

# CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

# **Faculty of Tropical AgriSciences**

# Morphological and genetic diversity of wild and domesticated populations of njansang (*Ricinodendron heudelotii* Baill Heckel) in Cameroon

MASTER'S THESIS

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# Declaration

I Martin Smetana declare that I have done this master's thesis entitled 'Morphological and genetic diversity of wild and domesticated populations of njansang (*Ricinodendron heudelotii* Baill Heckel) in Cameroon' independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague, 22<sup>nd</sup> April 2023

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Martin Smetana

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It was not easy!

### Abstract

Ricinodendron heudelotii (Baill.) Heckel (Euphorbiaceae) is a multipurpose tree species native to humid tropical forests of sub-Saharan Africa. The species is most valued for its oily kernels, which are an important source of food and income for many communities in West Africa, including Cameroon, where the kernels are known as njansang. The main goal of this study was to gather more information on the intraspecific variability of populations of R. heudelotii from different geographical sites in Cameroon and acquire insight of the local ethnobotanical knowledge of the species. This was achieved in 3 objectives. In the first one, intraspecific diversity of R. heudelotii was assessed by measuring morphological characteristics of, in total, 380 fruits and 696 seeds collected from 50 trees coming from 4 different sites in Cameroon. The resulting data did not show statistically significant difference between the 3 populations from sites found in the South region (Vallée-du-Ntem division), while the population found in the Centre region (Nyong-et-Mfoumou division) did differ significantly in terms of fruit length and seed weight. Thus, the second objective was accomplished through principal component analysis of the morphological data which showed the existence of 2 well-defined populations, 1 in each geographical region, while suggesting great variability within the populations as well. The third objective was done by questioning the local farmers, 31 in the South region and 35 in the Centre region. The ethnobotanical information provided insight into management and importance of the species to local communities. It is evident that R. heudelotii is of great value and there is interest in increasing cultivation and production of its kernels, although there are still certain obstacles delaying the domestication efforts. Further research on the species, especially in the field of genetics, and close cooperation with the local communities and researchers seems vital for achieving a successful outcome.

Key words: Farming practices, genetic diversity, morphology, njansang, R. heudelotii

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# List of the abbreviations used in the thesis

BUCREP - Bureau Central des Recensements et des Etudes de Population

- CFA Central African Franc
- ČZU Czech University of Life Sciences
- DBH Diameter at breast height
- ICRAF -- International Council for Research in Agroforestry
- ISSR Inter-simple sequence repeat.
- masl Metres above sea level
- NTFPs -Non-timber forest products
- PCA Principal component analysis

## **1.** Introduction

The term 'non-timber forest products' (NTFPs) is commonly used to describe natural products other than commercial timber which people harvest from forests and other, natural, or human-influenced, habitats. Such products are generally sourced from wild populations of both native and non-native organisms. NTFPs of animal origin can be game, honey, insects, snails, and venison, while those of plant and fungal origin include herbs, spices, fibre, rubber, firewood, essential oils, berries, nuts, and mushrooms (Suleiman et al. 2017; Epanda et al. 2020). Shackleton and de Vos (2022) estimated that approximately 3.5-5.8 billion people worldwide, both in rural and urban areas, use NTFPs to some degree. West Africa is often mentioned as a region in which many communities financially depend on selling NTFPs which can represent up to 80% of income for some rural households in Nigeria (Jimoh & Haruna 2007) or 56% in Cameroon (Endamana et al. 2016). It is assumed that with the right approach NTFPs represent a unique opportunity to help people fight poverty, improve their wellbeing, and offer valuable resources in times of scarcity, while also helping to protect the whole forest ecosystems from destruction (Shanley et al. 2015).

*R. heudelotii* (Baill.) Heckel is a dioecious tree belonging to the Euphorbiaceae family (Facheux et al. 2007), which is commonly known as njansang in Cameroon, and the species is a valuable multipurpose tree (Donfagsiteli Tchinda et al. 2013). Its native range includes humid and semi-deciduous forest habitats as well as wooded savannah regions of tropical Africa from Senegal to East Africa and Madagascar (Iyiola et al. 2019). The tree is economically and nutritionally appreciated for its oily kernels used in West-African cuisine, namely in Ivory Coast, Ghana, Nigeria, Cameroon, and Gabon (Mpeck et al. 2003). In Cameroon, the fallen fruits, a valued commodity, are traditionally collected from wild trees by women from July to October (Caspa et al. 2014). Ndumbe et al. (2019) claim that *R. heudelotii*, along with other Cameroonian native species (e.g., *Irvingia gabonensis* Aubry Lecomte ex O Rorke Baill or *Gnetum africanum* Welw.), is one of the most important non-timber forest products (NTFPs) of significant commercial value, as the fruits harvested in the Southwest Region alone were sold for 33,511 EUR in 2015.

Despite the fact, that *R. heudelotii* is widely utilized for food (fruits, protein-rich leaves), agroforestry (shade, erosion prevention, improvement of soil fertility), and fodder (leaves fed to goats and sheep), no descriptors to assess the morphological diversity of the species have been developed yet (Tchoundjeu & Atangana 2006). *R. heudelotii* has been recognised among the top priority tree species under the tree domestication program led by World Agroforestry Centre (ICRAF) over the past twenty years. Other key species are, among others: *Adansonia digitata* L., *Allanblackia floribunda* Oliv., *Dacryodes edulis* (G. Don) H.J. Lam., *Garcinia kola* Engl., and *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill (Franzel & Kindt 2012).

Since *R. heudelotii* represents an essential source of food and income for some rural families (Cosyns et al. 2011) it is beneficial to promote its domestication and cultivation to allow the people to be less dependent on the wild specimens growing in diminishing habitats (Fongnzossie et al. 2014) through creation of cultivars with more desirable characteristics such as stable yields, dwarfed growth, or better kernel qualities (e.g., nutritional properties or taste). Thus, domestication of *R. heudelotii* would be benefiting the rural communities both in terms of food and financial security (Jimoh & Haruna 2007). Acquiring better knowledge regarding the current *R. heudelotii* populations in Cameroon can prove helpful for the domestication efforts. Therefore, the main aim of this study was to describe and evaluate morphological differences, namely in fruits and kernels, between the populations of *R. heudelotii* growing in different geographical areas and land-use systems, as well as comparing the trees growing in their natural habitat (wild) and the trees found on farms or in agroforestry systems (undergoing domestication). Another goal of the study was to assess how the local farmers regard the species in terms of value, utilization, and gender-cultural aspects.

# 2. Literature review

### 2.1. Fruit tree domestication

At the end of the last major glacial period, approximately 12,000 years ago, originally hunter-gatherer societies in several different parts of the world started cultivating plants for food and other purposes. This period of human history is known as 'The Neolithic Revolution' and marks the beginning of rapid social-cultural progress (Purugganan 2019). Therefore, plant domestication is recognized as one of the most important milestones in the development of anthropological societies worldwide as cultivation of domesticated crops led to improved food security and at least partial independence on wild-growing food sources, consequently enabling people to establish permanent settlements and larger, more complex communities (Fuller & Stevens 2019). Although it is usually believed that perennial fruit crops (trees and vines) were generally cultivated and domesticated several millennia later than annual staple crops (cereals and pulses), their importance to humans is no less significant (Goldschmidt 2013). Moreover, it might be possible that certain fruit tree species underwent domestication equally or even sooner than annual crops, which could be the case of the common fig tree (*Ficus carica* L.) (Kislev et al. 2006). According to Miller and Gross (2011), perennial fruit crops originate from all major domestication centres on Earth. These centres are found in eastern Asia (Citrus L.), Central America (Carica papaya L., Casimiroa edulis La Llave, Persea americana Mill.,), the Middle East and Mediterranean basin (Ficus carica L., Phoenix dactylifera L., Pistacia vera L., Punica granatum L., Olea europaea L., Vitis vinifera L.), South America (Anacardium occidentale L., Annona L., Psidium guajava L.), and Central Asia (Malus spp Mill., Prunus dulcis D.A.Webb, Prunus persica (L.) Batsch, Pyrus communis L.). It is worth mentioning that early domesticated fruit species were among the first industrially processed foods (grape wine, olive oil). Dried or otherwise preserved fruits improved nutrition during times of scarcity (Goldschmidt 2013), and many fruit trees play a substantial role in modern human nutrition and global economy today (Schreckenberg et al. 2006).

