the Teaching and Research Farm at the University of Ibadan in Nigeria (from April 2017 to July 2018). This experiment followed the method described by Keerthisena and Gunawardana (1996). *Ricinodendron heudelotii* was used as the alleys while maize was utilised as the test crop due to its significant contribution to human survival. It equally served as input material for the production of many other food products (Rouf *et al*., 2016).

A plot of size 30 m x 10 m was demarcated for the alley treatments while a 15 m x 10 m dimension was used as the control plot. Two types of alley width were used. These include 6 m and 3 m alley widths. The difference between the alley plot and the control plot is the planting of *R. heudelotii* in alleys while the control plot had no tree at all. Hence, due to the flat surface of the experimental plot, a completely randomized design with three replications was utilised. A replicate is equivalent to an alley.

Soil sampling and planting

Soil samples were systematically collected from the plots before and after the experiment to determine the impact of agroforestry on the soil nutrients. This collection was achieved with the use of a soil auger. In preparation of the experimental plots for the planting of alleys and maize, the soil was first ploughed using a tractor after which linning-out and pegging were carried out.

A total of seventy-seven seedlings of *Ricinodendron heudelotii* obtained from the germination study were used to plant the alleys. The alleys were planted using the espacement of 1 m for between tree distances (Figure 3.3). The alley plot, therefore, contained seven rows of trees with each row containing 11 trees. The seven rows represent 6 alleys out of which the first three (3) alleys were 6 m while the remaining 3 were 3 m alleys. The trees were first managed for 6 months to give room for their stabilization

before the introduction of maize as a test crop. The same post-planting management of the plot was used for the control and the alley plots.

The test crop was cultivated in the wet and dry seasons of the year. Unfortunately, the incidence of pests impeded the survival of the dry season crop. Maize seeds were planted at a row distance of sixty (60) centimetres in the alleys of trees and plot without trees. Based on the espacement, there were seven rows of maize in the 6 meters' alley while the 3 meters alley contained two rows of maize as used by Reynolds *et al*. (2007). The distance of the maize rows to the alleys were four treatments; 120 cm, 180 cm, 240 cm, and 300 cm (Keerthisena and Gunawardana, 1996).

Data collection

During the wet season, the number of leaves, height and collar diameter of maize were obtained fortnightly for 3 months. Similarly, the trees' height, collar diameter, and the number of leaves were measured every month for a year to ascertain the growth rate of the species. The height and diameter were measured with a metre tape and a Vernier caliper, respectively, and the number of leaves was determined visually. After the tree species had been managed for twelve months, pollarding was done. This was to test the regeneration potential of the tree. The resultant leaves were applied to the soil as mulch. Coppice lengths and the leaf numbers on pollarded trees were determined for three months before the experiment was terminated.

Soil samples collected were dried and subjected to nutrient analysis; these include exchangeable cations such as Ca^{2+}, K^{+}, Na^{+}, Mg^{2+}, and Fe^{3+}. Soil pH, organic carbon, total nitrogen and available phosphorus were also determined according to Ogeh and Ogwurike (2006).

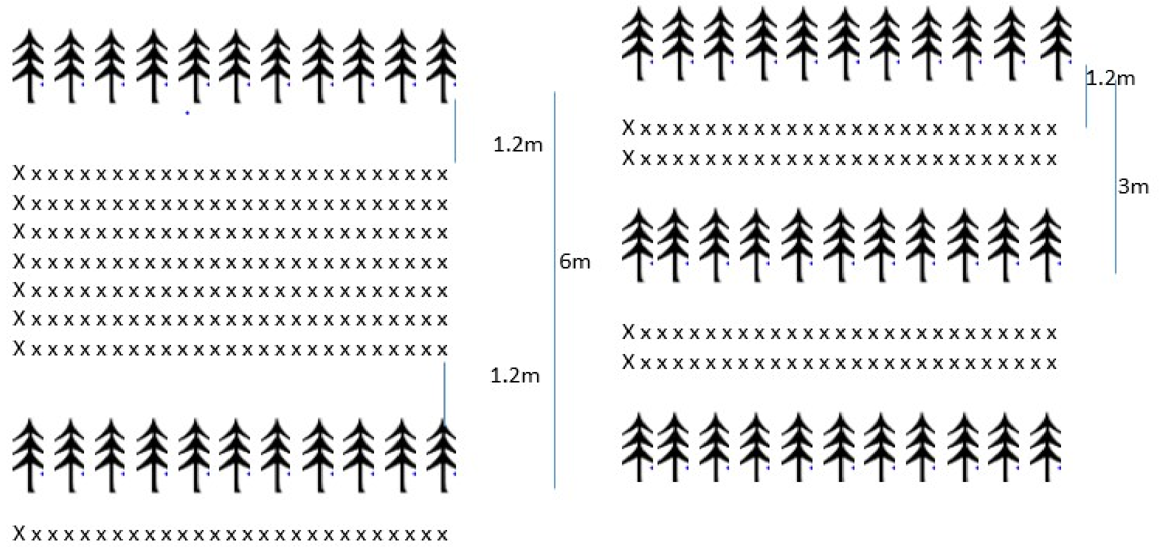


Figure 3.3. Field layout of the alleys

The statistical model for this experiment is given as:

$Yij = \mu + Tj + eij$

Yij = Individual observation

μ = Overall mean

Tj = Alley

eij = Experimental error

3.5.2 Data Analysis

Data were subjected to analysis of variance (ANOVA), t-test and non-parametric test for two or more treatment groups.

CHAPTER FOUR

RESULTS

4.1 Epidermal and Macro Morphological Characteristics of *Ricinodendron heudelotii* in southern Nigeria

The adaxial and abaxial epidermal layers of *Ricinodendron heudelotii* leaves from different operational taxonomic units (OTUs) in Nigeria were largely characterised by polygonal cell shape (Plates 4.1 and 4.2). The wall pattern for all the OTUs was periclinal except for the Osu, which was anticlinal on the adaxial surface. The entire anticlinal walls pattern was straight to arch pattern. The leaves of *R. heudelotii* for all the OTUs were amphistomatic (having stomata on the abaxial surface than the adaxial surface) with paracytic stomata (Plates 4.1 and 4.2). Striations were found on the epidermal surfaces of Osu, Ibadan, Oloruntele, Ile-Ife, Ikoyi, Onigambari and Akure but absent on Benin and Boki. Trichome was not observed in all the epidermal peels.

The epidermal characteristics of *Ricinodendron heudelotii* of different OTUs in southern Nigeria were significantly different ($p<0.05$) except for the number of stomata, number of epidermal cells, epidermal cell widths (ECW) and stomatal index of the adaxial surface and epidermal cell widths and stomatal length of the abaxial surface (Table 4.1 and 4.2) (Appendices 33 to 36). The highest number of stomata per microscopic field of view was 20 on the adaxial leaf surface of Oloruntele foliar epidermal peel and 80 on abaxial surfaces of Benin foliar epidermal peel (Table 4.1). The least number of stomata per field of view was discovered in Ikoyi for the adaxial surface (3) and the Ibadan for the abaxial surface (8). Epidermal cell count on the adaxial surface was predominant in the specimen from Ibadan and Oloruntele with 241 cells each. On the abaxial surface (Table 4.2), the highest number (255) of epidermal cells was discovered from Ikoyi and the least (93) was from Akure. The number of wall sides ranged from 4 to 11. Akure had the highest epidermal cell length (44.5±2.8 µm), stomatal length (34.9±2.0 µm) and pore size (273.5±103.3 μm^2) on the adaxial surface.

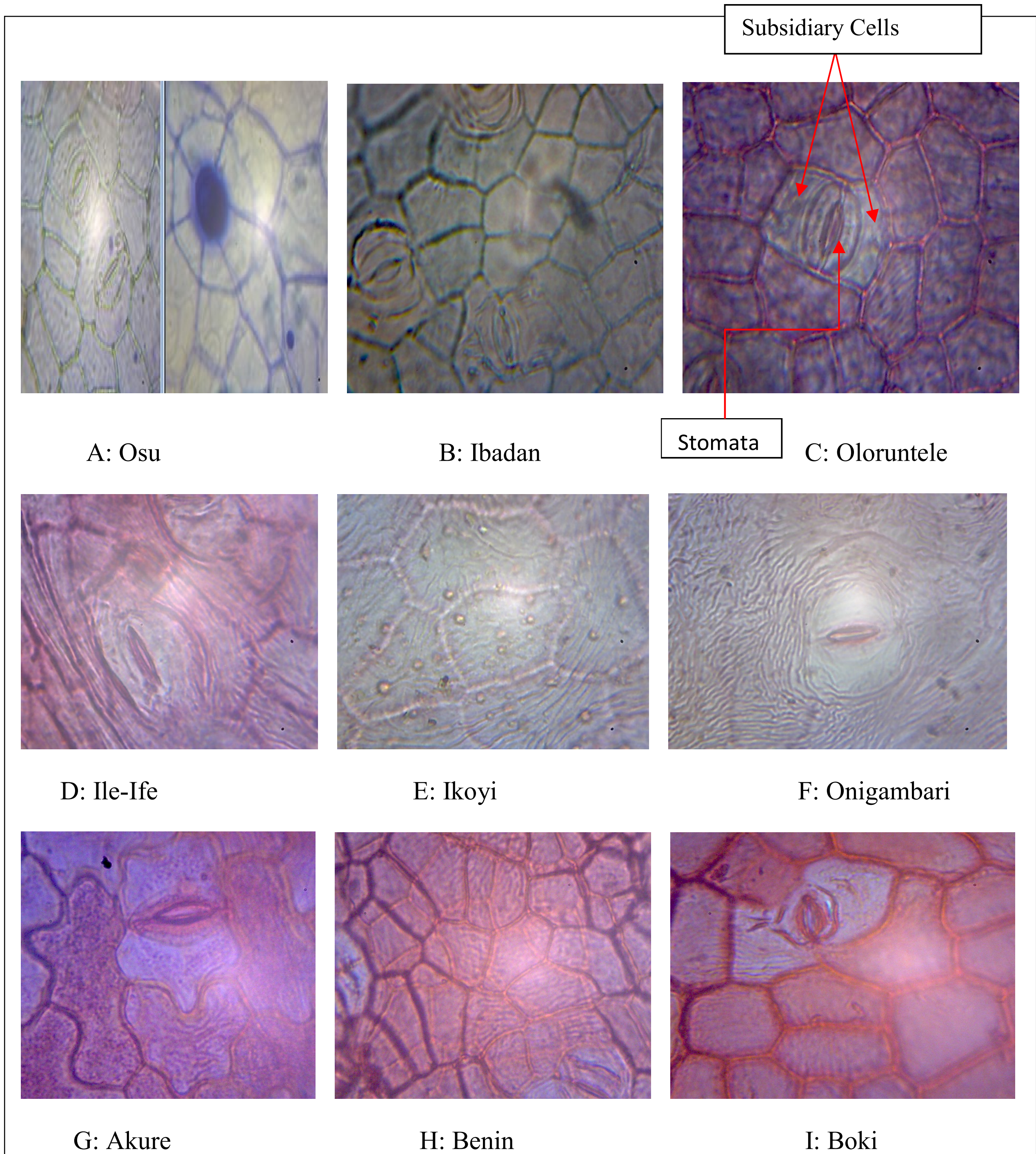


Plate 4.1. Photomicrographs (x400) of the adaxial epidermal layer of *Ricinodendron heudelotii* leaves from different operational taxonomic units

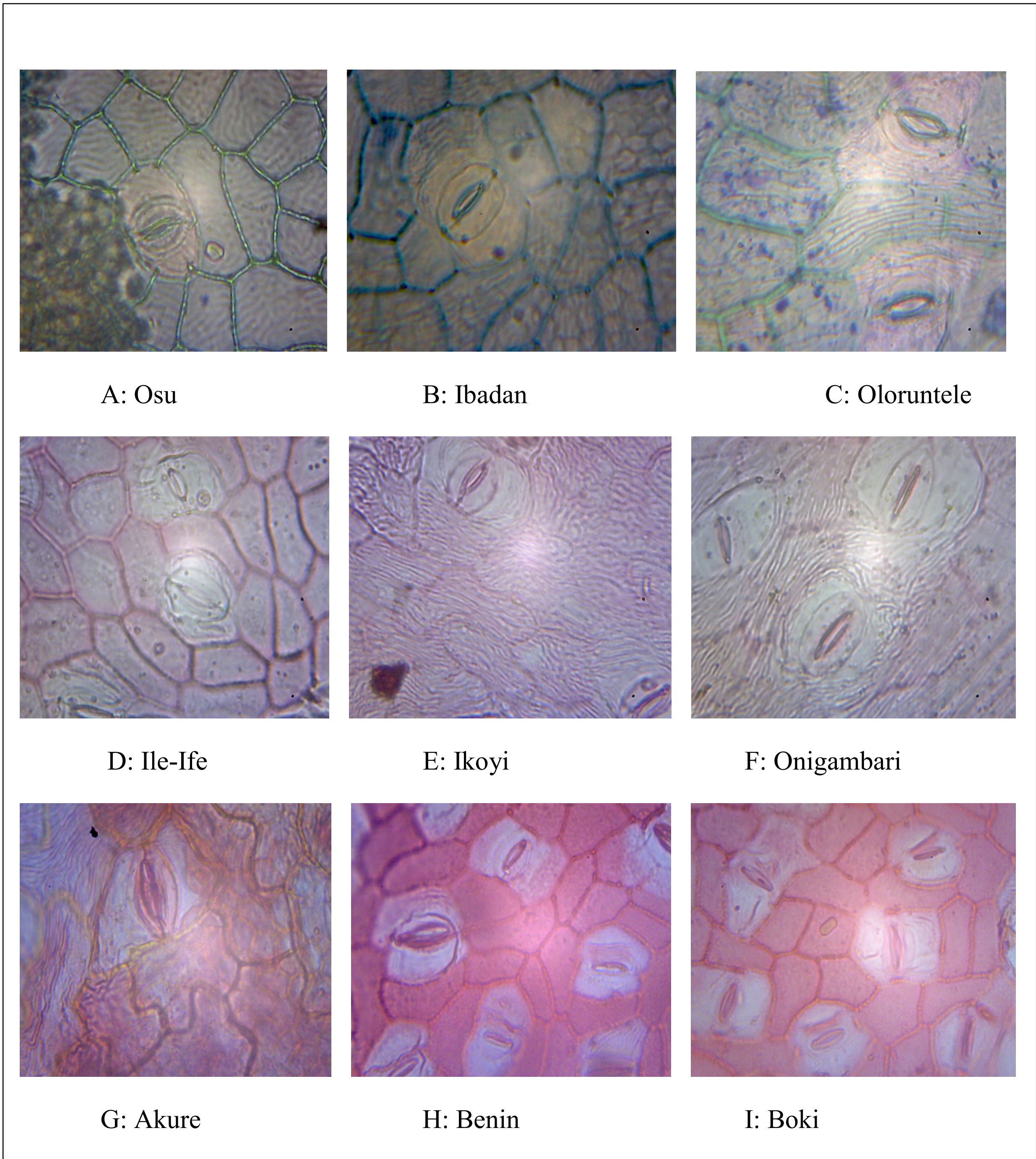


Plate 4.2. Photomicrographs (x400) of the abaxial epidermal layer of *Ricinodendron heudelotii* leaves from different operational taxonomic units

Table 4.1. Summary of adaxial epidermal characteristics of *Ricinodendron heudelotii* from different Operational Taxonomic Units (OTUs) in Nigeria

OTUs	NS	NEC	NWS	ECL (µm)	ECW (µm)	GCA (µm^2)	SL(µm)	SW (µm)	SI (%)	GCW(µm)	PS(µm^2)
Osu	18	164	5	37.6±5.5^{ab}	22.5±10.6	97.2±18.5^{bc}	22.0±1.8^{b}	14.6±0.8^{b}	11.4±11.2	5.6±0.6^{c}	44.4±7.6^{c}
Ibadan	14	241	5	22.4±7.0^{c}	17.6±5.5	118.2±71.3^{bc}	24.1±6.7^{b}	14.8±4.6^{b}	5.0±4.0	5.9±2.1^{c}	39.8±20.9^{c}
Oloruntele	20	241	5	31.3±4.0^{abc}	22.0±3.0	144.5±44.3^{bc}	22.0±4.5^{b}	21.2±2.6^{b}	7.9±6.8	8.2±0.9^{bc}	52.6±19.9^{c}
Ile-Ife	4	223	5	24.9±6.0^{bc}	20.7±5.0	96.9±16.0^{bc}	22.0±1.8^{b}	13.5±2.8^{b}	1.7±1.2	5.6±1.3^{c}	37.0±10.2^{c}
Ikoyi	3	194	7	19.6±5.4^{c}	13.7±1.1	86.4±12.7^{c}	23.3±2.6^{b}	18.8±6.7^{b}	1.7±1.1	4.7±0.1^{c}	24.3±4.1^{c}
Onigambari	8	189	5	37.6±5.5^{ab}	22.0±3.0	126.8±52.0^{bc}	22.5±2.7^{b}	16.5±4.8^{b}	4.2±4.2	7.0±2.1^{bc}	23.8±2.7^{c}
Akure	12	100	8	44.5±2.8^{a}	21.8±2.3	292.4±16.0^{a}	34.9±2.0^{a}	32.8±3.4^{a}	11.0±4.6	10.6±0.6^{ab}	273.5±103.3^{a}
Benin	11	195	5	28.2±3.2^{bc}	17.3±0.4	218.6±17.7^{ab}	26.6±2.8^{ab}	31.7±3.2^{a}	5.1±1.7	10.5±1.6^{ab}	172.3±33.9^{b}
Boki	9	187	5	31.7±3.2^{abc}	20.2±2.5	293.9±84.5^{a}	28.8±2.1^{ab}	36.2±6.4^{a}	4.4±1.5	12.9±3.1^{a}	204.3±10.4^{ab}
p-value	0.170ns	0.131ns	0.019*	0.000*	0.364ns	0.000*	0.002*	0.000*	0.240ns	0.000*	0.000*

NS=number of stomata, NEC=number of epidermal cells, NWS= number of wall sides, ECL= epidermal cell length, ECW= epidermal cell width, GCA= guard cell area, SL= stomatal length, SW= stomatal width, SI=stomal index, GCW= guard cell width, PS=pore size.