However, the majority of fruit tree species remains undomesticated or is at the beginning of the domestication process (Ingvarsson & Dahlberg 2019). Fruit trees are

plants which usually need to grow for years before becoming productive, often require pollination by an unrelated specimen to reproduce sexually, and their populations tend to be highly genetically diverse. Due to such characteristics, domestication of tree crops is often achieved through vegetative propagation of specimens with desirable characteristics (Miller & Gross 2011). While this is certainly true in modern fruit tree breeding, Fuller and Stevens (2019) argue that human interference with fruit tree genetics is still poorly understood, and evidence suggests that some fruit tree species were grown from seeds and selected similarly to annual corps, at least during the early stages of domestication. However, regardless of the means of breeding and propagation, the main objective of fruit tree domestication is to obtain cultivars which mature at younger age and have high, constant yields (Goldschmidt 2013). Since fruit tree species are often long-lived and propagated clonally, the genetic diversity of their wild or semi-domesticated populations tends to remain high (Pickersgill 2007; Goldschmidt 2013). Despite that, domestication still inevitably leads to selection of specimens with certain phenotypes, thus there is a risk of genetic erosion of the species through 'domestication bottleneck' (Doebley 1989). It is advised to keep the breeding populations large enough to avoid loss of genetic diversity through the domestication process (Harfouche et al. 2012).

Domestication of indigenous fruit trees is thought to be beneficial for small household farmers in many countries, especially in Africa, as well as Asia, Central and South America, and Oceania. The starting point of such programmes is to encourage farmers to replace collection of wild fruits with cultivation of the chosen species (Goldschmidt 2013). High genetic variability in wild populations of fruit trees and sitespecific adaptations of each provenance are the main obstacles in the beginning of the domestication efforts. Thus, it is recommended to start with testing large number of seedlings from different provenances of the target species under diverse conditions in several areas. The promising specimens can then be selected for further vegetative propagation and creation of clonal lines (Akinnifesi et al. 2004; Leakey 2004). Some examples of recently domesticated fruit trees are the pecan nut (Carya illinoiensis (Wangenh.) K. Koch) and macadamia hybrids (Macadamia F.Muell.) which have been domesticated about 200 and 100 years ago, respectively. Wild pecan trees irregularly exhibit masting behaviour in some years when the whole population flowers and produces larger than usual amounts of seeds. This survival strategy is undesirable under cultivation but can be supressed to some extent through the right agricultural management (Goldschmidt 2013). The Australian macadamia is also a successful case of a domesticated fruit tree which was not commercially utilized until the 20<sup>th</sup> century. In the wild, macadamias do not produce fruit regularly and some specimens do not flower until reaching age of more than 20 years. Thanks to cultivation and selection, the domesticated trees have stable, regular yields, and start producing fruit at the age of 5-8 years (Hardner et al. 2009; Goldschmidt 2013). Therefore, novel domestication of forest fruit tree species seems plausible and desirable.

### 2.2. Ricinodendron heudelotii

#### **2.2.1. Taxonomy**

*Ricinodendron heudelotti* (Baill.) Pierre ex Pax. is one of more than approximately 7,000 species belonging to the family Euphorbiaceae. There are about 300 genera in the family, thus making it the largest family of the order Euphorbiales. With such a number of genera and species the family Euphorbiaceae is represented by a great diversity of plant body planes including herbaceous and succulent forms and woody shrubs and trees, as well as vines. Despite the high variability of physical characteristics, the majority of the species belonging to the family Euphorbiaceae has the ability to produce a protective secondary metabolite, known as latex, in the laticiferous cell found in the leaves or stems (Tchoundjeu & Atangana 2006), and *R. heudelotii* is not an exception (Yakubu et al. 2019).

Many species in the family can be utilized by humans as a source of food, medicine, industrial material or for ornamental purposes. The most economically important species have been domesticated, notably cassava (*Manihot esculenta* Crantz.), castor bean (*Ricinus communis* L.), purging nut (*Jatropha spathulata* Müll.-Arg.), rubber (*Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg.) and tung oil tree (*Vernicia fordii* (Hemsl.) Airy-Shaw), while others are still being used in their rather wild state, such as largely medicinal species of the genera *Euphorbia*, *Hura*, *Jatropha*, *Pedilanthus* and *Phyllanthus* (Tchoundjeu & Atangana 2006; Rahman & Akter 2013).

According to the latest taxonomical classification, *R. heudelotii* is the only species in its genus, thus rendering the genus monotypic (Tchoundjeu & Atangana 2006). However, it is thought that based on distinct morphological properties and different areas of distribution two subspecies can be distinguished: *R. heudelotii* subsp. *heudelotii* and *R. heudelotii* subsp. *Africanum* (Plenderleith 1997; Tchoundjeu & Atangana 2006, 2007). The closest related species to *R. heudelotii* is *Schinziophyton rautanenii* (Schinz) Radl. Sm., commonly called mongongo, which was originally named *R. rautanenii* Schinz as the species used to be contained within in the *Ricinodendron* genus (Tchoundjeu & Atangana 2006; Jangid & Gupta 2016).

#### 2.2.2. Origin and distribution

The species is native to a large portion of tropical Africa (Figure 1), as such the geographical distribution of *R. heudelotii* encompasses Senegal, Guinea, Liberia, Ivory Coast, Ghana, Nigeria, Cameroon, Gabon, and Congo in the west, continues through Central African Republic and Democratic Republic of Congo to Uganda, Kenya, and Tanzania in the east and reaches to Tanzania, Angola, and Zambia in the south



**Figure 1.** Distribution of *R. heudelotii* subsp. *heudelotii* (orange) and *R. heudelotii* subsp. *africanum* (green). Based on: https://powo.science.kew.org/. Created using: https://www.mapchart.net/.

(Tchoundjeu & Atangana 2006; Iyiola et al. 2019). Subspecies *heudelotii* occupies the western part of the range from Ghana westwards, while subspecies *africanum* can be found from Nigeria eastwards and southwards (Plenderleith 1997; Tchoundjeu & Atangana 2006).

In its natural habitat, *R. heudelotii* is typically found throughout a range of environments including African semi-dry wooded savannahs, moist deciduous forests, and rainforests (Guillaume et al. 2022). *R. heudelotii* is a very light-demanding pioneer tree species able to colonize clearings in fractured forests, abandoned pastures and farmland, therefore it is a common tree of secondary and transitional forests as well as forest edges. Human-controlled habitats containing *R. heudelotii* include cocoa plantations, home gardens, fields, and fallows (Tchoundjeu & Atangana 2006; Ouédraogo et al. 2011).