*= Significant at 5% probability level, ns= not significant 5% probability level

Means of any set of treatments with the same superscripts along the same column are not significantly different

Table 4.2. Summary of abaxial epidermal characteristics of *Ricinodendron heudelotii* from different Operational Taxonomic Units (OTUs) in Nigeria

OTUs	NS	NEC	NWS	ECL (µm)	ECW (µm)	GCA (μm^2)	SL(µm)	SW (µm)	SI (%)	GCW(µm)	PS(μm^2)
Osu	19	200	5	38.8±15.2^{ab}	19.2±7.3	78.9±25.1^{c}	24.0±4.3	12.6±2.6^{b}	9.1±5.5^{b}	4.2±1.0^{b}	66.1±19.0^{bc}
Ibadan	8	242	5	19.2±8.7^{c}	14±6.6	99±33.0^{c}	21.0±3.4	16.6±2.7^{b}	2.8±2.0^{b}	5.9±1.1^{b}	65.8±39.1^{bc}
Oloruntele	59	170	5	28.4±14.3^{abc}	17.2±9.3	105.9±24.8^{c}	23.2±8.2	15.9±3.0^{b}	28±23.1^{a}	6.0±1.0^{b}	45.5±29.7^{c}
Ile-Ife	44	119	7	28.1±3.5^{abc}	16.7±3.0	97.7±18.8^{c}	25.6±5.5	13.5±1.3^{b}	26.3±8.7^{a}	4.9±0.1^{b}	43.7±13.8^{c}
Ikoyi	13	255	5	36.9±7.1^{abc}	24.4±3.0	72.7±7.0^{c}	20.4±3.6	14.8±0.5^{b}	5±0.9^{b}	4.6±0.9^{b}	76.4±17.9^{bc}
Onigambari	10	145	5	42.8±13.3^{a}	22.2±5.1	93.9±3.6^{c}	21.0±3.4	14.4±2.7^{b}	6.3±1.3^{b}	5.8±1.2^{b}	40.3±8.0^{c}
Akure	11	93	11	43.7±8.8^{a}	33.2±18.1	243.1±30.5^{a}	29.4±2.4	34.1±0.9^{a}	10.5±0.09^{b}	10.5±1.2^{a}	322.8±78.5^{a}
Benin	80	153	4	22.2±1.8^{bc}	12.9±1.6	183.3±27.7^{b}	21.7±4.1	29.2±5.1^{a}	34.4±1.6^{a}	10.9±2.2^{a}	112.1±17.0^{b}
Boki	75	223	5	27.1±2.1^{abc}	13.8±3.2	223.0±55.0^{ab}	22.6±2.3	29.8±5.2^{a}	25.2±0.4^{a}	12.5±2.6^{a}	75.4±9.6^{bc}
p-value	0.026*	0.013*	0.024*	0.046*	0.106ns	0.000*	0.338ns	0.000*	0.001*	0.000*	0.000*

NS=number of stomata, NEC=number of epidermal cells, NWS= number of wall sides, ECL= epidermal cell length, ECW= epidermal cell width, GCA= guard cell area, SL= stomatal length, SW= stomatal width, SI=stomal index, GCW= guard cell width, PS=pore size.

*= Significant at 5% probability level, ns= not significant 5% probability level

Means of any set of treatments with the same superscripts along the same column are not significantly different

The ECW (22.5±10.6 μm) and stomatal index (11.4±11.4 %) on the adaxial surface were highest in Osu while guard cell area (293.9±84.5 $μm^2$), stomatal width (36.2±6.4 μm) and guard cell width (12.9±3.1 μm) were highest in Boki. On the abaxial surface, Akure had the highest epidermal cell length (43.7±8.8 μm), epidermal cell width (33.2±18.1 μm), guard cell area (243.1±30.5 $μm^2$), stomatal length (29.4±2.4 μm), stomatal width (34.1±0.9 μm) and pore size (322.8±78.5 $μm^2$). Stomatal index (34.4±1.6 %) and guard cell width (12.5±2.6 μm) were highest in Benin and Boki respectively.

The leaf and fruit taxonomic characters of *Ricinodendron heudelotii* from different operational taxonomic units (Tables 4.3 and 4.4) were significantly different ($p<0.05$) (Appendices 37 to 40). The number of leaflets (NLL) varied from 4 to 7 among the operational taxonomic units while the number of secondary veins ranged between 8 and 15 with the highest from Benin and Ikoyi. Acumen length ranged from 0.9±0.2 cm (Boki) to 2.2±0.8 cm (Ikoyi). Osu had the least leaf total length (22.3±5.7 cm), lamina length (10.7±3.7 cm), petiole width (1.7±0.4 mm), leaf width at the base (3.2±0.7 cm), leaf width at the middle (4.8±0.7 cm), leaf width at the top (3.6±2.0 cm) and space between secondary veins (0.9±0.1 cm). The least petiole length (8.9±0.1 cm) was found in Boki, while, Onigambari had the highest leaf total length (53.0±5.8 cm) and petiole length (30.9±5.0 cm). Boki had the highest lamina length (22.9±4.1 cm), leaf width at the middle (12.2±6.3 cm) and leaf width at the top (9.4±5.4 cm). In the same vein, Ibadan had the highest petiole width (5.5±0.8 cm), leaf width at the base (5.7±1.3 cm) and space between secondary veins (3.7±0.7 cm).

Fruits taxonomic characteristics of *Ricinodendron heudelotii* from different OTUs (Table 4.4) were significantly different ($p<0.05$). The number of seeds per fruit varied from 2 to 3 with only the fruits from Osu and Boki having an average abortion of 1. The highest values for taxonomic characters such as fruit weight (40.5±7.4 g) and seed weight (10.9±2.5 g) were observed from the University of Ibadan. Oloruntele had the highest values for fruit smallest width (40.9±3.1 mm) and pulp weight (34.3±7.2 g) while seed diameter (16.3±0.6 mm) was highest in Ile-Ife. Akure had the highest seed length (17.1±0.7 mm) and roundness ratio (1.31) among the OTUs from Southern Nigeria. The highest fruit length (45.4±2.6 mm) was found in Boki.

Table 4.3. Leaf characters of *Ricinodendron heudelotii*

OTUs	NLL	NSV	AL (cm)	LTL (cm)	LL (cm)	PW (mm^2)	LWB (cm)	LWM (cm)	LWT (cm)	PL (cm)	SBSV (cm)
Benin	5	15	1.3±0.7^{b}	34.9±3.9^{c}	16.6±2.0^{abcd}	4.2±0.8^{ab}	4.9±0.6^{ab}	6.2±0.6^{bc}	3.7±0.5^{b}	16.4±3.3^{bc}	3.3±0.6^{a}
Ibadan	5	13	1.0±0.2^{b}	48.2±8.4^{a}	20.6±3.2^{abc}	5.5±0.8^{a}	5.7±1.3^{a}	8.0±1.2^{bc}	5.3±0.8^{b}	24.7±5.6^{ab}	3.7±0.7^{a}
Ile-Ife	5	12	1.5±1.0^{ab}	32.0±11.0^{c}	15.2±4.5^{cd}	3.8±0.7^{bc}	3.4±1.3^{b}	6.1±1.3^{bc}	4.3±2.4^{b}	16.7±6.6^{bc}	1.0±0.2^{b}
Onigambari	5	11	1.5±0.4^{ab}	53.0±5.8^{a}	22.0±2.4^{ab}	5.4±0.6^{a}	5.0±0.4^{ab}	8.7±0.5^{b}	5.5±0.6^{b}	30.9±5.0^{a}	1.6±0.2^{b}
Oloruntele	5	14	1.9±0.8^{ab}	30.0±7.2^{c}	15.9±3.6^{bcd}	3.4±0.8^{bcd}	4.7±1.1^{ab}	6.1±1.6^{bc}	3.8±1.8^{b}	14.1±4.1^{bc}	1.2±0.5^{b}
Ikoyi	4	15	2.2±0.8^{a}	39.4±4.5^{b}	18.2±1.3^{abc}	2.5±0.6^{cde}	3.8±0.2^{ab}	6.3±0.6^{bc}	3.7±0.2^{b}	18.3±6.4^{bc}	1.2±0.08^{b}
Osu	5	8	1.4±0.7^{b}	22.3±5.7^{c}	10.7±3.7^{d}	1.7±0.4^{e}	3.2±0.7^{b}	4.8±0.7^{b}	3.6±2.0^{b}	9.5±3.6^{c}	0.9±0.1^{b}
Boki	4	14	0.9±0.2^{b}	42.6±12.8^{b}	22.9±4.1^{a}	2.0±0.07^{de}	4.3±0.9^{ab}	12.2±6.3^{a}	9.4±5.4^{a}	8.9±0.1^{c}	1.1±0.1^{b}
Akure	7	8	1.8±0.4^{ab}	43.4±8.3^{b}	19.5±3.2^{abc}	2.5±0.5^{cde}	4.7±0.9^{ab}	7.4±1.7^{bc}	4.0±1.4^{b}	21.0±6.8^{b}	1.3±0.2^{b}
p-value	0.000*	0.006*	0.038*	0.000*	0.000*	0.000*	0.002*	0.000*	0.003*	0.000*	0.000*

NLL= number of leaflet, NSV= number of secondary veins, , AL= acumen length, LTL= leaf total length, LL= leaflet length, PW= petiole width, LWB= leaf width at the base, LWM= leaf width at the middle, LWT= leaf width at the top, PL= petiole length, SBSV= space between secondary veins

*= Significant at 5% probability level

Means of any set of treatments with the same superscripts along the same column are not significantly different

Table 4.4. Fruit taxonomic characters of *Ricinodendron heudelotii*

OTUs	NSF	NAS	FLW (mm)	FSW (mm)	PW (g)	FL (mm)	FW (g)	SL (mm)	SD (mm^2)	SW (g)	RR
Benin	2	0	40.9±3.7^{a}	34.8±5.4^{c}	20.5±4.4^{c}	30.6±1.4^{c}	27.7±7.1^{c}	16.6±0.9^{a}	15.2±1.0^{ab}	7.2±3.0^{b}	0.82±0.07^{b}
Ibadan	3	0	42.4±2.4^{a}	38.9±5.3^{ab}	29.6±7.4^{ab}	35.3±4.2^{bc}	40.5±7.4^{a}	16.9±2.6^{a}	15.9±1.2^{a}	10.9±2.5^{a}	0.85±0.10^{b}
Ile-Ife	2	0	43.5±2.9^{a}	31.4±2.2^{cd}	22.7±3.2^{bc}	30.2±11.5^{c}	26.7±3.9^{c}	16.8±0.8^{a}	16.3±0.6^{a}	3.9±0.8^{c}	0.82±0.05^{b}
Onigambari	2	0	44.0±4.6^{a}	31.5±1.4^{cd}	28.8±5.6^{ab}	32.5±3.0^{bc}	31.1±5.7^{bc}	16.7±1.4^{a}	16.0±0.5^{a}	2.3±0.3^{cd}	0.87±0.06^{b}
Oloruntele	3	0	44.2±4.0^{a}	40.9±3.1^{a}	34.3±7.2^{a}	32.2±3.3^{bc}	35.9±7.7^{ab}	14.4±1.7^{b}	14.1±1.4^{b}	1.6±0.4^{d}	0.77±0.03^{c}
Ikoyi	2	0	33.7±5.4^{b}	35.7±5.4^{abcd}	19.3±4.0b^{c}	42.5±1.8^{ab}	23.0±5.0^{c}	15.0±0.3^{b}	12.6±0.4^{c}	1.7±0.4^{cd}	1.2±0.13^{a}
Osu	2	1	35.3±4.4^{b}	33.8±4.4^{bcd}	21.4±3.7^{bc}	39.8±4.7^{ab}	26.3±5.4^{c}	15.2±0.9^{b}	13.3±1.1^{bc}	2.3±0.3^{cd}	1.1±0.05^{a}
Boki	2	1	34.9±2.6^{b}	32.9±2.7^{cd}	26.6±4.1^{b}	45.4±2.6^{a}	31.9±4.7^{bc}	17.0±0.8^{a}	12.8±0.3^{bc}	2.3±0.2^{cd}	1.3±0.07^{a}
Akure	2	0	31.2±1.3^{b}	29.6±1.8^{d}	18.4±3.3^{c}	40.1±3.4^{ab}	24.0±4.2^{c}	17.1±0.7^{a}	12.9±0.9^{bc}	2.4±0.4^{cd}	1.31±0.11^{a}
p-value	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*

NSF= number of seeds per fruit, NAS= number of aborted seeds, FLW= fruit largest width, FSW= fruit smallest width, PW= pulp weight, FL= fruit length, FW= fruit weight, SL= seed length, SD= seed diameter, SW= seed weight, RR= roundness ratio.

*= Significant at 5% probability level

Means of any set of treatments with the same superscripts along the same column are not significantly different

On the other hand, the least fruit largest width (31.2±1.3 mm), fruit smallest width (29.6±1.8 mm) and pulp weight (18.4±3.3 g) were recorded in Akure. Whereas, the least seed length (14.4±1.7 mm), seed weight (1.6±0.4 g) and roundness ratio (0.77±0.03) were discovered in Oloruntele. Ikoyi had the least fruit weight (23.0±5.0 g) and seed diameter (12.6±0.5 mm) while the least fruit length was obtained in Ile-Ife.

Figure 4.1 indicates the scree plot for principal component analysis of *Ricinodendron heudelotii* using epidermal characters. The plot flattens out at the second component, therefore only the first principal component is retained for the taxonomic delimitation of the OTUs. Approximately 77.3% of the total variation in the characters was explained by the first principal component having about 26522.5 eigenvalues (Table 4.5). Of all the epidermal characters subjected to principal component analysis (PCA), adaxial guard cell area, adaxial pore size, stomatal length for abaxial and pore size for abaxial with 0.47, 0.57, 0.38 and 0.47 loadings respectively were significant for the delimitation of the populations (Table 4.5).

The first three principal components were significant for the taxonomic delimitation of the species into groups (Figure 4.2) using leaf and fruit characters. The first three principal components (PC) accounted for about 89.1% of the total variations with 286.9 eigenvalues (Table 4.6). Out of the total percentage contributions, PC1, PC2 and PC3 accounted for 47.8%, 26.5% and 14.8% respectively of the variations in the population. Petiole length, leaf total length, leaflet length, leaf width at the middle, fruit length, fruit weight, fruit largest width, fruit smallest width and pulp weight having 0.48, 0.71, 0.24, 0.20, 0.49, 0.40, 0.43, 0.27 and 0.40 loadings respectively were highly loaded to the taxonomic delimitation of *R. heudelotii* populations based on leaf and fruit descriptors. The scatter plots (Figures 4.3, 4.4 and 4.5) derived from the PCA separated the *Ricinodendron heudelotii* in the study area into different groups by OTUs with some located close to each other. Adaxial guard cell area (0.48), pore size (0.57), abaxial stomatal length (0.39) and pore size were significantly loaded to the taxonomic variation among the OTUs using the pooled morphological data (Table 4.7). Four major clusters were produced according to the phylogenetic tree (Figure 4.6) based on the pooled morphological characters. Akure, Benin and Oloruntele were separated while Onigambari, Ile-Ife, Ikoyi, Ibadan and Osu populations formed a group that is more closely related.

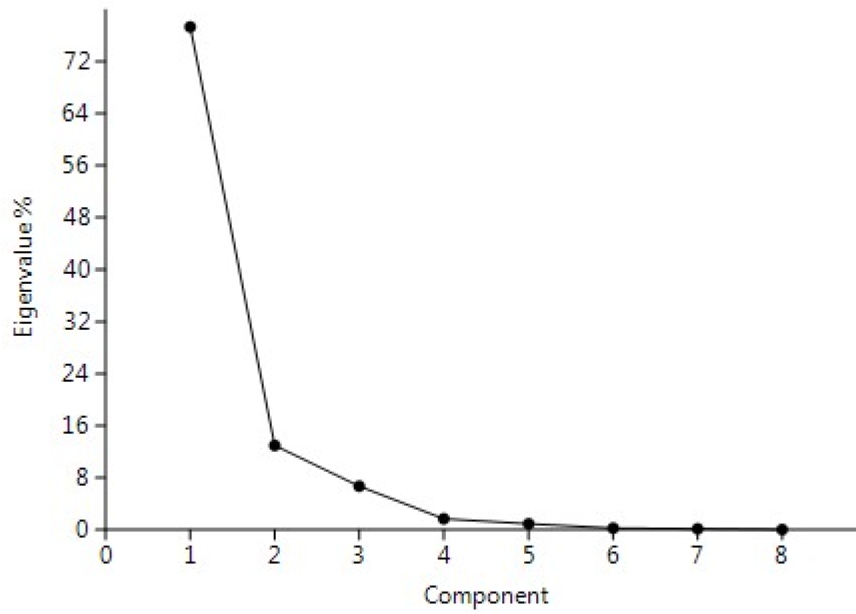


Figure 4.1. Scree plot for principal component analysis of *Ricinodendron heudelotii* using epidermal characters

Table 4.5. Eigenvalue, percentage variance and loadings for the principal components from epidermal characters of *Ricinodendron heudelotii*

	Principal components							
	1	2	3	4	5	6	7	8
Eigen value	26522.50	4449.21	2298.21	577.09	311.50	81.81	47.66	7.01
% variance	77.34	12.97	6.70	1.68	0.91	0.24	0.14	0.02
Characters				Loadings				
NSad	0.00	0.00	-0.00	0.03	-0.00	0.55	-0.42	-0.07
NECad	-0.19	0.30	-0.15	0.78	-0.33	0.06	-0.00	0.08
ECLad	0.02	-0.04	-0.03	-0.17	-0.07	0.35	-0.23	-0.02
ECWad	0.00	-0.00	-0.03	-0.04	-0.04	0.13	-0.16	0.32
GCAad	**0.47**	0.39	-0.00	-0.18	-0.47	0.34	0.26	-0.08
SLad	0.02	-0.00	0.01	0.01	-0.02	-0.04	0.01	0.01
SWad	0.04	0.05	0.01	-0.00	0.05	0.05	0.24	-0.29
SIad	0.00	-0.01	0.00	-0.02	0.02	0.26	-0.24	0.05
NWSad	0.00	-0.01	0.01	-0.00	0.00	0.00	0.05	0.02
GCWad	0.01	0.02	-0.00	-0.01	-0.01	0.03	0.01	-0.05
PSad	**0.57**	0.18	0.04	0.03	0.32	-0.18	-0.37	0.05
NSab	0.04	0.34	-0.25	0.13	0.64	0.26	0.28	-0.03
NECab	-0.16	0.36	0.89	0.04	0.12	0.05	0.02	0.07
ECLab	0.00	-0.01	-0.00	0.00	-0.01	-0.00	0.00	0.23
ECWab	0.00	-0.08	0.01	-0.23	-0.03	0.15	0.38	0.26
GCAab	0.01	-0.07	0.02	-0.04	-0.04	0.06	0.33	0.29
Slab	**0.38**	0.23	-0.05	0.02	-0.16	-0.40	-0.06	0.17
Swab	0.01	-0.02	-0.01	0.00	0.01	0.01	-0.07	0.52
SIab	0.04	0.02	0.00	0.02	0.01	-0.06	0.04	-0.41
NWSab	0.01	0.10	-0.15	0.10	0.27	0.09	0.12	0.21
GCWab	0.01	0.02	-0.00	-0.00	-0.00	-0.02	0.01	-0.16
PSab	**0.47**	-0.60	0.27	0.46	0.05	0.18	0.18	-0.02

NS=number of stomata, NEC=number of epidermal cells, ECL= epidermal cell length, ECW= epidermal cell width, GCA= guard cell area, SL= stomatal length, SW= stomatal width, SI=stomatal index, NWS= number of wall sides, GCW= guard cell width, PS=pore size, ad = adaxial, ab= abaxial.