*R. heudelotii* trees occupying the rainforest habitats of Cameroon and Ghana have been detected growing alongside other forest trees including *Alstonia boonei* De Wild., *Myristica angolensis* Welw., *Triplochiton scleroxylon* K.Schum., *Milicia excelsa* (Welw.) C.C.Berg, *Ceiba pentandra* (L.) Gaertn, *Terminalia superba* Engl. & Diels, *Cordia platythyrsa* Baker, and *Ficus exasperate* Vahl (Mapongmetsem et al. 1998; Kyereh et al. 1999; Ajayi 2019). While in Nigeria, the trees found together with *R. heudelotii* were represented by several other pioneering species, for instance *Ceiba pentandra* (L.) Gaertn, *Pterygota macrocarpa* K.Schum., *Milicia excelsa* (Welw.) C.C.Berg, and *Sterculia rhinopetala* K.Schum., and the species typical for established forests counting *Blighia sapida* K.D.Koenig, and *Strombosia pustulata* Oliv. (Riddoch et al. 1991; Adekunle 2006). The Cameroonian evergreen forests are dominated by tree species of the family Caesalpiniaceae (Neumann et al. 2012) and the deciduous forests are rich in trees from the Sterculiaceae and Ulmaceae families (Fayolle et al. 2014).

#### 2.2.3. Botanical description

*Ricinodendron heudelotii* is a medium-large tree with a cylindrical and erect trunk usually reaching from 20 to 30 m, sometimes up to 50 m, in height and commonly 150 cm, possibly up to 270 cm, in diameter. The base of the trunk is thickened as short and sturdy buttresses grow from there and further develop into heavy superficial roots. The outer bark is grey and smooth at first (Figure 2a), becoming coarser and cracked later

(Figure 2b). The inner bark is pink to red, mottled, and gritty. The wood is soft and white or lightly yellow in colour. Upward arching and whorled branches are typically seen in



**Figure 2.** Bark of *R. heudelotii*. **a:** a young individual (Ji-Elle 2017); **b:** a mature individual (Kalousová 2021).

young trees while adult trees have stout, broad branches growing at two-thirds of the trunk (Figure 3), forming the wide candelabra-like shaped crown often with many broken branches (Tchoundjeu & Atangana 2006, 2007).



Figure 3. R. heudelotii in its natural habitat (ICRAF 2013).

The leaves are alternate and compound, each leaf composed of 3-8 (usually 5-7) digitately arranged leaflets (Figure 4). Young leaves may have the leaflets united at the base; thus, the blade appears lobed. The young leaflets are pubescent, often shedding the hairs when mature. The mature leaflets are obovate to elliptical-lanceolate and measure from 6 to 30 cm in length and from 2.5 to 15 cm in width at the middle while becoming thinner near the top of the petiole. The central leaflets are slightly bigger than the lateral ones. There are 10-16 pairs of side veins as well as small glands at the leaf margins present in each leaflet.



Figure 4. Leaves of R. heudelotii (Jongkind 2007).

The petioles grow from 5 to 30 cm, possibly 40 cm, long and possess two lateral glands at the top. The stem-embracing stipules are persistent (Figure 5), 1-5 cm long and 1.5-4 cm wide, and (Tchoundjeu & Atangana 2006, 2007).



Figure 5. Stipules on a branch of *R. heudelotii* (Jongkind 2007).

*R. heudelotii* is a dioecious species and as such each tree produces inflorescences which bear either male or female flowers only. In both cases, the inflorescence is a terminal panicle (Figure 6). Male panicles are typically leaner and can grow up to 40 cm long, while female panicles are shorter and about 20 cm long. The unisexual flowers are greenish white to light yellow. Male flowers are characterized by 5 sepals with a 5-lobed corolla tube and typically bear 6-16 stamens. Female flowers have a superior, globose, star-shaped hairy ovary and possess 2-3 slender, bipartite styles (Tchoundjeu & Atangana 2006, 2007).



Figure 6. Young leaves and flowers of *R. heudelotii* (Latham 2001).

The fruits (Figure 7) are spherical, typically indehiscent drupes of yellow green (Figure 8) colour when unripe, turning black when mature, 2-5 cm long and 2.5-5 cm wide, weighting 19-47 g. Each drupe develops at least 1, but typically 2 or 3 lobes, where 2-lobed drupes are predominantly found in subsp. *heudelotii* and 3-lobed drupes in subsp. *africanum*.

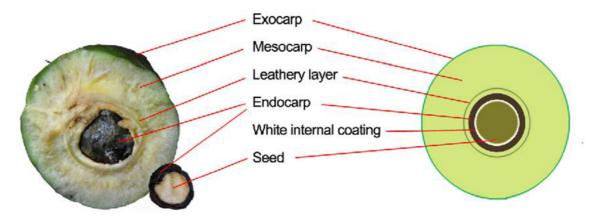


Figure 7. *R. heudelotii* fruit and seed anatomy (created by Cosyns 2013; based on descriptions by Plenderleith 2006; Tchoundjeu and Atangana 2006; Mundi et al. 2012).

Each lobe bears 1 stone in which the seed is encapsulated. The seeds are round, approximately 1.5 cm in diameter, with a reddish-brown or black protective coat (Figure 8) inside which there is a yellow, edible kernel (Tchoundjeu & Atangana 2006, 2007).



Figure 8. Seeds (left) and fruits (right) of R. heudelotii (Kyereh 2021).

The seedlings (Figure 9) germinate in an epigeal fashion with the hypocotyl reaching up to 20 cm in length, epicotyl is shorter. The cotyledons including the petiole are about 1.5-2.5 cm long. The first leaf has 3 lobes, is palmately veined, glandular at margins and its size is approximately 6-7 cm  $\times$  5-6 cm (Plenderleith 1997; Tchoundjeu & Atangana 2007).



Figure 9. A seedling of *R. heudelotii* (Ndangalasi 2011).

### 2.2.4. Environmental requirements

*R. heudelotii* is often labelled as a lowland species thriving at elevations from 100 to 500 m above sea level where it is usually limited to forested areas and absent in mountainous areas (Tchoundjeu & Atangana 2006, 2007), although other sources mention a broader range of 100-1,200 masl (Orwa et al. 2009). However, populations of *R. heudelotii* occurring at even higher altitudes were recorded, for example at above 1,200 m on the Ugandan plateaux described by Babweteera and Brown (2009) and at more than 1,600 m in Mahale Mountains in Tanzania mentioned by Nishida and Uehara (1981). On the other hand, a study carried out by (Fondoun et al. 1999) claims that no fruits were found in *R. heudelotii* trees growing at elevations lower than 130 m in coastal Cameroon.

The climatic and environmental conditions experienced across the large and diverse native range of *R. heudelotii* differ significantly. However, there are several

crucial characteristics common to all the regions and climate types which must be met to allow for successful existence of the species and can be determined as following:

The annual rainfall of at least 1,000 mm is critical for *R. heudelotii* (Tchoundjeu & Atangana 2007), parts of Kenya, Uganda, and Togo are good examples of places receiving approximately such levels of annual rainfall. Nevertheless, higher amounts of total rainfall are not uncommon throughout many regions of West Africa with 1,500-2,500 mm annually, or more, for instance the west coast of Cameroon or the east coast of Madagascar receive about 3,000 mm of annual rainfall (Sebastian 2014). It is worth mentioning that R. heudelotii can survive in environments with much higher amount of rainfall, for example in Debundscha, Cameroon, which is regarded as the rainiest place in Africa, receiving more than 10,000 mm of rainfall per year (Wanji et al. 2003; Sanwo & Arimoro 2005). R. heudelotii trees can tolerate drought and especially the ability to shed the leaves to avoid excessive water loss allows them to survive during the dry season (Danquah & Oppong 2007). In general, R. heudelotii grows in areas of tropical Africa which experience two distinguishable rainy seasons in a year, or one extensive rainy season only shortly interrupted by a drier period, and two dry seasons, one of which is hotter and longer, the other one cooler and shorter (Tchoundjeu & Atangana 2006; Nicholson 2018).

The mean annual temperatures in areas suitable for *R. heudelotii* are typically tropical between  $18^{\circ}$ C and  $32^{\circ}$ C. Depending on the season, the temperatures range from  $18^{\circ}$ C to  $23^{\circ}$  C during the coolest time of the year and from  $30^{\circ}$ C to  $32^{\circ}$  C during the warmest period (Tchoundjeu & Atangana 2006).

The species prefers acidic soils with a pH 5-6, but according to (Anigbogu et al. 1996) it can perform well at pH up to 7.7. The ideal soil types are those of medium texture and good drainage, e.g., loam and silt soils. If enough light is provided, *R. heudelotii* can tolerate a wide variety of soil types, moisture levels and drainage (Anigbogu et al. 1996; Orwa et al. 2009). Due to the softness of the wood, *R. heudelotii* trees cannot handle strong winds well as it will break their fragile branches (Tchoundjeu & Atangana 2007).

#### 2.2.5. Ecology

The flowering period of *R*. *heudelotii* can differ between regions as it is a highly seasonal event marked by the end of the dry season during which the trees were leafless.

The new leaves often appear while the trees are in bloom (Andrew et al. 2018). In Cameroon, the flowering happens from March to May, and the main fruiting season last from July till September, although in some areas, fruits can be found in December as well. The *R. heudelotii* trees are typically 6-10 years old when the fruit production begins, fruiting occurs once in every 2 or 3 years (Caspa et al. 2014).

The amount of fruit produced by a single tree is usually abundant, ranging between 1,500 and 2,300 fruits, depending on the area (Caspa et al. 2014). The seeds stay dormant for roughly 6 months. Animals such as mammals (e.g., rodents, fruit bats) and birds (e.g., hornbills) are responsible for the seed dispersal (Orwa et al. 2009). There are concerns that humans gathering too large quantities of the fruits together with the delayed seed germination may lead to unsustainability of the *R. heudelotii* population in the wild (Djeugap et al. 2014; N'Guessan et al. 2015). The fruits are considered to be indehiscent and as such the seeds are usually left undispersed under the mother tree as the endocarps decay if no animal dispersers (e.g., *Philantomba monticola*) are present (Babweteera & Brown 2009). The idea of spontaneously exploding fruits proposed by Katende et al. (1995) seems to be false.

#### 2.2.6. The use

#### 2.2.6.1. Fruit

The seeds of *R. heudelotii* are rich in oils, protein, and numerous minerals (Table 1) (Yeboah et al. 2011; Saad et al. 2019). The seeds, or kernels, are made of protein from about 21-24% (Saki et al. 2009; Coulibaly et al. 2018; Nikiema et al. 2019). The lipid content of the seed is approximately 47%, the oils are polyunsaturated. The content of cellulose, hemicellulose, and lignin is low, although the seeds are a suitable source of healthy fibre (Nikiema et al. 2019). Minerals such as calcium, magnesium, phosphorus, potassium, and sodium are found in the seeds in nutritionally significant amounts (Saki et al. 2009; Kinge et al. 2019).

Macronutrient	Mean	SD
Dry matter (%)	92.00	0.36
Protein (%)	24.00	0.94
Total carbohydrates (%)	2.90	0.09
Starch (%)	0.42	0.01
Sugars (%)	0.08	0.00
Cellulose (%)	2.40	0.08
Fat (%)	47.80	0.75
Mineral	Mean	SD
Mineral       Ash (%)	<b>Mean</b> 6.12	<b>SD</b> 0.50
Ash (%)	6.12	0.50
Ash (%) Ca <sup>2+</sup> ( g/l )	6.12 0.33	0.50 0.01
Ash (%) Ca <sup>2+</sup> ( g/l ) Mg <sup>2+</sup> (g/l)	6.12 0.33 0.20	0.50 0.01 0.01
Ash (%) Ca <sup>2+</sup> ( g/l ) Mg <sup>2+</sup> (g/l) Cl <sup>-</sup> (g/l)	6.12 0.33 0.20 0.03	0.50 0.01 0.01 0.00

**Table 1.** Mean amounts of nutrients in *R. heudelotii* seeds from 7 provinces of CôteD'Ivoire. Source: modified from Saki et al. (2009).

The harvested green *R. heudelotii* fruits are traditionally left for about 4-20 weeks to let the pulp rot away (Figure 10) so the seeds (Figure 11) are released and can be washed easily. Then, the seeds must be dehulled so the kernels can be extracted (Mbosso et al. 2013, 2015).



**Figure 10.** A pile of rotting *R. heudelotii* fruits (Kyereh 2021).



Figure 11. Collected *R. heudelotii* seeds (ICRAF 2014).

Dehulling can be achieved either through traditional treatment of the seeds or mechanically using a nutcracker (Nikiema et al. 2019) or a machine (Mbosso et al. 2015). The traditional way of dehulling the seeds is done in three phases. In the first one the seeds are soaked for at least 24 h (up to 48 h) in lukewarm water. Then they are boiled for 3 to 12 h, until the husks crack. And finally, the kernels are released from the broken husks simply by using a nail or knife. When dehulling the seeds mechanically, no pre-treatment is needed because the hulls are directly cracked with a nutcracker in a similar manner as walnuts. It must be done carefully so the kernel is not smashed inside, once the hull is cracked the kernel can be released just like when using the traditional method (Nikiema et al. 2019).

In 2007, a prototype of a njansang seed dehulling machine was introduced for the first time by ICRAF (Mbosso et al. 2013). The machine is powered by a diesel engine cooled by water. It cracks the hulls by spinning (up to 2,800 revolutions/min). One of the advantages of using the machine is that this method is more attractive to men than the traditional way, which is usually performed by women who would benefit from the lowered workload. Furthermore, the time to extract 1 kg of kernels is about 18 min faster than the usual process. The downside of this method appears to be a higher percentage of broken kernels compared to manual extraction (approximately 20% and 3%, respectively). Broken kernels have marketed for a lower value than intact ones (Mbosso et al. 2015).

After dehulling, the kernels (Figure 12) are most commonly dried in the sun for approximately 10 days or smoked. Sun-dried kernels acquire light-yellow colour which is popular with purchasers. Protecting the seeds from moisture is crucial during drying as excess wetness can cause the seeds to turn brownish or black in colour (Kinge et al. 2019).



Figure 12. Njansang kernels on the market (ICRAF 2014).

*R. heudelotii* kernels serve different purposes in different countries. In Cameroon, and other countries of West and Central Africa, the kernels are popular for their flavour, thus they are often used as spice in various meat and vegetable dishes, or to thicken up soups and seasonings. The kernels can be processed into a paste or boiled or roasted and eaten on their own (Plenderleith 1997; Mariod 2019).

Other ways in which the seeds are utilized include cultural, medicinal, and industrial purposes. *R. heudelotii* seeds are used to make rattling musical instruments used in dances in Sierra Leone or can act as counters in local board games in West Africa (Ogbuagu et al. 2019; Saad et al. 2019).

The cold-pressed oil is traditionally used to treat smallpox (Boko-Haya et al. 2017) and the latest research suggests it has features which could be helpful in prevention of diabetes and heart diseases (Ouédraogo et al. 2011; Mariod 2019). According to a study carried out by Olasehinde et al. (2016), *R. heudelotii* oil extracts show antimicrobial and antifungal properties, thus further research is suggested by the authors. As for industrial uses, *R. heudelotii* seed oil provides a high yield of polyunsaturated fats making it fit to

be processed into soaps (Ogbuagu et al. 2019) and (Danbature et al. 2018) claim that *R*. *heudelotii* oil is a so-called drying oil with an ability to form a solid film when exposed to oxygen, thus it could be used as a binder in paints for surface coating and resins as well. Even the seed husks could be utilized to produce activated carbon used as a purification agent in many industries (Urbain et al. 2017).