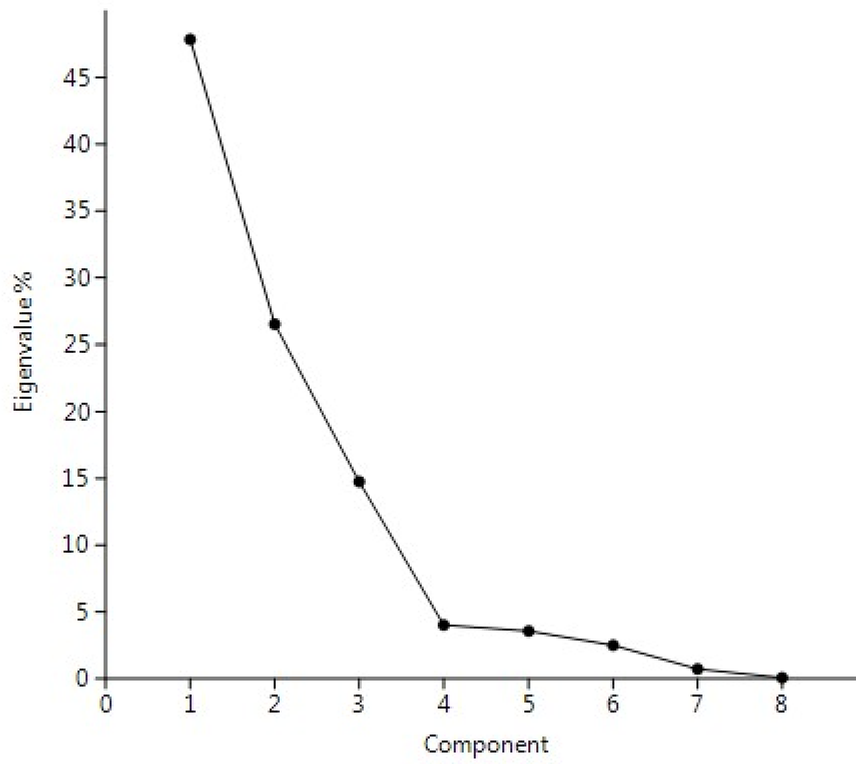


Figure 4.2. Scree plot for principal component analysis of *Ricinodendron heudelotii* using macromorphological characters

Table 4.6. Eigenvalue, percentage variance and loadings for the principal components from macromorphological characters of *Ricinodendron heudelotii*

	Principal components							
	1	2	3	4	5	6	7	8
Eigen value	154	85.4	47.5	12.9	11.5	8	2.3	0.2
% variance	47.8	26.5	14.8	4	3.6	2.5	0.7	0.1
Characters			Loadings					
NL	0.01	-0.01	-0.05	-0.02	0.04	-0.08	-0.30	-0.29
PL	**0.48**	-0.05	-0.46	-0.18	0.44	-0.04	0.17	-0.00
AL	-0.00	-0.00	-0.02	-0.05	0.06	0.08	-0.04	-0.18
LTL	**0.71**	-0.37	0.10	0.06	-0.07	0.07	-0.05	0.08
LL	**0.24**	-0.15	0.20	0.02	-0.22	0.20	-0.25	-0.26
PW	0.09	0.06	-0.07	0.06	-0.01	0.00	0.04	0.12
LWB	0.05	0.01	0.01	0.05	0.03	-0.01	-0.22	0.29
LWM	0.08	-0.08	**0.20**	-0.01	-0.29	-0.02	-0.01	0.17
LWT	0.04	-0.04	0.19	-0.01	-0.29	-0.06	0.15	0.12
NSV	0.03	0.07	0.09	0.29	-0.11	0.70	0.10	-0.04
SBSV	0.04	0.03	-0.01	0.21	0.04	-0.02	-0.08	0.48
FL	-0.10	-0.42	**0.49**	0.01	0.31	-0.13	0.56	-0.11
FW	0.24	**0.40**	0.40	0.13	0.11	-0.38	-0.11	-0.08
FLW	0.19	**0.43**	-0.16	0.01	-0.31	0.04	0.57	-0.05
FSW	0.01	**0.27**	0.20	0.18	0.53	0.38	-0.03	0.04
PW	0.19	**0.40**	0.36	-0.52	0.02	0.02	-0.06	0.02
NSF	0.00	0.03	0.01	0.01	0.03	0.04	-0.06	-0.12
NAS	-0.02	-0.00	0.02	0.00	-0.03	-0.07	0.04	0.52
SL	0.04	-0.03	-0.01	0.09	-0.17	-0.14	-0.07	-0.18
SD	0.06	0.09	-0.10	0.05	-0.13	-0.07	0.16	-0.19
SW	0.10	0.12	-0.03	0.69	0.04	-0.29	0.00	-0.12
RR	-0.00	-0.02	0.01	-0.00	0.00	-0.00	0.00	-0.01

NLL= number of leaflets, PL= petiole length, AL= acumen length, LTL= leaf total length, LL= leaflet length, PW= petiole width, LWB= leaf width at the base, LWM= leaf width at the middle, LWT= leaf width at the top, NSV= number of secondary veins, SBSV= space between secondary veins
NSF= number of seeds per fruit, NAS= number of aborted seeds, FLW= fruit largest width, FSW= fruit smallest width, PW= pulp weight, FL= fruit length, FW= fruit weight, SL= seed length, SD= seed diameter, SW= seed weight, RR= roundness ratio, PCs = principal components.

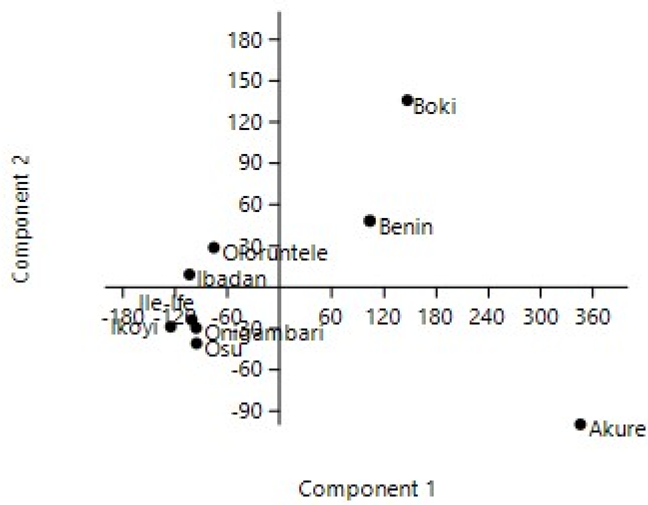


Figure 4.3. Scatter plot for *Ricinodendron heudelotii* from different operation taxonomic units using epidermal characters

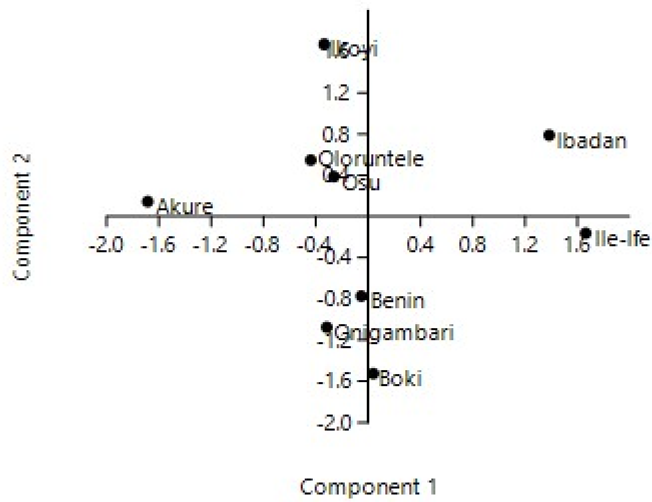


Figure 4.4. Scatter plot for *Ricinodendron heudelotii* from different operation taxonomic units using macromorphological characters

Figure 4.5. Scatter plot for *Ricinodendron heudelotii* from different operation taxonomic units using micro- and macro-morphological characters.

Table 4.7. Eigenvalue, percentage variance and loadings for the principal components from epidermal and macromorphological characters of *Ricinodendron heudelotii*

	Principal components				
	1	2	3	4	5
Eigen value	26593.3	4486.7	2331.3	621.2	326.4
% variance	76.8	13.0	6.7	1.8	0.9
Characters			Loadings		
NL	0.00	0.01	0.00	-0.01	-0.01
PL	-0.02	0.02	-0.03	0.03	-0.15
AL	0.00	0.00	0.00	-0.01	-0.01
LTL	-0.02	0.07	-0.07	0.06	-0.09
LL	-0.01	0.03	-0.02	0.02	0.02
PW	-0.01	0.00	0.00	0.02	-0.01
LWB	0.00	0.00	0.01	0.01	0.00
LWM	0.00	0.02	-0.01	0.03	0.04
LWT	0.00	0.01	-0.01	0.03	0.04
NSV	-0.01	-0.01	0.00	-0.02	0.03
SBSV	0.00	0.00	0.01	0.00	-0.01
FL	0.02	0.02	-0.02	-0.04	-0.06
FW	-0.01	-0.01	0.05	0.16	0.03
FLW	-0.02	-0.02	0.01	0.12	0.04
FSW	-0.01	-0.03	0.05	0.02	-0.02
PW	-0.01	-0.01	0.03	0.14	0.07
NSF	0.00	0.00	0.01	0.00	0.00
NAS	0.00	0.00	0.00	0.00	0.01
SL	0.00	0.01	-0.01	0.01	0.01
SD	-0.01	0.00	0.00	0.03	0.00
SW	-0.01	0.00	0.03	0.04	-0.04
RR	0.00	0.00	0.00	0.00	0.00
NSad	0.00	0.00	0.00	0.02	0.00
NECad	-0.20	0.31	-0.15	0.75	-0.35
ECLad	0.03	-0.04	-0.04	-0.17	-0.06
ECWad	0.00	-0.01	-0.03	-0.05	-0.04
GCAad	**0.48**	0.40	0.00	-0.19	-0.44
SLad	0.03	0.00	0.02	0.01	-0.03
SWad	0.05	0.06	0.01	-0.01	0.06
SIad	0.01	-0.02	0.01	-0.03	0.02
NWSad	0.01	-0.01	0.01	0.00	0.01
GCWad	0.01	0.02	-0.01	-0.01	-0.01
PSad	**0.57**	0.18	0.05	0.05	0.31
NSab	0.04	0.35	-0.25	0.14	0.64
NECab	-0.16	0.35	0.90	0.03	0.11
ECLab	0.01	-0.02	0.00	0.01	-0.02
ECWab	0.01	-0.09	0.01	-0.23	-0.01
GCAab	0.02	-0.08	0.02	-0.05	-0.03
Slab	**0.39**	0.24	-0.05	0.04	-0.17
Swab	0.01	-0.02	-0.02	0.01	0.01
SIab	0.05	0.03	0.01	0.03	0.01
NWSab	0.02	0.10	-0.16	0.10	0.27
GCWab	0.02	0.02	0.00	0.00	0.00
PSab	**0.47**	-0.61	0.26	0.45	0.04

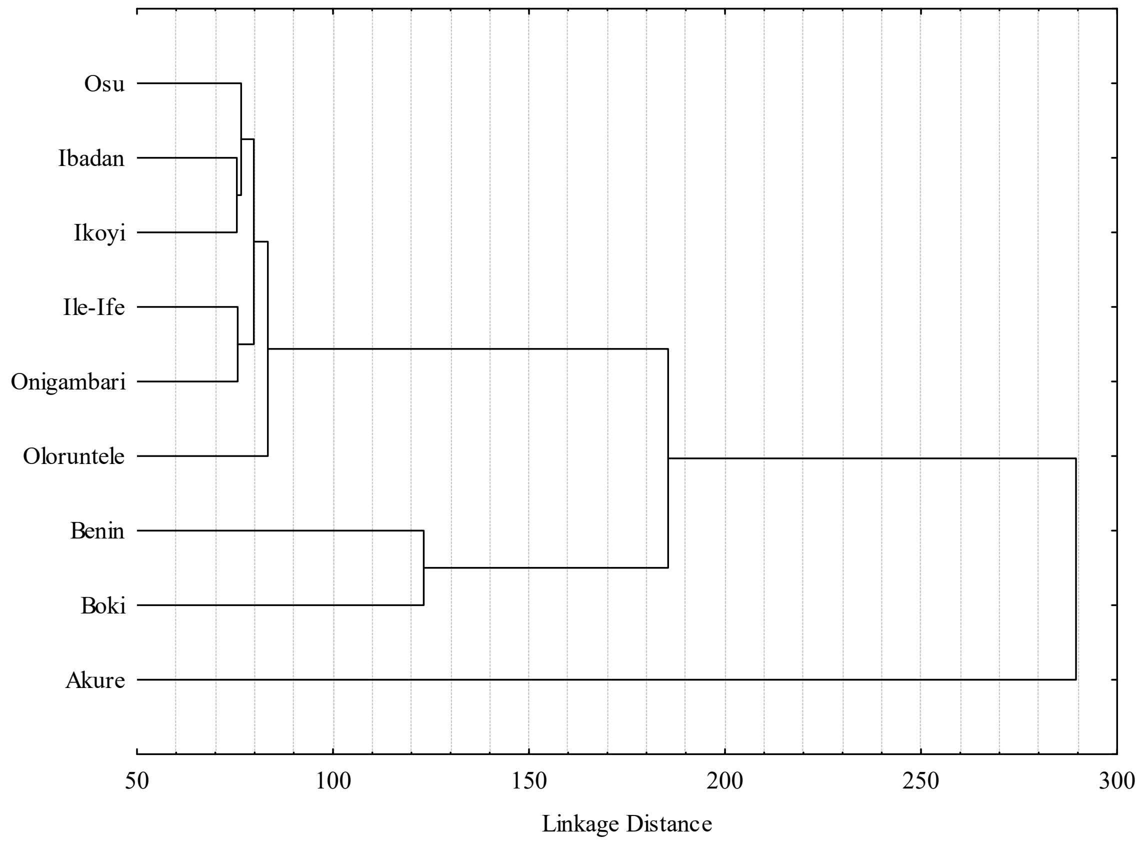


Figure 4.6. Dendrogram produced based on macro morphological and epidermal data from *Ricinodendron heudelotii*

4.2 Molecular Characterisation of *Ricinodendron heudelotii* from different Operational Taxonomic Units (OTUs)

Nineteen (19) out of the forty-eight leaf samples subjected to extraction resulted in good quality genomic DNA. The representative gel picture of the electrophoresis is presented in Plate 4.3.

4.2.1 Informativeness of ISSR Markers for *Ricinodendron heudelotii*

The summary of the amplified products from different OTUs of *R. heudelotii* using ISSR primers (Table 4.8) shows that 19 primers produced scorable alleles among all the 35 primers examined for the molecular characterisation. However, the 19 primers were 100% polymorphic for all the OTUs with each having considerable banding patterns (Plates 4.4 and 4.5).

The total number of polymorphic alleles generated at 19 ISSR loci was 111 with an average of 6 alleles across the OTUs. None of the scored fragments was monomorphic. The amplified DNA molecular weight ranged between 140bp and 2000bp.

Table 4.9 shows that the number of polymorphic alleles generated varied from 3 (UBC-848, UBC-817 and UBC-845 loci) to 9 (UBC-822 and ISSR-7). The highest Polymorphic Information Content (PIC) was discovered at locus UBC-840 (0.42) with 6 alleles. This was followed by ISSR-816 (0.41) having 5 alleles and the least observed at locus UBC-848 (0.21) with 3 alleles.

Three private alleles were generated at UBC-859, ISSR-816 and UBC-818 loci having 1600bp, 1800bp and 200bp molecular weight respectively (Figure 4.7).