#### 2.2.6.2. Bark and leaves

Both the leaves and bark of *R. heudelotii* were found to contain significant amounts of crude fibre, fat, and protein, as well as several nutritionally important minerals. Several types of bioactive compounds are also present in the leaves and bark, most of them being secondary metabolites such as alkaloids, cardiac glycosides, flavonoids, reducing sugars, saponins, steroids, tannins, and terpenoids (Uzoekwe & Hamilton-Amachree 2016; Yakubu et al. 2018).

Uzoekwe & Hamilton-Amachree (2016) recommend the leaves and bark of R. *heudelotii* as a suitable fodder for sheep and goats during the dry season as the leaves can provide a sufficient source of fibre, minerals (especially calcium, iron, magnesium, and phosphorus), and protein (about 5% of dry matter). The bark is an excellent source of fibre with its content being circa 64% of dry weight. However, the leaves and bark are poor energy sources for the animals due to low fat, sugar, and starch content.

Despite having limited nutritional characteristics, the *R. heudelotii* leaves and bark play a vital role in traditional medicine and seem to possess favourable qualities which are currently underutilized in conventional pharmacology but could be proven valuable in the future (Plenderleith 1997).Traditionally, leaf infusions are used for their antiseptic and analgesic properties, while bark is applied in remedies for rheumatism, diarrhoea and other gastrointestinal issues, mouth sores, various pains and more (Uzoekwe & Hamilton-Amachree 2016). It is worth mentioning that other uses of *R. heudelotii* leaves and bark against specific health problems were reported by several authors; ovarian cysts (Ngene et al. 2015), obstetric fistulas (Lagou et al. 2016), haemorrhoids (Dibong et al. 2015), and kidney issues (Nole et al. 2017). N'Guessan et al. (2009) claim that the leaves can act as a purgative. Yakubu et al. (2019) discovered that ethanol extract of *R. heudelotii* leaves shows cytotoxic activity when applied to a breast cancer cell line, suggesting further investigation of this subject. Research on antibacterial properties of *R. heudelotii* carried out by Yakubu et al. (2018) did not show any significant toxicity in rats when used in doses below 3,600 mg/kg of body weight, while being an effective antibiotic agent, thus implying a relative safety of *R. heudelotii* remedies in mammals. On the other hand, *R. heudelotii* leaf extracts were proven to repel certain pest species damaging stored grains (Udo & Epidi 2009). Apparently, leaves and bark of *R. heudelotii* already have a wide range of utilization, promising to be even more useful with more discoveries.

#### 2.2.6.3. Wood

The wood of *R. heudelotii* is relatively soft, light, fibrous, and not very durable, thus the timber is not suitable for use in heavy construction or as fuel because it burns fast. On the other hand, such characteristics make it useful in woodworking and production of pulp for paper making. Products made of *R. heudelotii* wood include planks, coffins, rafts, fishing net floats or more fine, carved items such as spoons, plates, bowls, chairs, and ritual masks (Yakubu et al. 2018). For example, the Yoruba of Benin, Nigeria, and Togo use the wood to carve masks used for ceremonies during the Guelede, an important cultural event (Boko-Haya et al. 2017). Another culturally important utilization of the wood is its processing into musical instruments. Drums are made from *R. heudelotii* in the Democratic Republic of Kongo as the wood exhibits great resonance abilities. In Nigeria, Gabon, and Angola it is utilized to make the resonating parts of or the entire musical instruments (Mariod 2019).

### 2.2.6.4. The use in agroforestry

*R. heudelotii* is a traditional part of agroforestry systems in West Africa. The trees are commonly left in fields and plantations after the forest has been cleared (Figure 13). *R. heudelotii* is especially useful as a shade tree in cocoa agroforestry and helps to increase soil fertility (Fondoun et al. 1999; Tchoundjeu & Atangana 2006) and to protect the soil against erosion as well (Anigbogu et al. 1996).

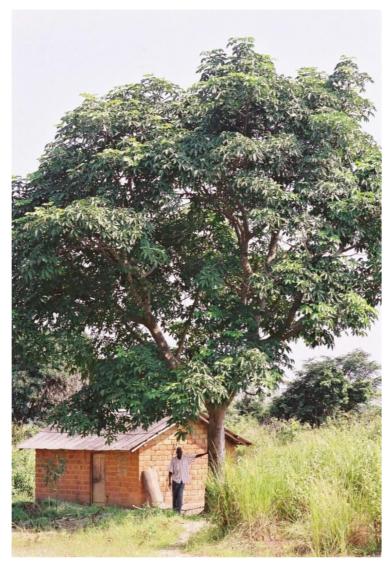


Figure 13. A smaller specimen of *R. heudelotii* in an agricultural landscape (Latham 2000).

Despite being an already popular multi-purpose tree species, *R. heudelotii* is still underutilized in many ways. The species seems to possess promising characteristics from which agroforestry systems would benefit (Onefeli et al. 2019). The ability to improve soil fertility probably comes from the ability of *R. heudelotii* to form symbiosis with

arbuscular mycorrhizae (Redhead 1960) with at least 14 species of fungi from three genera which act as a net spreading from the roots, collecting mineral nutrients from around the tree (Musoko et al. 1994). The fallen leaves, ash from burnt hulls, residual cakes left after oil extraction, and rotten pulp also act as valuable fertilizers rich in potassium and nitrogen (Mapongmetsem & Tchiegang 1996; Tchoundjeu & Atangana 2006). The flowers produce nectar, which is collected by bees, thus *R. heudelotii* can benefit farmers who produce honey (Tchoundjeu & Atangana 2006).

Onefeli et al. (2019) carried out a study assessing the suitability of *R. heudelotii* for alley cropping with maize in Nigeria. The trees were planted in 6 m wide alleys. The authors concluded that the combination of maize and *R. heudelotii* showed better performance in terms of both crop growth and soil fertility improvement compared to monoculture systems of only maize or *R. heudelotii*. Further research is required as agroforestry systems are highly site-specific, however *R. heudelotii* appears favourable as it is indigenous to West Africa and possess qualities desired in agroforestry trees such as fast growth, improving soil fertility and ability to regenerate well after pollarding (Tengnäs 1994).

### 2.2.7. Silvicultural management

### 2.2.7.1. Generative propagation

The seeds of *R. heudelotii* are found to be difficult to germinate due to the seed husk being impermeable to water if undamaged, causing the seed to stay dormant. This type of dormancy can be broken physically (by scarification) or physiologically (by stratification). The seeds can be scarified by carefully disrupting the coat integrity with a hammer (Boko-Haya et al. 2021). Djeugap et al. (2014) found out that mechanically scarified seeds of *R. heudelotii* germinate earlier and amply (88% success rate) if they are stratified by soaking in water for 24 hours. On average, seeds treated this way germinated 13 days after sowing. Furthermore, the authors recommend storing the seeds for 6 months to acquire the best germination results. Fungicide treatment of the stored seeds is also desirable as the emerging seedlings appear susceptible to fungal infection. Seedling performance seems to be related to seed mass, as seedlings grown from seeds weighting 1.5 g or more exhibit superior growth during the establishment phase (Guillaume et al. 2021).

According to a study carried out at the Azaguié fruit research station in Côte d'Ivoire (Djaha & Gnahoua 2014), it is advisable to cultivate the seedlings of *R. heudelotii* in polyethylene bags with rich substrate composed of equal parts of loam, sand and manure and let the young plants develop for about 3-6 months to grow 30-40 centimetres before being transplanted to the field. The cultivated trees can be expected to grow approximately 12 metres tall in 7 years after planting and bear fruits at the age of 12 years.