Table 4.8. Summary of the amplified products from different OTUs of *R. heudelotii* using ISSR primers

S/N	Variables	Value
1	Number of primers used	35
2	Number of ISSR primers with scorable DNA fragments	19
3	Number of ISSR primers with polymorphic alleles	19
4	Mean number of polymorphic alleles per primer	6
5	Percentage of polymorphism	100
6	Number of monomorphic alleles	0
7	Range of amplified DNA molecular weight	140-2000bp

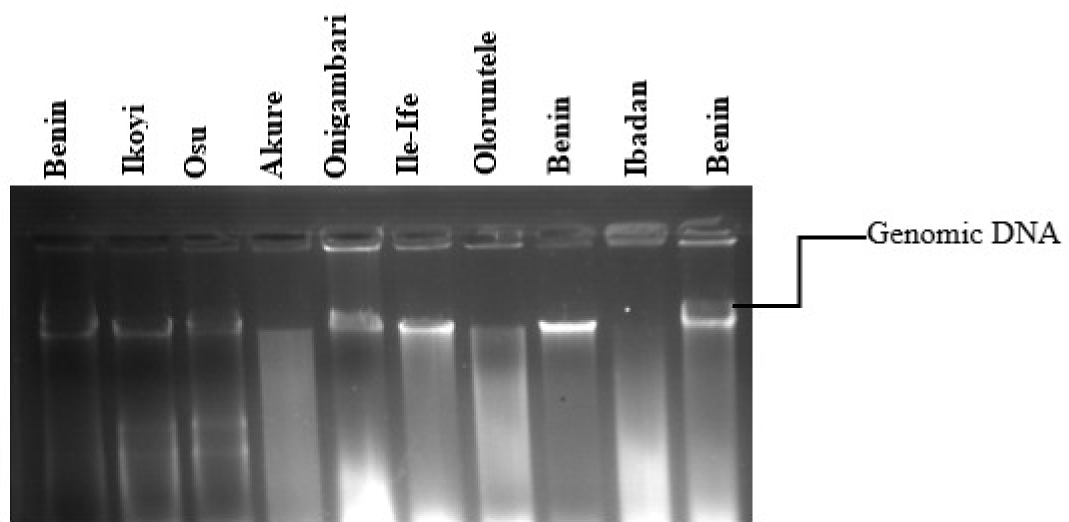


Plate 4.3. Gel picture of genomic DNA extracted from *Ricinodendron heudelotii*

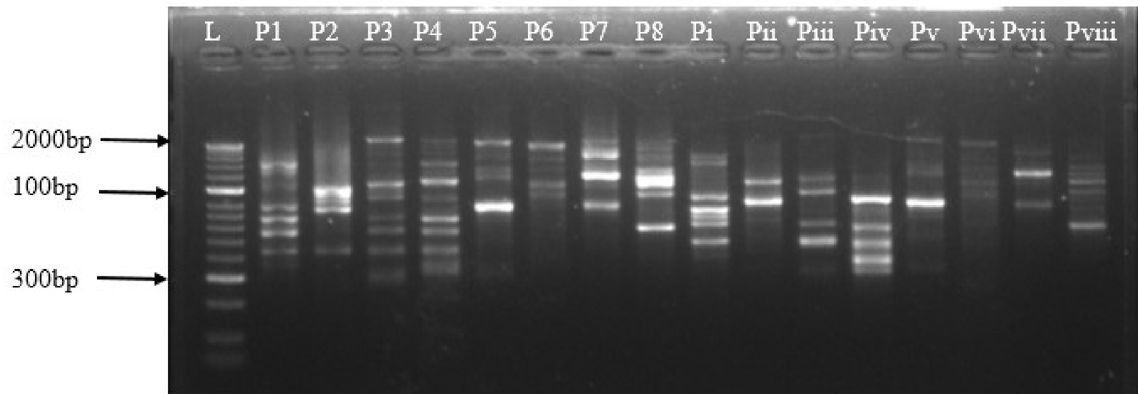


Plate 4.4. ISSR Profile of *Ricinodendron heudelotii* with eight representative primers

L= Ladder, P1/Pi= ISSR 808, P2/Pii= UBC 818, P3/Pii= ISSR 815, P4/Piv= UBC 840, P5/Pv= UBC 859, P6/Pvi= UBC 848, P7/Pvii= UBC 844, P8/Pviii= UBC 857

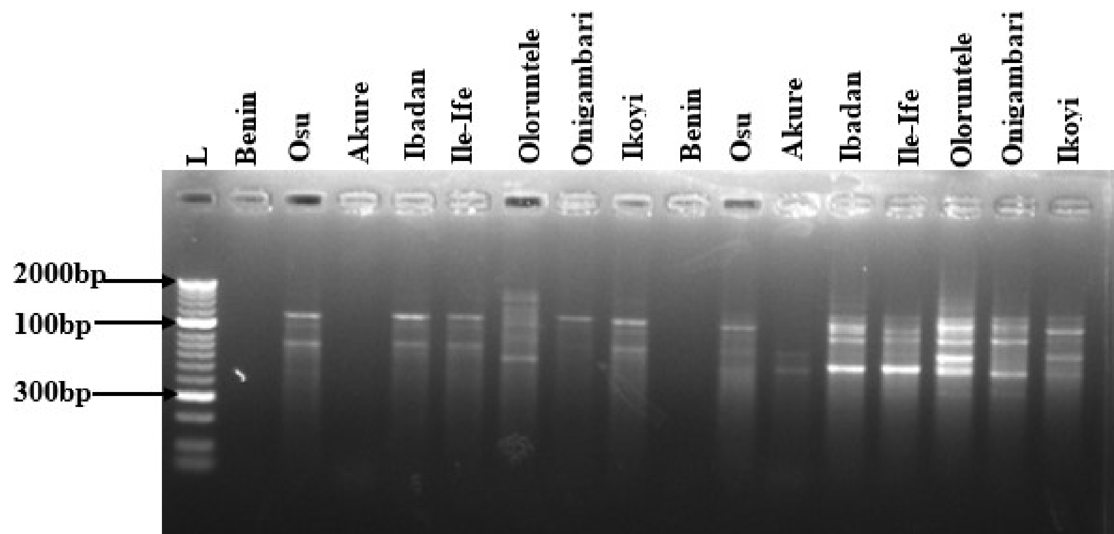


Plate 4.5. Gel Profile of eight different OTUs of *Ricinodendron heudelotii* using UBC 844 and UBC 857

L= Ladder

Table 4.9. Number of amplified DNA fragments, the size range of the alleles and Polymorphic Information Content (PIC) from each primer

S/N	Primer name	Primer sequences (5' - 3')	Total number of alleles	Number of polymorphic alleles	band size range (bp)	PIC
1	ISSR 808	AGAGAGAGAGAGAGAGC	7	**7**	350-1500	0.35
2	UBC 818	CACACACACACACACAG	7	**7**	300-2000	0.37
3	ISSR 815	CTCTCTCTCTCTCTCTG	4	4	400-1000	0.35
4	UBC 840	GAGAGAGAGAGAGAGAYT	6	**6**	250-700	**0.42**
5	UBC 859	TGTGTGTGTGTGTGTGRC	5	5	450-1600	0.29
6	UBC 848	CACACACACACACACARG	3	3	900-1600	**0.21**
7	UBC 844	CTCTCTCTCTCTCTCTRC	5	5	550-1400	0.32
8	UBC 857	ACACACACACACACACYG	8	**8**	400-1200	0.39
9	UBC 812	GAGAGAGAGAGAGAGAA	6	**6**	400-1100	0.39
10	UBC 817	CACACACACACACACAT	3	3	350-700	0.40
11	UBC 825	ACACACACACACACACT	8	**8**	450-1500	0.3
12	UBC 822	TCTCTCTCTCTCTCTCA	9	**9**	400-1400	0.37
13	ISSR 7	ACA CAC ACA CAC ACA CT	9	**9**	140-750	0.36
14	ISSR 816	CACACACACACACACAA	5	**5**	400-1800	0.41
15	UBC 842	GAGAGAGAGAGAGAGAYG	8	8	300-1200	0.30
16	UBC 845	CTCTCTCTCTCTCTCTRG	3	3	550-650	0.31
17	UBC 834	AGAGAGAGAGAGAGYT	4	4	350-600	0.36
18	UBC 836	AGAGAGAGAGAGAGYA	6	6	350-1000	0.31
19	UBC 885	HBHAGAGAGAGAGAGAG	5	5	250-600	0.30
	Total		111	111	140-2000	0.35

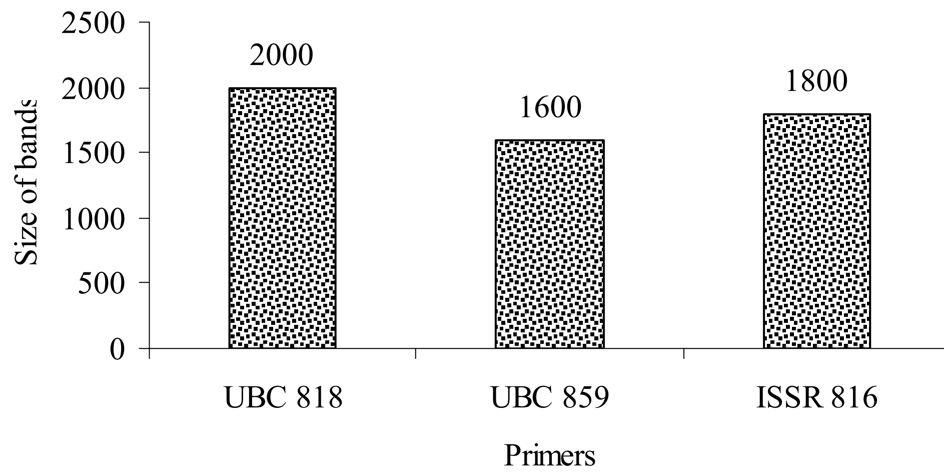


Figure 4.7. Private alleles produced by UBC 818, UBC 859 and ISSR 816 in *Ricinodendron heudelotii* from Oloruntele

4.2.2 Molecular taxonomic delimitation among populations *Ricinodendron heudelotii*

Figure 4.8 indicates that the highest genetic diversity was found in the population of *Ricinodendron heudelotii* from Oloruntele (0.24). This was followed by Ikoyi (0.23). Ibadan and Onigambari had approximately the same genetic diversity of 0.22. Osu, Ile-Ife and Benin had the genetic diversity of 0.21, 0.20 and 0.12 respectively while Akure had the least diversity.

Considering the genetic similarity among the populations (Table 4.10), Ibadan and Osu were more similar (60.3%). Closely following this was Ibadan and Ikoyi (60.0%); Ikoyi and Osu (59.1%) and Ibadan and Ile-Ife (57.6%). Akure and Benin had the least genetic similarity of 1.0%.

The visualised PCA scatter plot generated from the molecular data (Figure 4.9) indicates the diverseness of the *Ricinodendron heudelotii* from southern Nigeria. Four major clusters were formed. These include Oloruntele, Benin and Akure while Onigambari, Ikoyi, Ife, Osu and Ibadan formed a group. The clusters observed in the PCA supported the phylogenetic tree (Figure 4.10).

The phylogenetic tree indicates that Benin is the earliest population of *Ricinodendron heudelotii* in southern Nigeria, which had branch support of 87% with Akure. Evolutionarily also, the population of *Ricinodendron heudelotii* from Onigambari, Ile-Ife, Osu, Ikoyi and Ibadan were more recent and are having 100% branch supported with Oloruntele.

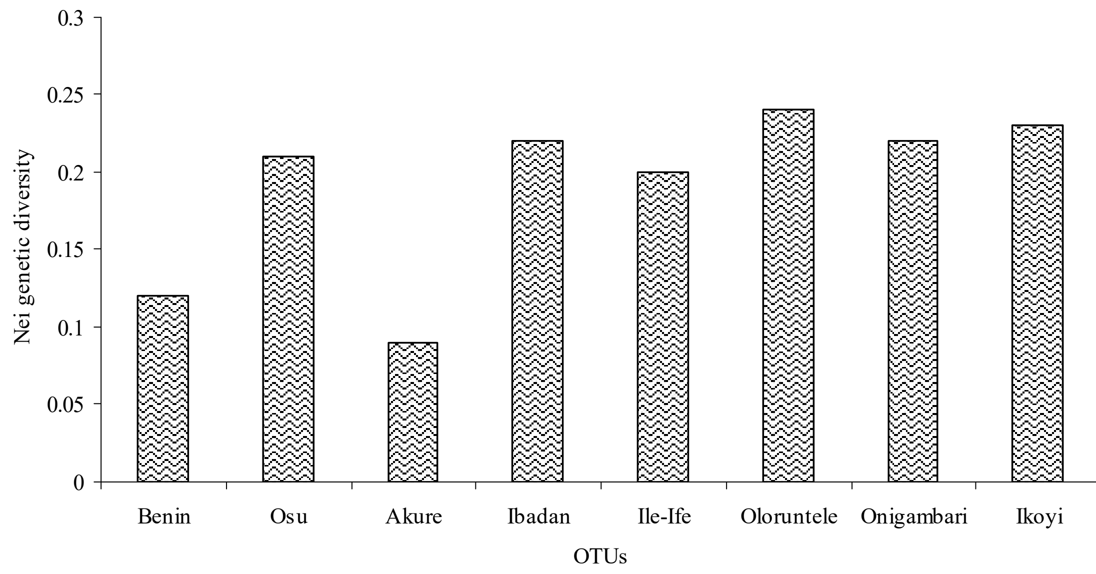


Figure 4.8. Genetic diversity among the OTUs of *Ricinodendron heudelotii*

Table 4.10. Similarity index matrix among the OTUs of *Ricinodendron heudelotii*

	Benin	Osu	Akure	Ibadan	Ile-Ife	Oloruntele	Onigambari	Ikoyi
Benin	100.0							
Osu	3.3	100.0						
Akure	1.0	3.6	100.0					
Ibadan	1.4	60.3	1.6	100.0				
Ile-Ife	4.1	54.0	2.3	57.6	100.0			
Oloruntele	2.8	26.0	1.5	33.3	35.0	100.0		
Onigambari	2.0	40.6	2.3	48.6	52.7	31.7	100.0	
Ikoyi	3.0	59.1	1.6	60.0	51.5	28.3	45.1	100.0

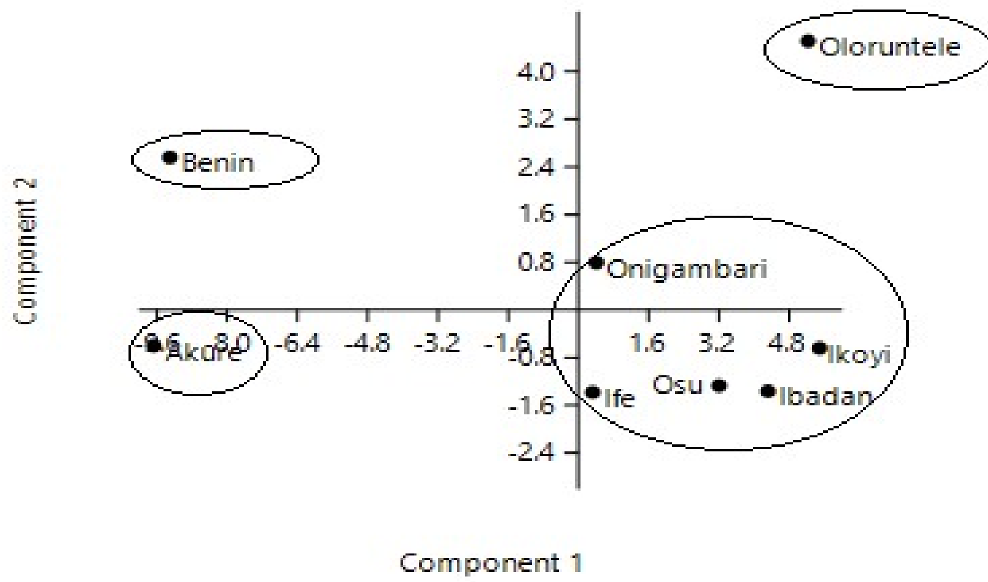


Figure 4.9. Scatter plot for *Ricinodendron heudelotii* from different operation taxonomic units using molecular markers

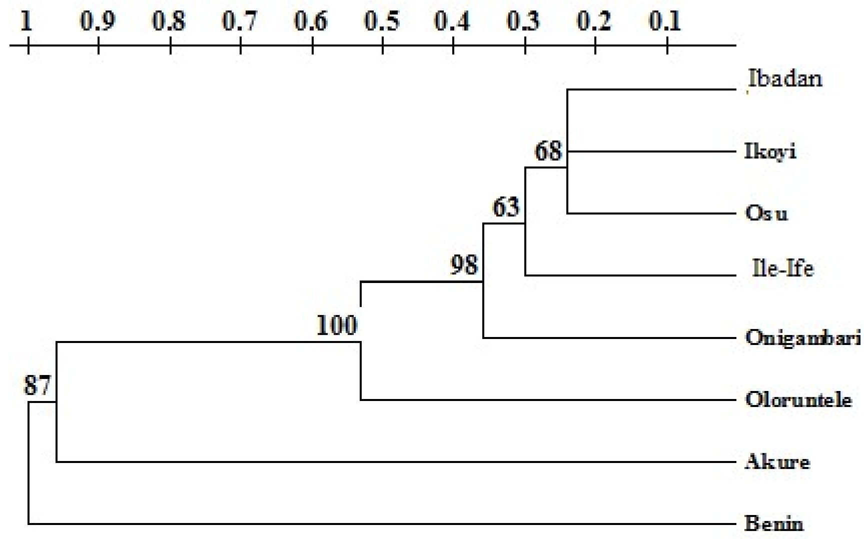


Figure 4.10. The phylogenetic tree produced using molecular data from *Ricinodendron heudelotii*

4.3 Germination Potential of Seeds of *Ricinodendron heudelotii*

of the effects of pre-germination treatments and sowing media on the germination percentage of seeds of *R. heudelotii*. The effect of pre-germination treatments was significant ($p<0.05$) (Appendix 41) on germination percentage with the scarified and soaked seeds having the highest germination of 35.0±2.5%. This was closely followed by scarified without soaking (33.3±2.1%) while the least was found with the control (11.7±0.8%). The effect of sowing media treatment was also significant ($p<0.05$) on seed germination of *R. heudelotii* (Table 4.11). Hence, seeds in topsoil (41.1±1.9%) germinated better than those in river sand (12.2±0.9%).

The interaction effects of pre-germination treatments and sowing media treatments on the germination of seeds of *R. heudelotii* was significant [($p<0.05$); (Table 4.12); (Appendix 41)]. Here, the individual effect of the treatments used was revealed. This is based on the fact that seeds that were scarified, soaked in cold water and sown in topsoil germinated best with a germination percentage of 56.7±0.4%. Closely following this group were the scarified seeds but without soaking and sown in topsoil (50.0±0.4%). Scarified seeds sown in river sand germinated equally with control seeds in topsoil having 16.7±0.4% germination each, followed by scarified and soaked seeds sown in river sand (13.3±0.9%) while control seeds sown in river sand had the least germination (6.7±0.9%).

In terms of germination rate, all the pretreated seeds commenced germination after a week of sowing (Figure 4.11). About 23.4% scarified with soaking seeds sown in topsoil germinated after a week and reached the peak after three (3) weeks of sowing with a 56.7% germination percentage (Figure 4.11). Approximately 20.0% of scarified seeds without soaking sown in topsoil germinated after a week while the peak of germination (50%) was recorded after four (4) weeks of sowing (Figure 4.11). Seeds without pre-germination treatment (control) did not commence germination until after eight (8) weeks of sowing, where only about 1.7% and 3.3% germination were observed for the ones sown in river sand and topsoil respectively.

Table 4.11. Effects of pre-germination treatments and sowing media on germination percentage of seeds of *Ricinodendron heudelotii*

Treatments	Mean±SD
Control	$11.7±0.8^{b}$
Scarified	$33.3±2.1^{a}$
Scarified & Soaked	$35.0±2.5^{a}$
p-value	0.015*
Sowing Media	Mean±SD
River sand	$12.2±0.9^{b}$
Topsoil	$41.1±1.9^{a}$
p-value	0.001*

*= Significant at 5% probability level

Means of any set of treatments with the same superscripts along the same column are not significantly different

Table 4.12. Interaction effects of pre-germination treatments and sowing media on the germination of seeds of *Ricinodendron heudelotii*

Treatments	Sowing Media	Mean±SD	p-value
control	River sand	$6.7±0.9^{c}$	0.004*
Scarified & Soaked	River sand	$13.3±0.9^{c}$	
control	Topsoil	$16.7±0.4^{b}$	
Scarified	River sand	$16.7±1.4^{b}$	
Scarified	Topsoil	$50.0±0.4^{a}$	
Scarified & Soaked	Topsoil	$56.7±0.4^{a}$	

*= Significant at 5% probability level

Means of any set of treatments with the same superscripts along the same column are not significantly different

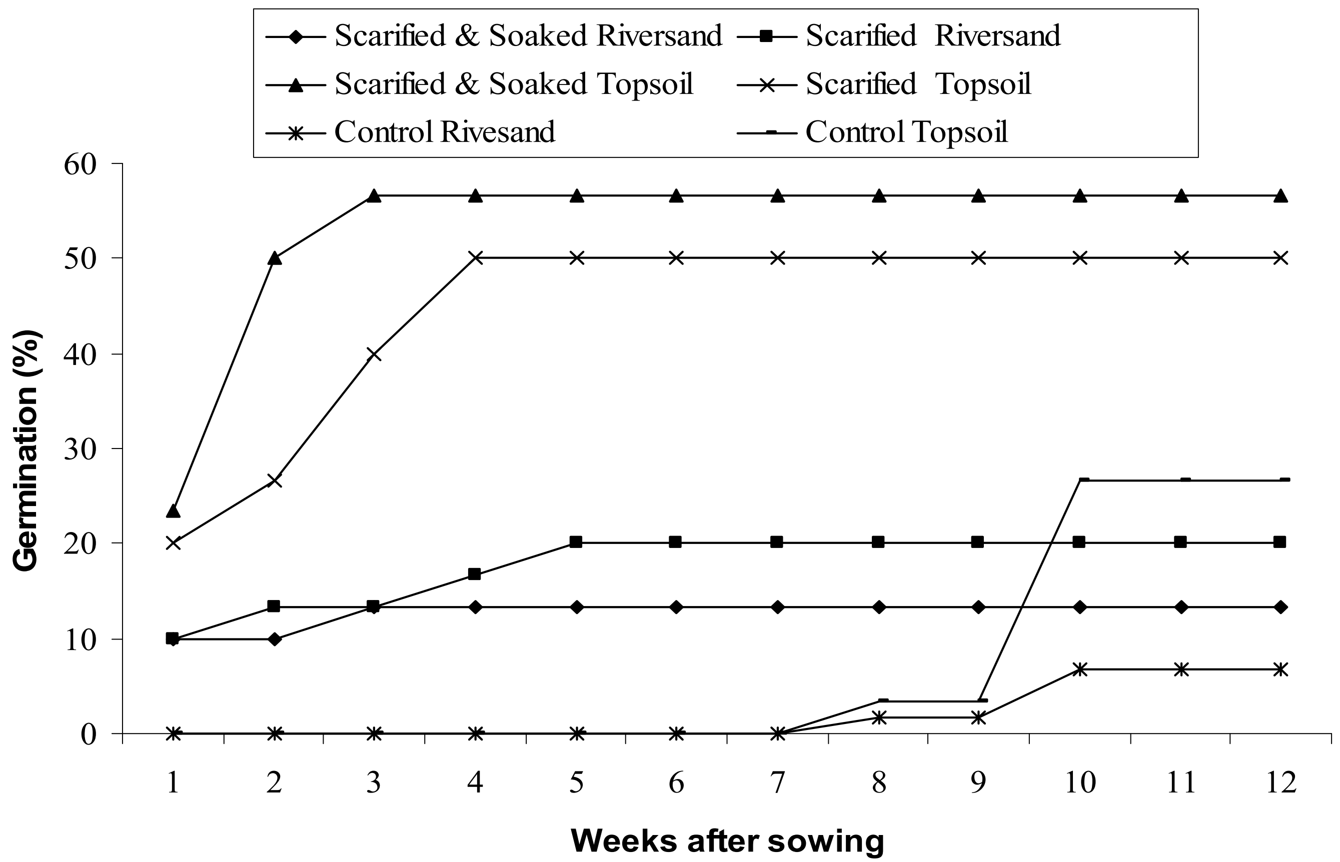


Figure 4.11. Cumulative germination of seeds of *Ricinodendron heudelotii* after sowing

4.4 Early growth of *Ricinodendron heudelotii* and its effect on the growth of maize and soil nutrients

Table 4.13 presents the result of the height, collar diameter and the number of leaves of the species. *Ricinodendron heudelotii* planted at 6m alley width (Plate 4.6) had a higher average height (255.7±66.5 cm) than those planted at 3m (170.5±73.6 cm) at the end of a year growth assessment (Plate 4.7). In terms of collar diameter, the 6m (38.1±12.0 cm) alley width outperformed the 3m (20.1±12.1 cm). The 6m alley trees produced an average of 31 leaves, while the 3m alley trees produced an average of 24 leaves. T-test analysis revealed that the alley width effect on tree height and collar diameter was significant ($p<0.05$) (Table 4.13). In the same vein, the Mann-Whitney U test revealed that the number of leaves produced by 6m alley trees differed significantly ($p<0.05$) from those produced by 3m alley trees (Table 4.13).

The height, collar diameter and the number of leaves produced by maize are shown in Table 4.14. Maize average heights in 6m alley, 3m alley and plot without tree were 118.7±13.8 cm, 105.1±29.2 cm, and 74.3±19.1 cm, respectively. The average collar diameter of maize in 6m alley was 12.1±4.0 mm, in 3m alley it was 12.4±3.2 mm, and in control maize, it was 9.73.1 mm. The alley width treatments had 7 leaves, while the plot without trees had six leaves. The alleys had a significant effect on height, collar diameter and the number of leaves produced by maize ($p<0.05$) (Appendices 42 & 44).

The results (Table 4.15) show that the distance to the trees did not significantly affect maize height, collar diameter and number of leaves ($p>0.05$) (Appendices 43 to 44). However, maize planted 300cm from the trees grew to the greatest height (109.8±30.8 cm). This was followed by those planted at 240cm, 180cm, and 120cm to trees with average heights of 103.3±32.5 cm, 103.129.2 cm, and 90.6±26.5 cm, respectively. Maize planted at 300cm and 120cm to the trees produced the same number of leaves (7), while those planted at 180cm and 240 cm produced 8 leaves each.

Plate 4.6. The 6m Alley farm showing *Ricinodendron heudelotii* trees and the seven rows of maize within the alley

Plate 4.7. The 3m Alley farm showing *Ricinodendron heudelotii* trees and the two rows of maize within the alley

Table 4.13. Height, collar diameter and number of leaves of the species

Alley width	Height±SD (cm)	Collar diameter±SD (mm)	Number of leaves	Mann-Whitney U value
3m	170.5±73.6	20.1±12.1	24	262.500
6m	255.7±66.5	38.1±12.0	31	
t-value	4.972	6.054		
df	66	66		
p-value	0.000*	0.000*	0.000*	

*= Significant at 5% probability level

Df= Degree of freedom

Table 4.14. Height, collar diameter and number of leaves produced by maize

Treatments	Height (cm)	Collar diameter (mm)	Number of Leaves
6m	118.7±13.8^{a}	12.1±4.0^{a}	7
3m	105.1±29.2^{a}	12.4±3.2^{a}	7
control	74.3±19.1^{b}	9.7±3.1^{b}	6
p-value	0.015*	0.000*	0.006*

*= Significant at 5% probability level

Means of any set of treatments with the same superscripts along the same column are not significantly different

Table 4.15. Effect of planting distance to the tree on the height, collar diameter and number of leaves of maize

Distance to the tree	Height (cm)	Collar diameter (mm)	Number of Leaves
120cm	90.6±26.5	11.1±3.1	7
180cm	103.1±29.2	12.5±3.2	8
240cm	103.3±32.5	12.2±3.2	8
300cm	109.8±30.8	12.4±4.1	7
p-value	0.153ns	0.399ns	0.102ns

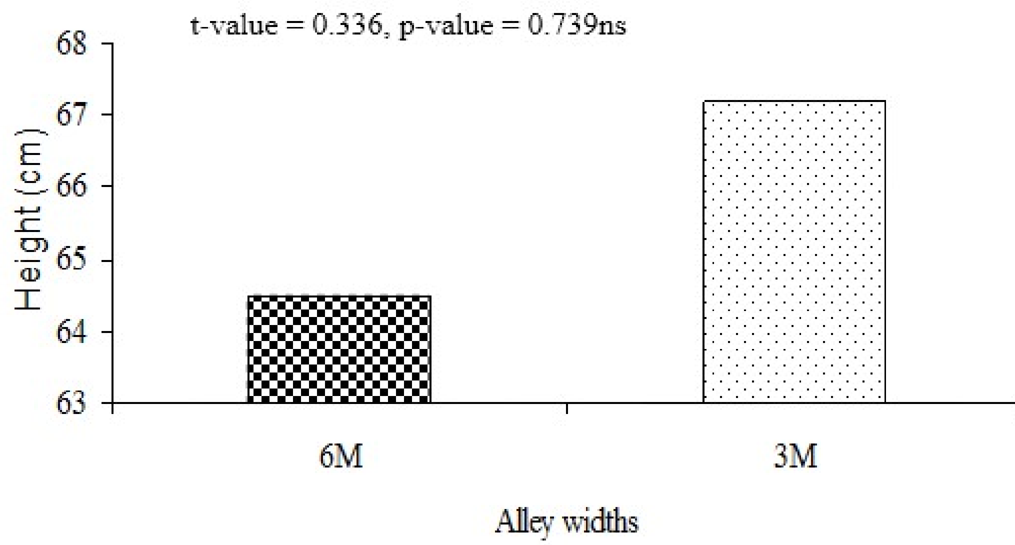
ns= not significant 5% probability level

Figures 4.12 and 4.13 show the height and number of leaves of *R. heudelotii* coppices. *R. heudelotii* coppiced length (67.2cn) in 3m alley was greater than that of 6m alley. The results (Figure 4.12) show that the coppiced height did not differ significantly ($p>0.05$). However, the number of tree leaves in 6m alley was greater than those in 3m alley. The Mann-Whitney U test result (Figure 4.13) revealed a significant difference ($p<0.05$) between the two alley widths according to the number of leaves produced.

The organic carbon (0.95±0.30%), nitrogen (0.10±0.02%), pH (6.140.25), and available phosphorus (19.75±5.25 mg kg-1) of the plot when the experiment was yet to commence were lower than the post-experiment results (1.23±0.47 %, 0.13±0.05 %, 6.36±0.16 and 23.91±1.98 mg kg-1) (Table 4.16). Ca 0.95±0.24, Mg 0.41±0.05, K 0.85±0.09, and Na 0.34±0.05 cmol kg-1) were also lower when the experiment was yet to commence than the post-experiment values (Ca 1.27±0.3, Mg 0.49±0.08, K 0.88±0.06, and Na 0.39±0.01 cmol kg-1). Similarly, the soil iron (Fe) concentration was higher in the post-experimental period (187.44±57.6 mg kg-1) than in the pre-experimental period (131.80±18.9 mg kg-1).

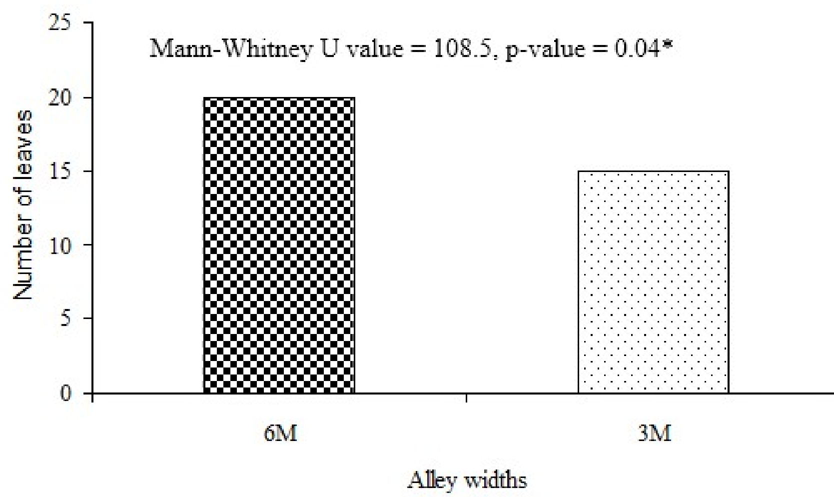
T-test results (Table 4.16) show that agroforestry had a significant ($p<0.05$) impact on soil pH, available phosphorus, magnesium, and sodium, but had no effect ($p>0.05$) on carbon, nitrogen, calcium, potassium, and iron.

The alley treatments had a significant ($p<0.05$) effect on all soil properties except organic carbon, nitrogen, calcium, and iron (Table 4.17) (Appendix 45). The least soil properties were obtained in the plot without trees. The pH (6.35), organic carbon (1.27 %), nitrogen (0.14 %), calcium (1.26 cmol kg^{-1}) and magnesium (0.51 cmol kg-1) were highest in 3 m alley width. On the other hand, highest values for phosphorus (24.61 mg kg^{-1}), potassium (0.89 cmol kg^{-1}), sodium (0.39 cmol kg^{-1}) and iron (191.29 mg kg^{-1}) were obtained in 6 m alley width.



ns= not significant 5% probability level

Figure 4.12. Height of *Ricinodendron heudelotii* coppices at 6 and 3 meters apart



*= Significant at 5% probability level

Figure 4.13. Number of leaves of *Ricinodendron heudelotii* coppices 6 and 3 meters apart

Table 4.16. Soil nutrient characteristics of the alley cropping plot

Analysis period	pH	C %	N %	P (mg kg^{-1})	Ca (cmol kg^{-1})	Mg (cmol kg^{-1})	K (cmol kg^{-1})	Na (cmol kg^{-1})	Fe (mg kg^{-1})
Before experiment	6.14±0.25	0.95±0.30	0.10±0.02	19.75±5.25	0.95±0.24	0.41±0.05	0.85±0.09	0.34±0.05	131.80±18.9
After experiment	6.36±0.16	1.23±0.47	0.13±0.05	23.91±1.98	1.27±0.37	0.49±0.08	0.88±0.06	0.39±0.01	187.44±57.6
p-value	0.013*	0.230^{ns}	0.209^{ns}	0.014*	0.098^{ns}	0.039*	0.362^{ns}	0.006*	0.050^{ns}

*= Significant at 5% probability level, ns= not significant 5% probability level

Table 4.17. Soil nutrient characteristics of the different treatments

Treatments	pH	O.C %	N %	P (mg kg^{-1})	Ca (cmol kg^{-1})	Mg (cmol kg^{-1})	K (cmol kg^{-1})	Na (cmol kg^{-1})	Fe (mg kg^{-1})
3 m	6.35±0.18^{a}	1.27±0.46	0.14±0.05	23.39±1.23^{a}	1.26±0.38	0.51±0.08^{a}	0.88±0.06^{a}	0.38±0.01^{a}	174.25±40.11
6 m	6.34±0.12^{a}	1.11±0.43	0.12±0.04	24.61±2.69^{a}	1.22±0.31	0.45±0.03^{ab}	0.89±0.07^{a}	0.39±0.01^{a}	191.29±77.54
Control	6.00±0.00^{b}	0.70±0.00	0.08±0.00	14.2±0.00^{b}	0.71±0.00	0.35±0.00^{b}	0.76±0.00^{b}	0.29±0.00^{b}	114.00±0.00
p-value	0.036*	0.236ns	0.220ns	0.000*	0.144ns	0.015*	0.044*	0.000*	0.238ns

O.C = organic carbon, *= Significant at 5% probability level, ns= not significant 5% probability level
Means of any set of treatments with the same superscripts along the same column are not significantly different

CHAPTER FIVE

DISCUSSION

5.1 Epidermal and Macro Morphological Characteristics of *Ricinodendron heudelotii* in southern Nigeria

The predominant qualitative epidermal characters observed in this species such as the polygonal cell shape, periclinal wall and amphistomatic nature of the stomata are unique taxonomic evidence, which may be utilised for the delimitation of populations of *Ricinodendron heudelotii* from southern Nigeria. The existence of polygonal cell shape in both abaxial and adaxial surfaces makes the epidermal surfaces of this species homomorphic. This homomorphic leaf epidermis identified in *R. heudelotii* has equally been reported for some members of Euphorbiaceae, especially for *Jatropha* species (Olowokudejo, 1993) and *Euphorbia* species (Talebi *et al.*, 2017) by previous workers. According to Talebi *et al.* (2017), virtually all the *Euphorbia* species are characterised by homomorphic epidermal cell shapes with only a few being heteromorphic. Raju and Rao (2008) equally reported similar findings after a taxonomic investigation of some selected species of *Euphorbia*. This is therefore an indication of the kind of affinity *Ricinodendron* shares with other members of the family Euphorbiaceae. The presence of striation on the epidermal surfaces of the populations of *R. heudelotii* from Osu, Ibadan, Oloruntele, Ile-Ife, Ikoyi, Onigambari and Akure is of taxonomic significance. This cuticular striation may be used in separating the taxa from others. Curtivular striation has equally been discovered to be taxonomically significant in some other species of Euphorbiaceae such as *Euphorbia hirta* (Aworinde *et al.*, 2009) and *Jatropha* species (Olowokudejo, 1993). However, utilisation of this character for delimitation at the level of the population should be taken with caution. This is because Wilkinson (1979) emphasised that striations may discriminate some species but sometimes be variable in others in the systematic application.