#### 2.2.7.2. Vegetative propagation

While there have been several optimised methods of vegetative propagation proposed as suitable for mass production of R. heudelotii clones, the feasibility of such techniques in commercial settings remains to be revealed (Yapo et al. 2020). Although, experimental propagation of R. heudelotii by cuttings, done by Shiembo et al. (1997) and Donfagsiteli Tchinda et al. (2013), proved as successful. According to the authors, the best results were obtained using softwood basal cuttings with a single node rooted in sand/sawdust-based substrate, which was shown by a relatively high survival rate of the cuttings in both studies (80-90%). Major factors influencing successful rooting seem to be the cutting type, genotype of the mother tree, season of the year, leaf area of the cuttings and pre-treatment of the propagation substrate and the cuttings to prevent fungal infection (Donfagsiteli Tchinda et al. 2013). Addition of plant growth regulators proved beneficial (0.04 mg of indole-3-butyric acid/cutting), improving survival rate. Precautions to prevent rot should be taken as fungus-induced decay was the main danger to the newly developing plants. The cuttings are susceptible to drying out, thus it is advisable to pay attention to the leaf area of the cutting so the ratio between perspiration and assimilation is balanced (Shiembo et al. 1997).

Donfagsiteli Tchinda et al. (2013) reported that acclimatization of young plants regenerated from cuttings was the most successful when a potting mix composed from equal parts of black soil and sand was used as the survival rate of the plantlets was 67.8%. The young individuals of *R. heudelotii* were grown in polyethene pots for 2 months before the acclimatization success rate was assessed.

According to Fotso et al. (2007), an *in vitro* method known as micropropagation, offers an alternative approach to vegetative propagation of *R. heudelotii* as was proved by the authors who used *in vitro* tissue culture to obtain plantlets of the species. In the study, the young plants were acclimatized for 15 weeks in an equal mix of soil and

vermiculite with survival rate of 52.7%. Single-nodal cuttings and internodal sections taken from a mature tree are suitable for direct regeneration of a whole new plant or regeneration through callus culture, respectively. To promote callus formation, production of new buds, and overall regeneration, plant growth regulators are used, especially auxins and cytokines. Although *in vitro* rooting of woody species can be challenging (Youmbi 2000), the method could be used for mass production of *R*. *heudelotii* clones with superior characteristics or to obtain individuals with interesting new traits developed via somaclonal variation during callus regeneration (Fotso et al. 2007).

Grafting might be another option useful in vegetative propagation of the species as shown by Tsobeng et al. (2021) in their grafting experiment. In *R. heudelotii*, the researchers observed survival rate slightly above 50% in scions which were grafted on rootstocks using the top cleft method.

#### **2.2.8. Domestication efforts**

The International Centre for Research in Agroforestry (ICRAF) pursuits domestication efforts of native valuable multipurpose tree species through developing cultivars from vegetatively propagated lines of exceptional individuals selected by farmers and scientists alike (Leakey 2014). The start of a modern fruit tree domestication process is often marked by preliminary assessment of the species' morphological and genetic attributes. Despite selection based on molecular markers has been proven efficient, it can be difficult to apply such methods in some developing countries, although morphological characteristics alone can be very efficient as well (Tsobeng et al. 2020). However, as Maňourová et al. (2023) argue, it is advisable to use both approaches, if possible, since morphological characteristics can be affected by environmental factors. Outcrossing tree species, such as *R. heudelotii*, often exhibit high intraspecific variability (Hamrick et al. 1992), thus evaluation of the variability within and between populations is essential (Achten et al. 2010). Mpeck et al. (2003) studied the variability of R. heudelotii populations based on the assessment of morphological traits of the fruits, seeds, and kernels. The authors found significant variability among the studied trees, especially in kernel mass which is one of the two most important traits in terms of domestication, a great potential for phenotypic selection for quantitative improvement of the species is indicated. The other important trait is the self-cracking seed, but as this characteristic seems to be connected to the seed's physiology, rather than morphology, physiological examination is needed. Since no relationship between fruit traits and kernel traits was found, the domestication efforts should primarily emphasise selection based on the kernel traits. A tree suitable for commercial cultivation would produce fruits with thin flesh and self-cracking seeds with big kernels.

Although domestication of novel fruit woody species can enhance food safety, create new commercial opportunities, and assist in establishment of more sustainable agricultural methods, there are also many obstacles to overcome. The major challenges are often connected to biology of the chosen species and socio-economic reasons (Shelef et al. 2017). The biological constrains and economic issues hindering the domestication process of R. heudelotii appear like the obstacles seen in the Brazil nut (Bertholletia excelsa Bonpl.), which is a species with an already established global market, yet there are essentially no cultivars or large-scale plantations of the species (Baldoni et al. 2019). However, it appears that a species does not have to be fully domesticated to benefit people and the cultural landscape. The case of Brazil nut can be such an example of a tree species which has been used by humans as a food source and in agroforestry systems for centuries, perhaps even entering the first stages of the domestication process, while remaining untamed (Shepard & Ramirez 2011). On the other hand, the domestication story of pecan [Carya illinoinensis) (Wangenh.) K. Koch] could serve as a source of viable information on how to successfully bring wild forest tree species into cultivation settings. While the domesticated pecan nut trees are not so drastically different from their wild-growing relatives, the most undesirable characteristics (for example irregular yielding, nuts with difficult-to-crack shells, long time to reach productive age etc.), were overcome or at least significantly diminished by selective breeding, development of vegetive propagation methods, and proper management techniques. Thus, a great commercial success has been achieved (Santerre 1994).

#### 2.2.9. Genetic diversity

Trees, as a widespread group of various woody perennial plants, are often associated with longevity, while simultaneously lacking the ability to move, hence they are presented with a set of unique challenges requiring them to possess prominent levels of adaptability to be able to cope with the changes in the environment throughout their extensive lives. Consequently, tree populations usually possess increased genetic diversity which is influenced by factors such as the way the tree species reproduce, natural selection, or gene-flow. The genetic composition in trees is also significantly shaped by the distance pollen or seeds can reach from their parents (Degen & Sebbenn 2014). Thus, it is thought that research aiming to gain a profound understanding of the variability found in tree genetics and epigenetics is extremely beneficial for improvement of tree species through breeding and in conservation of biodiversity (Amaral et al. 2020). Unfortunately, the knowledge on changes in the genome structure and its function in tree species undergoing selective breeding is lacking, at least in comparison to annual crops (Groppi et al. 2021).

Since a well-understood genetic diversity and population structure are immensely helpful in any attempt on a domestication of a fruit tree species, it is difficult to effectively evaluate the usefulness of the germplasm in question in question if such information is absent (Liu et al. 2019). For example, Buckley et al. (1988) found a huge genetic diversity within the populations of the Brazil nut trees with minimum variation between different populations. This observation suggests that the species is able to spread its pollen at very long distances. Thus, there is a possibility that just one population of the Brazil nut trees encompasses a substantial number of various genes of the whole species, therefore such knowledge can be impactful in terms of germplasm conservation. It seems that while proper expertise on the genetic makeup of R. heudelotii is yet to be described, the recognition of unique inter-simple sequence repeats markers (ISSR) in a Nigerian population of the species made by Onefeli (2021) is a truly remarkable feat as the information could serve in marker-assisted identification of distinct populations of R. heudelotii. If not used for domestication purposes, assessment of genetic diversity is still crucial in both in situ and ex situ conservation of plant diversity, especially in the fragmented landscapes of today's world. It could be suggested that while globalization is at the roots of many issues causing decline in plant species abundance, the potential for ex situ conservation of genetic resources has never been better (Huang 2022).