The paracytic type of stomata identified in this species is congruent with the findings of some previous authors that studied Euphorbiaceae (Hidayat and Kusdianti, 2009; Essiett *et al.*, 2012). This has been reported as one of the most common stomata type in the family, which could either be amphistomatic or hypostomatic. However, the amphistomatic leaf is the commonest in most Euphorbiaceae (Hidayat and Kusdianti, 2009). On the other hand, Zahra *et al.*, (2014) established the fact that anisocytic, diacytic and anomocytic stomata are predominant in the genus *Euphorbia*. This, however, makes stomatal type a considerable taxonomic significance for *R. heudelotii* within the family Euphorbiaceae. Hidayat and Kusdianti (2009) reiterated the fact that characters derived from stomata in Euphorbiaceae, such as stomatal size, type and shape have an advantage for use as a phylogenetic marker due to its relative stability in size and shape. Therefore, the large stomata of the Akure population may however be useful for taxonomic discrimination.

As expected in many angiosperms, stomata were more densely distributed at the abaxial surface than the adaxial surface of the leaf samples. This distribution pattern serves as an adaptive mechanism that helps to prevent abundant loss of water from the plant.

The pattern of epidermal cell wall observed in this study is similar to the work of Ullah *et al.* (2018), where irregular cell shapes were reported for some species in the subfamily Alsinoideae. The fact that only *R. heudelotii* from Akure exhibited a wavy epidermal cell wall pattern may be ascribed to the environmental influence. It was reported by Essiett *et al.*, (2012) that environmental conditions such as humidity significantly contribute to the cell wall pattern of epidermal peels. According to Ayodele and Olowokudejo (2006), plants with undulate walls are usually found in an area with high humidity while curved and straight walls were notable characteristics of plant species habituating in a drier condition. Comparing the altitudinal position of the populations in the present study, Akure samples were obtained at a higher altitude than the others, which may have necessitated the lower humidity in the population compared to other populations. Consequently, this may have caused the wavy cell wall pattern observed in Akure's population.

The significant variation in the guard cell area, stomatal width and abaxial stomatal index observed in this study is of taxonomic interest and can, therefore, be reasonably applied to

the taxonomic classification of the species into different taxa. Reports have shown that the stomatal index is constant for any given species (Essiett *et al*., 2012; Essiett and Archibong, 2014). The applicability and importance of the stomatal index in taxonomic studies of plants taxa have also been reported by Ayodele and Olowokudejo (2006), Essiett *et al.* (2012), Oyegoke *et al.* (2014) and Ullah *et al.* (2018). The existing variation in the guard cell area can be linked to the differences in pore size.

In terms of macro-morphological characterisation, the number of leaflets per leaf identified in this study aligns with the report by Tchoundjeu and Atangana (2006) which stated that *Ricinodendron heudelotii* subspecies *africanum* that was mentioned to be endemic to Nigeria, Mozambique, Angola, Sudan, Uganda and Tanzania has 3-8 leaflets per leaf. It was equally reported that *Ricinodendron heudelotii* subspecies *heudelotii* has about 3-5 leaflets per leaf and they are domicile only in Ghana and Guinea-Bissau. To some extent, the number of leaflets in some of the populations such as Oloruntele corresponded to that of subspecies *heudelotii*. Based on this classification, therefore, there is a high tendency of having the subspecies *heudelotii* in Nigeria, which may be as a result of the evolution of subspecies *africanum* (Soladoye *et al.,* 2010). The number of secondary veins recorded from this study falls with the range that was reported in the previous study (Tchoundjeu and Atangana, 2006). Nevertheless, the maximum petiole and lamina length encountered in this study were beyond what has been reported in literature (Tchoundjeu and Atangana, 2006).

Some of the findings on fruit characteristics from this study are in line with the past findings (Tchoundjeu and Atangana, 2006; Vihotogbe, 2012). For instance, it was reported that the fruit size was about 20 to 50 cm long having about 1 to 3 seeds, which corresponded to the results of the present study. Specifically for Oloruntele, virtually all the quantitative and qualitative fruit and seed characters assessed in this study fall within the range recorded for the subspecies *heudelotii,* which equally point to the fact that this population has some affinities to subspecies *heudelotii.*

The epidermal traits that mostly contributed significantly to the separation of the OTUs into different clusters happened to be the stomatal variables. The implication of this is those stomatal characteristics are more important than any other leaf anatomical characters

for taxonomic description and classification of *Ricinodendron heudelotii* into different possible groups. This is consistent with the findings of Hameed *et al.,* (2008), Essiett *et al.* (2012) and Dadegnon *et al.* (2015), where stomatal descriptors were presented as an efficient tool that provides significant and striking taxonomic differences for separating species and a higher level of a taxonomic hierarchy of plants.

The result of leaf and fruit principal component analysis implies that all the assessed taxonomic characters could be minimized into three components to provide high significant affinity and differences among the population of *Ricinodendron heudelotii*. Hence, only petiole length, leaf total length, fruit length, fruit weight, fruit largest width and pulp weight are more important to produce the existing groupings.

The different clusters obtained from the pooled morphological characters of *Ricinodendron heudelotii* indicated that there could be more than one taxonomic group in Nigeria, which are delimited into different taxa based on the taxonomic distance provided by the phylogenetic tree.

5.2 Molecular Taxonomy of *Ricinodendron heudelotii*

The highly polymorphic different banding pattern exhibited by the ISSR makers utilised in this study signifies a considerable molecular taxonomic delimitation among the OTUs of *Ricinodendron heudelotii*. This agrees with the findings from the recent studies on the molecular taxonomy of plants (Yegenoglu and Sesli, 2017; Escobar-Saucedo *et al*., 2018; Siew *et al.*, 2018).

Yegenoglu and Sesli (2017) studied *Olea europaea* (Olives) with ISSR and scored 92 visible bands, which were 100 % polymorphic. In the case of Escobar-Saucedo *et al.* (2018), where the genetic diversity of *Malus domestica* (Apple tree) was analysed using nine ISSR primers, a lesser number of polymorphic DNA fragments was identified from which about 63 % polymorphism was recorded out of 124 visible bands. According to Siew *et al.* (2018), who assessed the molecular taxonomy of Malaysian Durian varieties, 91.73 % (122) polymorphic bands out of the 133 reproducible DNA fragments were observed.

High molecular taxonomic differentiation was also reported among the operational taxonomic units of *Ricinus communis* (Goodarzi *et al.*, 2015), which shares the

Euphorbiaceae family with the present tree species but with lower polymorphism using ISSR markers. Approximately 99.3 % polymorphism was detected among the species of *Jatropha* cultivated at Tamil Nadu Agricultural University, India using the ISSR technique (Vijayanand *et al.*, 2009). This is undoubtedly similar to the extent of polymorphism identified in the present study. In the same vein, Gupta *et al.* (2008) discovered a similar finding in 13 accessions of *Jatropha curcas* obtained from four growing regions in India. However, 100 % polymorphism observed in the taxonomic grouping of *Ricinodendron heudelotii* may be ascribed to the limited ISSR sites in *R. heudelotii* from Benin. Gupta *et al.* (2008) emphasised the fact that polymorphism existing in different operational taxonomic units of plants popularly occur as a result of the presence of genetic diversities encouraged by the number of alleles existing in a locus vis-à-vis their distributional frequency in a population. This further indicates that spatial distribution in euphorbiaceous trees encourages their taxonomic delimitation.

The PIC obtained from this study implies that ISSR markers are effective for the molecular taxonomic characterisation of *R. heudelotii.* This was emphasised by Najafzadeh *et al.* (2014) that polymorphic information content is a good parameter for determining the effectiveness of primers for molecular taxonomic characteristics of plants. According to Nagy *et al.* (2012), the maximum PIC values that are obtainable from dominant marker analysis like ISSR is 0.5. In the characterisation of *R. heudelotii*, up to 0.42 PIC was obtained at locus UBC-840 in this study, which equally translates to the fact that ISSR markers are informative for the molecular study of *R. heudelotii.*

The three private alleles identified with Olorunlete from the analysis of UBC 818, UBC 859 and ISSR 816 indicated that the OTU is molecularly different from the others. This finding strengthens the fact that ISSR is one of the most significant methods for identifying bands that are unique to plants. This unique band attribute has also been previously discovered in some taxa of *Ricinus communis* (Goodarzi *et al.*, 2015) and *Jatropha* spp. (Vijayanand *et al.*, 2009). According to Goodarzi *et al.* (2015), this uniqueness in banding pattern could be transformed as a distinct fingerprint into sequence-tagged site and sequence characterised amplified regions markers to develop the species-specific marker for proper delimitation of a group of plants.

The clusters of *R. heudelotii* produced by the phylogenetic trees in one way or the other corresponded to the geographic distribution of the trees. This genetic relatedness within each cluster of *R. heudelotii* may have occurred as a result of seed movement and gene flow occasioned by the short distance that exists between the mother trees.

The close relationship exhibited by the OTUs of *R. heudelotii* in Ibadan and Osu based on the phylogenetic tree may be due to the fact that the two OTUs are sister groups. This implies that they share a lot of evolutionary history occasioned by the virtue of a common ancestor and therefore have a little evolutionary history that is peculiar to either one of the two sisters OTUs. These two OTUs can, therefore, be considered as the most recent taxa of *R. heudelotii* in Nigeria, considering the shortest edge length of the tree branch possessed by them.

The OTU from Benin, which is one of the OTUs that was isolated from other clusters can be regarded as the oldest group in the phylogenetic tree. This is due to its branching pattern, which stemmed from the base of the phylogenetic tree and separated from the major cluster.

The evolutionary pattern of *R. heudelotii* from Oloruntele, which got separated from Onigambari, Ile-Ife, Osu, Ikoyi and Ibadan may, therefore, be associated with the three private alleles identified from the group at UBC-818, UBC-859 and ISSR-816 loci.

5.3 Germination Potential of Seeds of *Ricinodendron heudelotii*

Pre-germination treatment methods utilised in this study have a remarkable role to play in the germination of the seeds of *Ricinodendron heudelotii*. However, some are more efficient than others at improving the rate of germination. The significant differences discovered in the effects of pre-germination treatments on the germination of *R. heudelotii* is congruent with the findings of Chebouti-Meziou *et al.* (2014) and Trivedi *et al.,* 2016).

It was revealed in this study that the seeds, which were scarified and soaked in cold water germinated best. This agrees with the literature. According to Mawahib (2004), mechanical scarification of seeds with hard coat through filing takes time and is very difficult to achieve but improves seed germination more than any other mechanical method. Nevertheless, a better germination result was achieved when the mechanical scarification with filing was carried out at the micropylar end of the seed (Mawahib,

2004). Aside from the scarification, environmental factors such as water, light, temperature and pH have been reported repeatedly (Trivedi *et al.,* 2016) to contribute to the germination of seeds. In this study, the soaking of seeds in cold water played a significant role in activating the germination rate of *Ricinodendron heudelotii*, which aligns with Chachalis and Reddy (2000), that water is one of the most important environmental factors affecting the germination of seeds. Concerning the sowing media, seeds sown in topsoil had better germination than those of river sand. This finding may be due to the higher water retention capacity of topsoil water than the river sand. This is based on the fact that seed germination commences with the absorption of water by the seeds and culminates with the appearance of the first true leaves.

The rate of germination was greatly influenced by the pre-germination treatments employed in this study, which produced the peak of germination after about three weeks of sowing while those seeds without scarification were not germinated at all until after eight weeks of sowing. This discovery agrees with Mawahib (2004), who reported a similar finding after a pre-germination treatments study on *Delonix regia*. In his study, about 90% of germination was recorded for mechanically scarified seeds after approximately two weeks of the experiment.

5.4 Early growth of *Ricinodendron heudelotii* and its agroforestry potential

The choice of tree species and its selection for any agroforestry purpose is an important aspect of agroforestry design that must be taken with good care. This is based on the fact that trees play a major role in the component of agroforestry. Based on the analysis of He *et al.* (2015), tree to be considered for alley cropping should be fast-growing ones so that it attains a big size within the shortest period. This would enable the farmer to get a good monetary return after selling the products achievable from it. However, going by the height and diameter growth measured within a year in this study as compared with other exotic tree species in Nigeria (Onefeli and Adesoye, 2014), it can be asserted that *Ricinodendron heudelotii* is a relatively fast-growing species. Unfortunately, it is a general belief that the indigenous tree species in Nigeria are slow-growing. This notion has variously restricted in one way or the other the application and use of the said species for agroforestry activities as well as general plantation projects in the country.

The ability to resprout quickly after pollarding or pruning is another significant criterion considered while choosing an agroforestry tree as the alley (He *et al.,* 2015). In this study, *R. heudelotii* was discovered to coppice effectively with fast growth after being pollarded. In as much that the leaves are consumed as fodder for goats and other domestic animals, this makes the species a good candidate for silvopastoral agroforestry. Also, the branches that resulted from pollarding could be used as a source of firewood for the farmers while the residual leaves and twigs may be utilised as mulch for the replenishment of the soil nutrients.

In this study, it was discovered that alley widths significantly influenced the leaf production observed on the tree component after the pollarding was done with 6m alleys having the highest quantity of leaf produced. The reason for this can be linked to the greater number of shoots and branches produced by trees under 6m alleys. This was probably occasioned by the agroforestry spatial arrangement that necessitated the spreading of the branches compared to the trees in 3m alleys. It could also be inferred from this fact that wider alley widths would encourage the production of flowers, fruits as well as general biomass in alley agroforestry practice.

Other economic benefits highlighted by He *et al.* (2015) as criteria for choosing agroforestry trees include the production of fruit and edible seeds as well as the medicinal effectiveness of the trees. Hence the seeds of *R. heudelotii* are edible and are usually prepared in various ways for consumption (Assanvo and Baruah, 2015). According to Assanvo and Baruah (2015), *R. heudelotii* has proven to be useful for the treatment of various ailments such as ulcer, cough, sexual issues, menstrual pain and infertility.

This study was unable to record the maize yield in terms of the average quantity of grain produced per hectare because the maize cobs were poached prematurely by unknown people from an unknown source. However, an improved height and diameter growth of maize obtained before the incidence from the two alley widths as compared to the control plot is an indication that the trees may have had a complementary contribution to the agroforestry. In other words, the growth records may be used as a surrogate for the yield. The relationship between the maize height, stalk diameter and grain yield has been established (Kelly, 2011; Tandzi and Mutengwa, 2020). Kelly (2011) however stated that

the height and diameter of maize stalk correlated positively with the grain yield, which implies that an increase in height and diameter of maize stalk tends to be associated with grain yield.

CHAPTER SIX

SUMMARY AND CONCLUSIONS

6.1 Summary of Results

This study was executed to provide necessary information for the taxonomic delimitation and optimise the utilisation of *Ricinodendron* species in Southern Nigeria.

The leaves of *R. heudelotii* from Nigeria are generally amphistomatic with polygonal epidermal cells. All the Operational Taxonomic Units (OTUs) of the species had periclinal cell wall alignment except Osu, which was anticlinal on the adaxial layer. *R. heudelotii* from Nigeria is characterised by Paracytic stomata. The number of stomata varied from Ikoyi (3) to 20 (Oloruntele) on the adaxial surface. All the epidermal characteristics of the *Ricinodendron heudelotii* were significantly different ($p<0.05$) except for the number of stomata, number of epidermal cells, epidermal cell widths (ECW) and stomatal index of the adaxial surface and epidermal cell widths and stomata length of the abaxial surface. Oloruntele and Ibadan had the predominant number of the cell on the adaxial layer, which was 241 cells each. The highest Guard Cell Area (243.1±30.5 μm^2), Pore size (322.8±78.5 μm^2), Stomatal length (29.4±2.4 µm) and epidermal cell length (43.7±8.8 µm) on abaxial leaf layer were observed in Akure while the least was in Ikoyi (72.7±7.0 μm^2), Onigambari (40.3±8.0 μm^2), Ikoyi (20.4±3.6 µm) and Ibadan (19.2±8.7 μm^2), respectively. The locations significantly ($p<0.05$) influenced leaf and fruit taxonomic characters. Onigambari had the highest leaf length (53.0±5.8 cm) while Osu had the least (22.3±5.7 cm). Petiole length varied from 8.9±0.1 cm (Boki) to 30.9±5.0 cm (Onigambari). Boki had the highest Fruit Length (45.4±2.6 mm) while Ile-Ife had the least (30.2±11.5 mm). The Fruit Pulp Weight and Fruit Largest Width were highest at Oloruntele (34.3±7.2 g; 44.2±4.0 mm) and least at Akure (18.4±3.3 g; 31.2±1.3 mm). The Fruit Rounness Ratio and seed length varied from Oloruntele (0.77±0.03;14.4±1.7 mm) to Akure (1.31±0.11; 17.1±0.7 mm) while Seed Diameter ranged from 12.6±0.4 mm^2 (Ikoyi)

to 16.3±0.6 mm^2 (Ile-Ife). Based on the Principal Component Analysis for epidermal characteristics, adaxial guard cell area, adaxial pore size, stomatal length for abaxial and pore size for abaxial with 0.47, 0.57, 0.38 and 0.47 loadings respectively are significant for the delimitation of the OTUs of *R. heudelotii* at 77.3 % of the total variation. For leaf and fruit characters, Petiole length, leaf total length, leaflet length, leaf width at the middle, fruit length, fruit weight, fruit largest width, fruit smallest width and pulp weight having 0.48, 0.71, 0.24, 0.20, 0.49, 0.40, 0.43, 0.27 and 0.40 loadings respectively were highly loaded to the taxonomic delimitation of *R. heudelotii.* Pooled morphological characters delimited the OTUs of *R. heudelotii* into four major clusters.