### 2.3. Study area

The Republic of Cameroon is a West African country situated between latitudes 2° and 13° N and 8° and 16° E, covering 475,442 km<sup>2</sup> (UNEP 2008). It is a lower-middle-income country with more than 27 million inhabitants (The World Bank 2021) of which over 2.6 million live in Yaoundé, the capital city (Ayeah et al. 2022). The country has access to the Atlantic Ocean in the west and is surrounded by the Central African Republic, Chad, Equatorial Guinea, Gabon, and Nigeria. Cameroon consists of 10 regions Adamawa, Centre, East, Far North, Littoral, North, Northwest, West, South, and Southwest (Page et al. 2010). While French is the prevalent official language in Cameroon, the Northwest and Southwest regions are anglophone. There are plenty of natural resources found in Cameroon, namely oil and gas, mineral ores, valuable timber, and various cash-crops (The World Bank 2021).

Cameroon is known for its highly diverse geography, resulting in a wide range of climatic conditions and biodiversity. Generally, the climate is hot and tropical, changing from mostly arid conditions in the northernmost parts of the country in proximity to the Lake Chad Basin to the humid southern parts near the equator. A volcanic mountain range known as the Cameroon Line runs through the country (Molua & Lambi 2006). The southern regions of the country host a significant portion of the Congo Basin tropical rainforest (Brown et al. 2010), the second largest rainforest in the world(White et al. 2021). As extensive areas of the rainforest remain mostly unharmed by human activity, the biodiversity found there is outstanding. The rainforests host numerous species of plants, animals, and other organisms, many of them endemic, including the bonobo (Pan paniscus). Also, the region has been an ancestral homeland of many indigenous groups of people for millennia (Pyhälä et al. 2016). The diverse nature of Cameroonian landscape makes it agriculturally the most variable country of the whole African continent. Based on the environmental conditions, five distinct agro-ecological zones can be described: Sudano-Sahel, Guinea Savanna, Western Highlands, Bi-modal Equatorial, and Mono-modal Equatorial (Yengoh et al. 2010) (Figure 14).

#### Legend

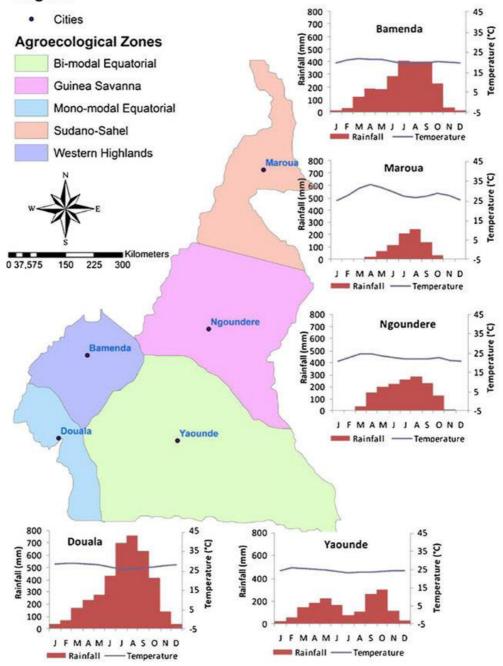


Figure 14. Major agro-ecological zones in Cameroon (Yengoh et al. 2010).

In Cameroon, the agricultural sector employs approximately 44% of the working population (women predominate at about 52%) and accounts for roughly 17% of the country's GDP (The World Bank 2021). Approximately 90% of people living in rural areas are dependent on agriculture financially, in terms of food security, and development in general. Cameroon is also becoming a key exporter of food products in the region. This fact benefits farmers, especially those in the southern regions of the country from which

the major cash-crops (cocoa, coffee, rubber, and palm oil) come from, as the humid forest zone has conditions favouring agricultural activities (Robiglio et al. 2010). In the humid forest zone, the farmers commonly practice shifting cultivation with a fallow period to regenerate soil fertility. Typical crops of the humid areas involve perennials (cocoa, coffee, fruit trees) and annuals (sugar cane, maize, vegetables, tobacco, plantains, and tubers), other food sources are fish, sheep, and goats (Ngondjeb 2013).

The present study was carried out in the Centre and South regions of Cameroon. The Centre region covers an area of 68,953 km<sup>2</sup> and its population is estimated to be around 4 million inhabitants. The administrative and political capital, Yaoundé, lies in this region. The South region covers an area of 47,191 km<sup>2</sup>, lying directly southwards of the Centre region, sharing its southern border with Equatorial Guinea, Gabon, and Congo. The western part of this region has access to the Atlantic Ocean. The capital city is Ebolowa and there are approximately 749,600 people living in the South region (BUCREP 2005). The Bimodal Rainforest zone of Cameroon encompasses the area between latitudes from 2°6" to 4°54"/5°48" N and longitudes from 10°30" to 16°12" E, hence it covers the southern half of the Centre region and the entire South region, apart from the coast. The altitude averages between 500 and 1,000 masl. The climate is warm and humid, with average annual temperature of 25° C and annual rainfall of 1,500-2,000 mm. There are two separated wet seasons, the longer one lasts from March to June, while the shorter one lasts from September to November. The natural forests are dense, semi-deciduous or evergreen (Ngondjeb 2013; Mbog et al. 2020).

# **3. Objectives**

The main aim of this study was to describe and evaluate morphological characteristics of *Ricinodendron heudelotii* to assess intraspecific diversity between different populations and individuals in the Centre and South regions of Cameroon. The main goal of the thesis was divided into three specific objectives:

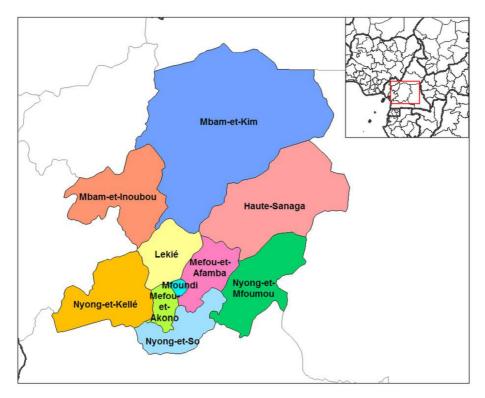
- To measure and compare morphological characteristics of fruits and seeds of *R. heudelotii* obtained from different geographical sites;
- 2) To evaluate intraspecific diversity of *R. heudelotii* based on morphological variations;
- 3) To gather ethnobotanical information about *R. heudelotii* using questionaries to assess local farmers' relationship with the species.

## 4. Materials and methods

#### 4.1. Study site

The choice of the study site was based on the information obtained from previous studies of the region carried out by ICRAF. Thus, the divisions of Nyong-et-Mfoumou and Vallée-du-Ntem, found in the Centre and South regions, respectively, were selected. The sampling was realized at seven study sites found in the proximity of rural settlements, i.e., Akontemba, Bissabifomo, Ngolebomo, and Ondeck in Nyong-et-Mfoumou and Alen, Mviili Mengale, and Tya' Assono in Vallée-du-Ntem. In each site, there was a member of the local association of njansang producers to provide our team with additional assistance.

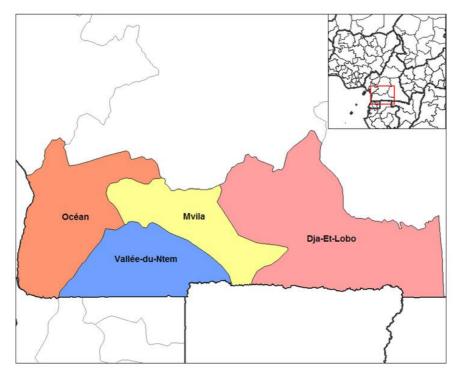
The division of Nyong-et-Mfoumou (Figure 15), named after the Nyong and Mfoumou rivers, lies in the Centre region at 4° N and 12° E and covers 6172 km<sup>2</sup>. As such, the division is found in the Bimodal Equatorial agro-ecological zone and experiences average annual rainfall of 2,000 mm with mean annual temperature of 24°.