Nineteen (19) of the 35 primers produced scorable alleles and were all polymorphic. A total of 111 polymorphic alleles with an average of 6 were generated across the OTUs. The highest Polymorphic Information Content was discovered at locus UBC-840 (0.42), followed by ISSR-816 (0.41) while UBC-848 (0.21) had the least value. Three private alleles were generated at UBC-859, ISSR-816 and UBC-818 loci with 1600bp, 1800bp and 200bp molecular weight respectively. Oloruntele was more genetically diverse (0.24) than Ikoyi (0.23), Ibadan and Onigambari (0.22) Osu (0.21), Ile-Ife (0.20), Benin (0.12) and Akure (0.08). Four major clusters were formed based on the molecular taxonomic differences in the OTUs.

Effects of pre-germination treatment, sowing media and interaction were significant ($p<0.05$) on the germination percentage of *R. heudelotii.* Scarified and soaked seeds germinated best (35.0±2.5 %) followed by scarified without soaking (33.3±2.1 %) and control had the least germination (11.7±0.8 %). Maximum germination of the seeds occurred within a week of sowing.

The alley widths had a significant ($p<0.05$) effect on the growth *R. heudelotii.* Six (6m) alley width had a better average height (255.7±66.5 cm) than 3 m (170.5±73.6 cm) while the collar diameter of why the 6m alley width performed better (38.1±12.0 cm) than the 3 m (20.1±12.1 cm). The average height of maize in 6m alley, 3m alley and plot without tree were 118.7±13.8 cm, 105.1±29.2 cm and 74.3±19.1 cm respectively. The average collar diameter of maize in 6 m alley was 12.1±4.0 cm, for 3 m alley, 12.4±3.2 cm. Maize grown in the plot without tree had an average collar diameter of 9.7±3.1 cm. Distance to

the trees was not significant ($p>0.05$) on the collar diameter, height and number of leaves of the maize. Coppice height (67.2 cm) of *R. heudelotii* in 3 m alley was higher than those in 6 m alley. Hence, coppice height for both treatments were not significantly different ($p>0.05$). Soil properties of the agroforestry plot, the organic carbon (0.95±0.30 %), nitrogen (0.10±0.02 %), pH (6.14±0.25) and available phosphorus (19.75±5.25 mg kg^{-1}) before experiment were lower compared to after experiment (1.23 %, 0.13 %, 6.36 and 23.91 mg kg^{-1}) respectively.

6.2 Conclusions

Generally, there is considerable variation and some similarities in the taxonomic features of *Ricinodendron heudelotii* in Southern Nigeria. *R. heudelotii* leaves are amphistomatic, while the epidermal cells are distinguished by polygonal cells with mainly arch wall pattern and periclinal alignment. Only paracytic stomata have presently been discovered from Nigerian *Ricinodendron*. All the operational taxonomic units of *Ricinodendron heudelotii* considered in this study are uniquely different from each other as far as the fruit and leaf taxonomic characters are concerned. Findings from this study have revealed that the first principal component of the multivariate application of epidermal characteristics in *Ricinodendron heudelotii* can explain the important differences that exist in the operational taxonomic units as a result of the characters. A similar taxonomic contribution was discovered for the fruit and leaf morphological characters but with a higher percentage, which was explained by the first three principal components. Nevertheless, out of all the forty-four (44) epidermal and macro morphological descriptors considered in this study, ten (10) are the most significant characters that could be utilised for the taxonomic delimitation of the species into different taxa. These include adaxial guard cell area, adaxial pore size, abaxial stomatal length, abaxial pore size, petiole length, leaf total length, fruit length, fruit weight, fruit largest width and pulp weight. These taxonomic characters were able to group all the operational taxonomic units into four major clusters.

It was discovered from this study that the ISSR marker is highly informative for the molecular characterisation of *Ricinodendron heudelotii* at the molecular level. A 100 % polymorphism were produced by the markers with reproducible bands, which indicated the high discriminating power recorded in this study. On average, each marker was able to

amplify approximately six polymorphic DNA fragments from the genomic DNA of the studied tree species. The finding showed that only the first principal component can explain the molecular variation existing in *R. heudelotii*. The OTUs of this tree species in Nigeria have been discovered to have four major groups. To some extent, the clusters produced by molecular markers conformed to that of the morphological marker, which confirmed the fact that the *R. heudelotii* is more than a taxon as previously classified.

The germination of *Ricinodendron heudelotii* seeds up to the tune of about 56 % can be greatly achieved through the application of pre-germination treatments. Based on the findings from this study, the optimum seed germination of this species can only be actualized when the seeds are first mechanically scarified through filing at the micropylar end coupled with soaking in water at room temperature for twenty-four hours and then sown in topsoil.

This study provided important information on the potential of *R. heudelotii* for alley cropping. This includes the determination of its early growth rate, which was considered to be rapid. It was also established that the growth of the species can favourably be compared to that of *Gmelina arborea,* which was reported to be one of the exotic trees in Nigeria with rapid growth. Therefore, the fact that the indigenous trees in Nigeria are slow-growing may have to be reconsidered. Considering the growth characteristics of maize assessed in this study, it may be subjected to further studies in order to determine its compatibility with the studied perennial tree. However, based on the growth rate of *Ricinodendron* recorded in this study, it is advisable to incorporate the test plant before the trees are 6 months old.

6.3 Contribution to Knowledge

This study provided information on the taxonomy and alley cropping potential of *Ricinodendron heudelotii*. Although there had been previous studies on the systematics of indigenous trees in Nigeria, few have documented the taxonomic discrimination and relatedness of members of euphorbiaceous taxa from the population point of view. Most importantly, this study considered the Operational Taxonomic Units (OTUs) of *Ricinodendron*, which happened to be one of the most important fruit plants in its family in Nigeria. References were made to the macromorphology, epidermal characteristics and

molecular differentiation among the units. Therefore, the study provided baseline information on the macromorphology and epidermal taxonomic variation in *Ricinodendron heudelotii* in Southern Nigeria.

The molecular basis for the morphological delimitation of *Ricinodendron heudelotii* was revealed as ISSR markers are informative for the taxonomic description and delimitation of the various OTUs assessed. More importantly, the unique alleles identified in the population of *Ricinodendron heudelotii* from Oloruntele is a novel in as much that they could be used for marker-assisted identification of the population.

A significant breakthrough in the production of seedlings from this species, which has hitherto been problematic is a novelty in the field of silviculture. Hence, seedlings of *Ricinodendron* were successfully produced through scarification at the micropylar region of the seeds.

It was established that one major reason why some indigenous species are not being used for agroforestry and plantation purpose is that they have a slow growth rate because there is limited information on their growth ex-situ. Meanwhile, this study was able to monitor the growth of *Ricinodendron* for one year, which have not been done before but very important in selecting agroforestry tree. With regards to the agroforestry potential of the *Ricinodendron*, this study is the first of its kind to practically carry out the alley cropping study by intercropping maize with *Ricinodendron* on the field. The study demonstrated that *R. heudelotii* is a fast-growing species and can be incorporated into alley cropping with some precautions.

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Appendix 1: Straight trunk of *Ricinodendron heudelotii*

Appendix 2: Compound leaf of *Ricinodendron heudelotii*

Appendix 3: Indehiscent fruits of *Ricinodendron heudelotii*

Appendix 4: Seeds of *Ricinodendron heudelotii*

Appendix 5: Kernel of *Ricinodendron heudelotii*

Appendix 6: Different fruit types of *Ricinodendron heudelotii*

3-seeded fruits

2-seeded fruits

2-seeded fruits with one abortion

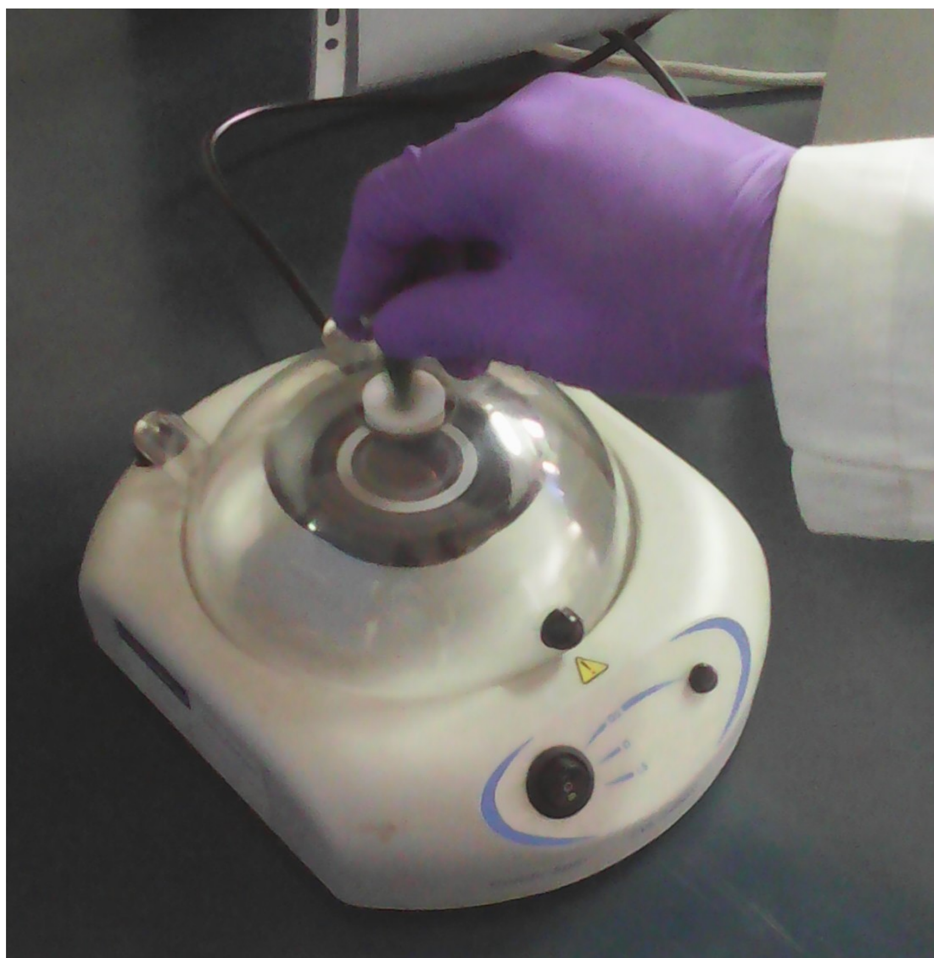
1-seeded fruits with one abortion

Appendix 7: Stands of *Ricinodendron heudelotii* that are deliberately retained in Maize farm

Appendix 8: Ring-barked *Ricinodendron heudelotii* in plantation of *Theobroma cacao*

Appendix 9: Coppiced *Ricinodendron heudelotii* after felling

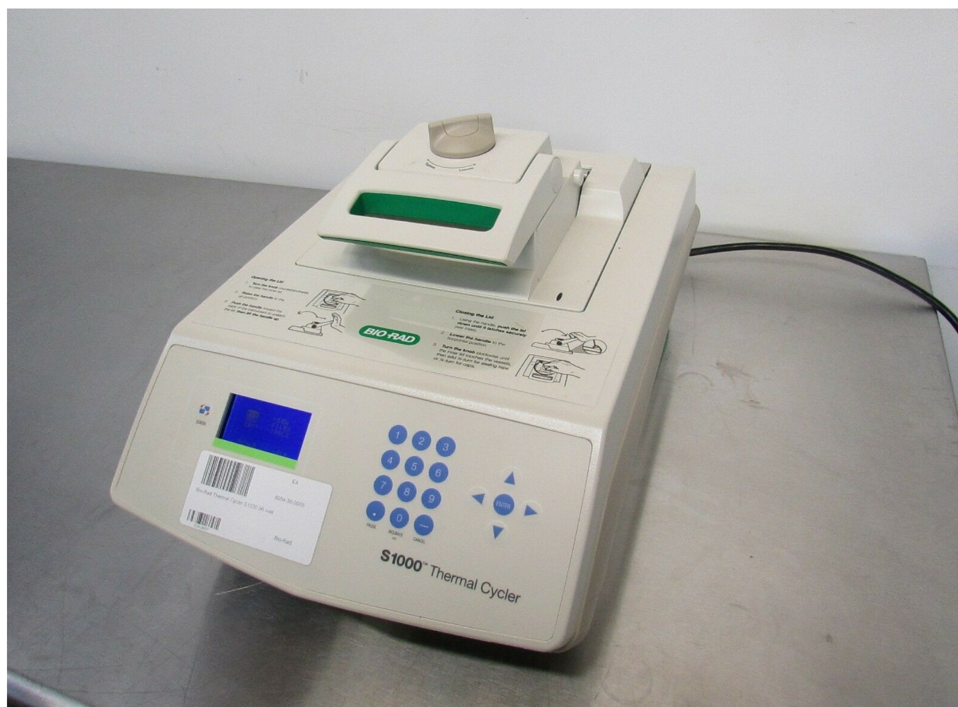
Appendix 10: Vortexing of the ground **s**ample in a microfuge tube using a Biosan MSC-3000 Vortex



Appendix 11: Setting of the incubated ground samples into an Eppendorf refrigerated Centrifuge 5425R for centrifugation

Appendix 12: Showing a NanoPhotometer indicating the concentration of one of the DNA samples

Appendix 13: Showing a 96-well S1000 BIO-RAD thermal cycler used for the Polymerase Chain Reaction (PCR)



Appendix 14: Optimum PCR condition for Primer1 (ISSR 808)

	Master Mix	**x1**	**x14**
	2 x taq	6.25 µl	87.5 µl
	Primer	0.3 µl	4.2 µl
	$MgCl_2$	0.1 µl	1.4 µl
	H_2O	4.85 µl	67.9 µl
	DNA Template	1 µl	
	Rxn Volume	12.5 µl	

Conditions	**Temperature**	**Time**	
	94 ℃	4 mins	
	98 ℃	30 secs	35 Cycles
	48 ℃	1 min	35 Cycles
	72 ℃	3 mins	35 Cycles
	72 ℃	10 mins	
	10 ℃	∞	

Appendix 15: Optimum PCR condition for Primer 2 (UBC 818)

Master Mix	x1	x14
2 x taq	6.25 µl	87.5 µl
Primer	0.3 µl	4.2 µl
$MgCl_2$	0.1 µl	1.4 µl
H_2O	4.85 µl	67.9 µl
DNA Template	1 µl	
Rxn Volume	12.5 µl	

Conditions	Temperature	Time	
	94 °C	4 mins	
	98 °C	30 secs	35 Cycles
	48 °C	1 min	
	72 °C	3 mins	
	72 °C	10 mins	
	10 °C	∞	

Appendix 16: Optimum PCR condition for Primer 3 (ISSR 815)

	Master Mix	**x1**	**x14**
	2 x taq	6.25 µl	87.5 µl
	Primer	0.3 µl	4.2 µl
	$MgCl_2$	0.1 µl	1.4 µl
	H_2O	4.85 µl	67.9 µl
	DNA Template	1 µl	
	Rxn Volume	12.5 µl	
Conditions	**Temperature**	**Time**	
	94 °C	4 mins	
	98 °C	30 secs	35 Cycles
	48 °C	1 min	
	72 °C	3 mins	
	72 °C	10 mins	
	10 °C	∞	

Appendix 17: Optimum PCR condition for Primer 4 (UBC 840)

Master Mix	x1	x14
2 x taq	6.25 µl	87.5 µl
Primer	0.3 µl	4.2 µl
$MgCl_2$	0.1 µl	1.4 µl
H_2O	4.85 µl	67.9 µl
DNA Template	1 µl	
Rxn Volume	12.5 µl	

Conditions	Temperature	Time	
	94 ℃	4 mins	
	98 ℃	30 secs	35 Cycles
	45 ℃	1 min	35 Cycles
	72 ℃	3 mins	35 Cycles
	72 ℃	10 mins	
	10 ℃	∞	

Appendix 18: Optimum PCR condition for Primer 5 (UBC 859)

Master Mix	**x1**	**x14**
2 x taq	6.25 µl	87.5 µl
Primer	0.3 µl	4.2 µl
$MgCl_2$	0.1 µl	1.4 µl
H_2O	4.85 µl	67.9 µl
DNA Template	1 µl	
Rxn Volume	12.5 µl	

Conditions	**Temperature**	**Time**	
	94 ℃	4 mins	
	98 ℃	30 secs	35 Cycles
	47 ℃	1 min	35 Cycles
	72 ℃	3 mins	35 Cycles
	72 ℃	10 mins	
	10 ℃	∞	

Appendix 19: Optimum PCR condition for Primer 6 (UBC 848)

Master Mix	x1	x14
2 x taq	6.25 µl	87.5 µl
Primer	0.3 µl	4.2 µl
$MgCl_2$	0.1 µl	1.4 µl
H_2O	4.85 µl	67.9 µl
DNA Template	1 µl	
Rxn Volume	12.5 µl	

Conditions	Temperature	Time	
	94 ℃	4 mins	
	98 ℃	30 secs	35 Cycles
	49 ℃	1 min	
	72 ℃	3 mins	
	72 ℃	10 mins	
	10 ℃	∞	

Appendix 20: Optimum PCR condition for Primer 7 (UBC 844)

	Master Mix	x1	x14
	2 x taq	6.25 µl	87.5 µl
	Primer	0.3 µl	4.2 µl
	$MgCl_2$	0.1 µl	1.4 µl
	H_2O	4.85 µl	67.9 µl
	DNA Template	1 µl	
	Rxn Volume	12.5 µl	
Conditions	**Temperature**	**Time**	
	94 °C	4 mins	
	98 °C	30 secs	35 Cycles
	43 °C	1 min	35 Cycles
	72 °C	3 mins	35 Cycles
	72 °C	10 mins	
	10 °C	∞	

Appendix 21: Optimum PCR condition for Primer 8 (UBC 857)

	Master Mix	**x1**	**x14**
	2 x taq	6.25 µl	87.5 µl
	Primer	0.3 µl	4.2 µl
	$MgCl_2$	0.1 µl	1.4 µl
	H_2O	4.85 µl	67.9 µl
	DNA Template	1 µl	
	Rxn Volume	12.5 µl	
Conditions	**Temperature**	**Time**	
	94 °C	4 mins	
	98 °C	30 secs	35 Cycles
	42 °C	1 min	35 Cycles
	72 °C	3 mins	35 Cycles
	72 °C	10 mins	
	10 °C	∞	

Appendix 22: Optimum PCR condition for Primer 9 (UBC 812)

Master Mix	x1	x14
2 x taq	6.25 µl	87.5 µl
Primer	0.3 µl	4.2 µl
$MgCl_2$	0.1 µl	1.4 µl
H_2O	4.85 µl	67.9 µl
DNA Template	1 µl	
Rxn Volume	12.5 µl	

Conditions	Temperature	Time	
	94 ℃	4 mins	
	98 ℃	30 secs	35 Cycles
	47 ℃	1 min	35 Cycles
	72 ℃	3 mins	35 Cycles
	72 ℃	10 mins	
	10 ℃	∞	

Appendix 23: Optimum PCR condition for Primer 10 (UBC 817)

Master Mix	x1	x14
2 x taq	6.25 µl	87.5 µl
Primer	0.3 µl	4.2 µl
$MgCl_2$	0.1 µl	1.4 µl
H_2O	4.85 µl	67.9 µl
DNA Template	1 µl	
Rxn Volume	12.5 µl	

Conditions	Temperature	Time	
	94 ℃	4 mins	
	98 ℃	30 secs	35 Cycles
	50 ℃	1 min	35 Cycles
	72 ℃	3 mins	35 Cycles
	72 ℃	10 mins	
	10 ℃	∞	

Appendix 24: Optimum PCR condition for Primer 11 (UBC 825)

Master Mix	x1	x14
2 x taq	6.25 µl	87.5 µl
Primer	0.3 µl	4.2 µl
$MgCl_2$	0.1 µl	1.4 µl
H_2O	4.85 µl	67.9 µl
DNA Template	1 µl	
Rxn Volume	12.5 µl	

Conditions	Temperature	Time	
	94 °C	4 mins	
	98 °C	30 secs	35 Cycles
	45 °C	1 min	35 Cycles
	72 °C	3 mins	35 Cycles
	72 °C	10 mins	
	10 °C	∞	

Appendix 25: Optimum PCR condition for Primer 12 (UBC 822)

	Master Mix	x1	x14
	2 x taq	6.25 µl	87.5 µl
	Primer	0.3 µl	4.2 µl
	$MgCl_2$	0.1 µl	1.4 µl
	H_2O	4.85 µl	67.9 µl
	DNA Template	1 µl	
	Rxn Volume	12.5 µl	
Conditions	**Temperature**	**Time**	
	94 ℃	4 mins	
	98 ℃	30 secs	35 Cycles
	44 ℃	1 min	35 Cycles
	72 ℃	3 mins	35 Cycles
	72 ℃	10 mins	
	10 ℃	∞	

Appendix 26: Optimum PCR condition for Primer 13 (ISSR 7)

	Master Mix	**x1**	**x14**
	2 x taq	6.25 µl	87.5 µl
	Primer	0.3 µl	4.2 µl
	$MgCl_2$	0.1 µl	1.4 µl
	H_2O	4.85 µl	67.9 µl
	DNA Template	1 µl	
	Rxn Volume	12.5 µl	
Conditions	**Temperature**	**Time**	
	94 ℃	4 mins	
	98 ℃	30 secs	35 Cycles
	43 ℃	1 min	35 Cycles
	72 ℃	3 mins	35 Cycles
	72 ℃	10 mins	
	10 ℃	∞	

Appendix 27: Optimum PCR condition for Primer 14 (ISSR 816)

	Master Mix	x1	x14
	2 x taq	6.25 µl	87.5 µl
	Primer	0.3 µl	4.2 µl
	$MgCl_2$	0.1 µl	1.4 µl
	H_2O	4.85 µl	67.9 µl
	DNA Template	1 µl	
	Rxn Volume	12.5 µl	
Conditions	**Temperature**	**Time**	
	94 ℃	4 mins	
	98 ℃	30 secs	35 Cycles
	42 ℃	1 min	35 Cycles
	72 ℃	3 mins	35 Cycles
	72 ℃	10 mins	
	10 ℃	∞	

Appendix 28: Optimum PCR condition for Primer 15 (UBC 842)

Master Mix	x1	x14
2 x taq	6.25 µl	87.5 µl
Primer	0.3 µl	4.2 µl
$MgCl_2$	0.1 µl	1.4 µl
H_2O	4.85 µl	67.9 µl
DNA Template	1 µl	
Rxn Volume	12.5 µl	

Conditions	Temperature	Time	
	94 ℃	4 mins	
	98 ℃	30 secs	35 Cycles
	51 ℃	1 min	35 Cycles
	72 ℃	3 mins	35 Cycles
	72 ℃	10 mins	
	10 ℃	∞	

Appendix 29: Optimum PCR condition for Primer 16 (UBC 845)

	Master Mix	**x1**	**x14**
	2 x taq	6.25 µl	87.5 µl
	Primer	0.3 µl	4.2 µl
	$MgCl_2$	0.1 µl	1.4 µl
	H_2O	4.85 µl	67.9 µl
	DNA Template	1 µl	
	Rxn Volume	12.5 µl	
Conditions	**Temperature**	**Time**	
	94 ℃	4 mins	
	98 ℃	30 secs	35 Cycles
	50 ℃	1 min	
	72 ℃	3 mins	
	72 ℃	10 mins	
	10 ℃	∞	

Appendix 30: Optimum PCR condition for Primer 17 (UBC 834)

Master Mix	x1	x14
2 x taq	6.25 µl	87.5 µl
Primer	0.3 µl	4.2 µl
$MgCl_2$	0.1 µl	1.4 µl
H_2O	4.85 µl	67.9 µl
DNA Template	1 µl	
Rxn Volume	12.5 µl	

Conditions	Temperature	Time	
	94 ℃	4 mins	
	98 ℃	30 secs	35 Cycles
	48 ℃	1 min	35 Cycles
	72 ℃	3 mins	35 Cycles
	72 ℃	10 mins	
	10 ℃	∞	

Appendix 31: Optimum PCR condition for Primer 18 (UBC 836)

Master Mix	x1	x14
2 x taq	6.25 µl	87.5 µl
Primer	0.3 µl	4.2 µl
$MgCl_2$	0.1 µl	1.4 µl
H_2O	4.85 µl	67.9 µl
DNA Template	1 µl	
Rxn Volume	12.5 µl	

Conditions	Temperature	Time	
	94 ℃	4 mins	
	98 ℃	30 secs	35 Cycles
	46 ℃	1 min	35 Cycles
	72 ℃	3 mins	35 Cycles
	72 ℃	10 mins	
	10 ℃	∞	

Appendix 32: Optimum PCR condition for Primer 19 (UBC 885)

	Master Mix	**x1**	**x14**
	2 x taq	6.25 µl	87.5 µl
	Primer	0.3 µl	4.2 µl
	$MgCl_2$	0.1 µl	1.4 µl
	H_2O	4.85 µl	67.9 µl
	DNA Template	1 µl	
	Rxn Volume	12.5 µl	
Conditions	**Temperature**	**Time**	
	94 °C	4 mins	
	98 °C	30 secs	35 Cycles
	47 °C	1 min	35 Cycles
	72 °C	3 mins	35 Cycles
	72 °C	10 mins	
	10 °C	∞	

Appendix 33: Analysis of Variance for effect of OTUs on quantitative adaxial epidermal characteristics of *Ricinodendron heudelotii*

Variables	Source of Variation	Sum of Squares	Df	Mean Square	F	Sig.
ECL	OTUs	1556.681	8	194.585	7.911	0.000
	Error	1992.30	81	24.596		
	Total	3548.98	89			
ECW	OTUs	210.597	8	26.325	1.178	0.364
	Error	1810.46	81	22.351		
	Total	2021.06	89			
GCA	OTUs	165646.800	8	20705.850	10.210	0.000
	Error	164267.83	81	2027.998		
	Total	329914.63	89			
SL	OTUs	456.699	8	57.087	4.950	0.002
	Error	934.11	81	11.532		
	Total	1390.81	89			
SW	OTUs	1892.819	8	236.602	12.396	0.000
	Error	1546.07	81	19.087		
	Total	3438.89	89			
SI	OTUs	309.245	8	38.656	1.457	0.240
	Error	2148.50	81	26.525		
	Total	2457.75	89			
GCW	OTUs	195.567	8	24.446	8.709	0.000
	Error	227.38	81	2.807		
	Total	422.94	89			
PS	OTUs	211811.140	8	26476.392	18.371	0.000
	Error	116736.51	81	1441.192		
	Total	328547.65	89			

Appendix 34: Kruskal Wallis Test for effect of OTUs on nominal adaxial epidermal characteristics of *Ricinodendron heudelotii*

Statistics	NS	NEC	NWS
Chi-Square	11.595	12.482	18.290
df	8	8	8
Sig.	0.170	0.131	0.019

Appendix 35: Analysis of Variance for effect of OTUs on quantitative abaxial epidermal characteristics of *Ricinodendron heudelotii*

Variables	Source of Variation	Sum of Squares	Df	Mean Square	F	Sig.
ECL	OTUs	1882.74	8	235.343	2.566	0.046
	Error	7428.00	81	91.704		
	Total	9310.74	89			
ECW	OTUs	1033.33	8	129.167	2.041	0.106
	Error	5127.00	81	63.296		
	Total	6160.33	89			
GCA	OTUs	101970.728	8	12746.341	15.334	0.000
	Error	67330.36	81	831.239		
	Total	169301.09	89			
SL	OTUs	198.074	8	24.759	1.207	0.338
	Error	1662.00	81	20.519		
	Total	1860.07	89			
SW	OTUs	1696.215	8	212.027	22.000	0.000
	Error	780.64	81	9.638		
	Total	2476.85	89			
SI	OTUs	3439.073	8	429.884	5.979	0.001
	Error	5824.28	81	71.905		
	Total	9263.36	89			
GCW	OTUs	232.296	8	29.037	15.680	0.000
	Error	150.00	81	1.852		
	Total	382.30	89			
PS	OTUs	188047.979	8	23505.997	21.360	0.000
	Error	89136.67	81	1100.453		
	Total	277184.65	89			

Appendix 36: Kruskal Wallis Test for effect of OTUs on nominal abaxial epidermal characteristics of *Ricinodendron heudelotii*

Statistics	NS	NWS	NEC
Chi-Square	17.402	16.346	19.346
df	8	8	8
Sig.	0.026	0.024	0.013

Appendix 37: Analysis of Variance for effect of OTUs on fruit taxonomic characters of *Ricinodendron heudelotii*

Variables	Source of Variation	Sum of Squares	Df	Mean Square	F	Sig.
AL	OTUs	74.065	8	9.258	24.414	0.038
	Error	167.231	441	0.379		
	Total	241.296	449			
LTL	OTUs	37058.170	8	4632.271	95.990	0.000
	Error	21281.708	441	48.258		
	Total	58339.878	449			
LL	OTUs	5855.029	8	731.879	87.060	0.000
	Error	3707.320	441	8.407		
	Total	9562.349	449			
PW	OTUs	777.622	8	97.203	256.957	0.000
	Error	166.823	441	0.378		
	Total	944.445	449			
LWB	OTUs	253.005	8	31.626	43.913	0.002
	Error	317.602	441	0.720		
	Total	570.606	449			
LWM	OTUs	1886.075	8	235.759	71.012	0.000
	Error	1464.126	441	3.320		
	Total	3350.201	449			
LWT	OTUs	1383.199	8	172.900	53.863	0.003
	Error	1415.601	441	3.210		
	Total	2798.800	449			
PL	OTUs	19840.445	8	2480.056	115.528	0.000
	Error	9466.986	441	21.467		
	Total	29307.431	449			
SBSV	OTUs	419.337	8	52.417	341.661	0.000
	Error	67.658	441	0.153		
	Total	486.994	449			

Appendix 38: Kruskal Wallis Test for effect of OTUs on nominal fruit characteristics of *Ricinodendron heudelotii*

Statistics	NLL	NSV
Chi-Square	307.144	218.339
df	8	8
Sig.	0.000	0.006

Appendix 39: Analysis of Variance for effect of OTUs on fruit taxonomic characters of *Ricinodendron heudelotii*

Variables	Source of Variation	Sum of Squares	Df	Mean Square	F	Sig.
FLW	OTUs	2258.246	8	282.281	22.372	0.000
	Error	5564.30225	441	12.617		
	Total	7822.548	449			
FSW	OTUs	1859.710	8	232.464	16.570	0.000
	Error	6186.70266	441	14.029		
	Total	8046.412	449			
PW	OTUs	3441.419	8	430.177	14.392	0.000
	Error	13181.2975	441	29.890		
	Total	16622.717	449			
FL	OTUs	2743.154	8	342.894	36.956	0.000
	Error	4091.82999	441	9.279		
	Total	6834.984	449			
FW	OTUs	3716.230	8	464.529	12.048	0.000
	Error	17003.9479	441	38.558		
	Total	20720.177	449			
SL	OTUs	111.154	8	13.894	6.013	0.000
	Error	1019.00853	441	2.311		
	Total	1130.162	449			
SD	OTUs	210.798	8	26.350	25.411	0.000
	Error	457.285536	441	1.037		
	Total	668.084	449			
SW	OTUs	1365.998	8	170.750	65.960	0.000
	Error	1141.61333	441	2.589		
	Total	2507.611	449			
RR	OTUs	5.022	8	.628	105.337	0.000
	Error	2.62817485	441	.006		
	Total	7.650	449			

Appendix 40: Kruskal Wallis Test for effect of OTUs on nominal fruit characteristics of *Ricinodendron heudelotii*

Statistics	NSF	NAS
Chi-Square	65.249	70.264
df	8	8
Sig.	0.000	0.000

Appendix 41: Analysis of Variance for effect of Pre-germination treatments and sowing media on germination of seeds of *Ricinodendron heudelotii*

Sources of Variation	Sum of Squares	df	Mean Square	F	Sig.
Pretreatments	1355.56	2	677.778	9.15	0.015
Sowing media	2503.7	1	2503.7	33.8	0.001
Pretreatments * Sowing media	585.185	2	292.593	3.95	0.004
Error	444.444	6	74.074		
Total	4888.89	11			

Appendix 42: Analysis of Variance for effect of alley treatments on collar diameter and height of maize

Variables	Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Collar diameter	Treatments	100.821	2	50.410	4.369	0.015
	Error	1476.884	128	11.538		
	Total	1577.704	130			
height	Treatments	19629.260	2	9814.630	14.543	0.000
	Error	86381.366	128	674.854		
	Total	106010.626	130			

Appendix 43: Analysis of Variance for effect of planting distance on collar diameter and height of maize

Variables	Treatments	Sum of Squares	df	Mean Square	F	Sig.
Collar diameter	Planting distance to the alley	33.681	3	11.227	0.993	0.399
	Error	1152.852	102	11.302		
	Total	1186.533	105			
height	Between Groups	4745.852	3	1581.951	1.794	0.153
	Within Groups	89926.497	102	881.632		
	Total	94672.349	105			

Appendix 44: Kruskal Wallis Test for effect of number of alley treatments and planting distance on leaves produced maize

Statistics	Alley treatments	Planting distance
Chi-Square	10.206	6.214
df	2	3
Sig.	0.006	0.102

Appendix 45: Analysis of Variance for effect of alley treatments on the soil properties

Variables	Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
pH	Alley treatments	0.219	2	0.109	4.038	0.036
	Error	0.487	18	0.027		
	Total	0.706	20			
% O.C	Alley treatments	0.604	2	0.302	1.565	0.236
	Error	3.476	18	0.193		
	Total	4.080	20			
% Nitrogen	Alley treatments	0.007	2	0.004	1.650	0.220
	Error	0.039	18	0.002		
	Total	0.046	20			
Av. P (mg/kg	Alley treatments	174.925	2	87.462	26.013	0.000
	Error	60.521	18	3.362		
	Total	235.446	20			
Ca(Cmol/kg)	Alley treatments	0.534	2	0.267	2.160	0.144
	Error	2.226	18	0.124		
	Total	2.760	20			
Mg (Cmol/kg)	Alley treatments	0.054	2	0.027	5.376	0.015
	Error	0.090	18	0.005		
	Total	0.144	20			
K(Cmol/kg)	Alley treatments	0.030	2	0.015	3.725	0.044
	Error	0.073	18	0.004		
	Total	0.103	20			
Na(Cmol/kg)	Alley treatments	0.018	2	0.009	51.049	0.000
	Error	0.003	18	0.000		
	Total	0.022	20			
Fe (mg/kg)	Alley treatments	9291.560	2	4645.780	1.555	0.238
	Error	53783.679	18	2987.982		
	Total	63075.238	20			