**Figure 15.** Nyong-et-Mfoumou (green) and other divisions of the Centre region. Source: Wikipedia Commons.

The estimated elevation is 726 masl. The capital town is called Akonolinga. More than 130,000 people live in the division and rely on agriculture, livestock farming, hunting, fishing, and family businesses as primary sources of income (Oyono et al. 2019; Florence 2021).

Vallée-du-Ntem is a landlocked division (Figure 16) of the South region covering 7,303 km<sup>2</sup> found along the Ntem river at 2° N and 10° E. In the south, it shares borders with Equatorial Guinea and Gabon. The altitude varies between 450 and 650 metres above sea level. The average annual temperature is 25 °C and the annual rainfall of 1,700 mm is delivered during two rainy seasons (the longer last from September to November, while the shorter one from March to May). Overall, the climatic conditions are typical for the Bimodal Equatorial agro-ecological zone. The capital city is Ambam. Over 80,000 people inhabit the division. Subsistence farming is the major form of livelihood. Secondary forests formed by traditional slash-and-burn agricultural methods are a source of NTFPs valued by the locals (Pagezy et al. 2000; BUCREP 2005).

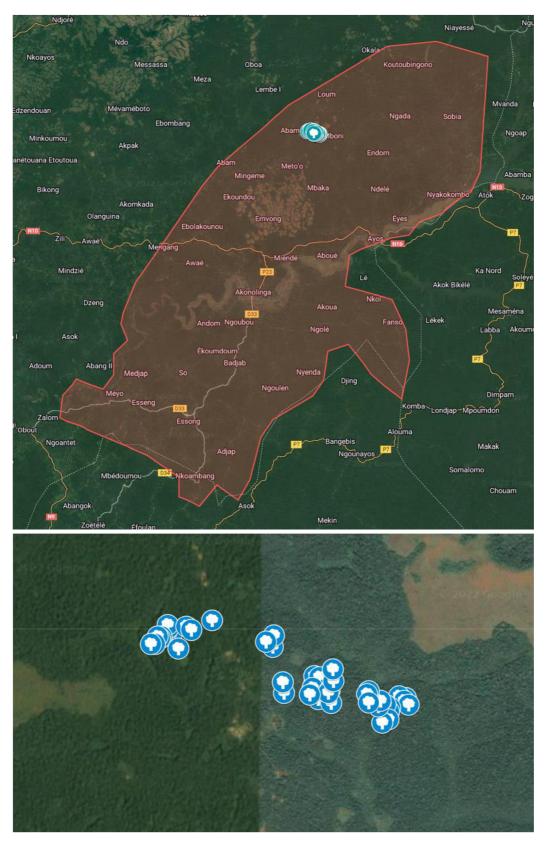


**Figure 16.** Vallée-du-Ntem (blue) and other divisions of South Region. Source: Wikipedia Commons.

#### 4.2. Sampling and data collection

The data collection was carried out from July to September 2021. A structured questionnaire was used to obtain ethnobotanical information about *R. heudelotii* from 66 anonymous farmers selected by the snowball method in 7 villages. The farmers were asked questions focused on the importance, utilization, harvest, storage, and other aspects of exploitation of the species (Appendix A). In the Nyong-et-Mfoumou division of the Centre region, 3 farmers were from Akontemba, 13 came from Bissabifomo, 9 were based in Ngolebomo, and 6 lived in Ondeck. In the Vallée-du-Ntem division of the South region, 13 respondents came from Alen, 16 people were from Mviili Mengale, and 6 from Tya' Assono.

In the present study, there were 116 mature trees, including 50 fruiting individuals, examined, and sampled in total. The trees growing at least 100 metres from each other were selected randomly at 4 study sites (Figure 17 and 18) From the fruiting individuals, up to 10 fallen fruits per tree were gathered. The fruits (drupes) were measured and morphologically described on-site. From each fruit, seeds (stones) were extracted, dried, packed, and sent to Czech University of Life Sciences (ČZU) for further morphological evaluation. Table 2 shows the overall number of examined trees and samples taken from each study site.



**Figure 17.** A map showing the Nyong-et-Mfoumou division (red area) and the locations of the sampled trees belonging to the Ondeck (blue pictograms) population. Source: Modified from Google Maps using the My Maps tool.



**Figure 18.** A map showing the Vallée-du-Ntem division (yellow area) and the locations of the sampled trees belonging to the Mviili Mengale (green pictograms), Alen (red pictograms), and Tya' Assono (purple pictograms) populations. Source: Modified from Google Maps using the My Maps tool.

Region	Division	Site	No	No	No	No	No
			farmers	all trees	fruiting trees	fruits	seeds
Centre	Nyong-	Akontemba	3	-	-	-	-
	et-	Bissabifomo	13	-	-	-	-
	Mfoumou	Ngolebomo	9	-	-	-	-
		Ondeck	6	42	25	222	419
		Centre total	31	42	25	222	419
South	Vallée-	Alen	13	10	7	57	101
	du-Ntem	Mviili Mengale	16	14	7	57	96
		Tya' Assono	6	50	11	44	80
		South total	35	74	25	158	277
Total			66	116	50	380	696

**Table 2.** The number of farmers questioned, trees sampled, and fruits/seeds collected per site.

#### 4.3. Morphological evaluation

Information recorded about each of the 116 evaluated individuals of *R. heudelotii* included basic morphological characteristics (height, circumference, diameter at breast heigh, crown diameter), brief description of the soil conditions (soil type, soil colour, stoniness, drainage), general environmental and ecological attributes of the surrounding area (elevation, topography, habitat, species occurrence, associated species), and the exact GPS location.

Height of the trees was estimated by naked eye due to technical constraints. Circumference and DBH of the trunk were determined using measuring tape at the height of 137 cm above the ground. Crown diameter was obtained by measuring tape laid on the ground under the tree, determining the projected length and width of the branches. Morphological evaluation of the drupes gathered from the 50 fruiting individuals was carried out on-site. The fruits were weighted with the use of portable scales (Figure 19).



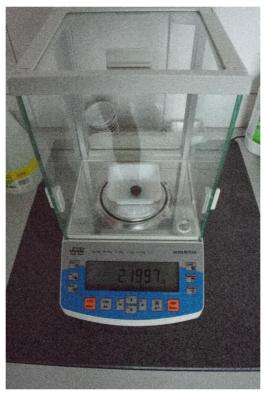
Figure 19 Weighting the *R. heudelotii* fruits *in situ* using portable scales

Fruit length and width of the fruits were measured by a calliper (Figure 20).



**Figure 20.** Taking the seed measurements *in situ* with a calliper (Kalousová 2021).

Every stone present in each drupe was extracted. After the stones were delivered to ČZU in Prague, they were weighted using laboratory scales (Figure 21).



**Figure 21.** The laboratory scales used to weight the collected seeds (author).

Stone length and width were measured with a calliper (Figure 22).

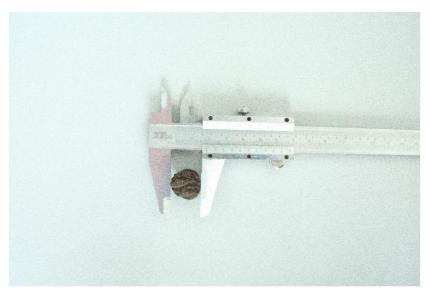


Figure 22. Measuring the seed in a laboratory setting (author).

#### 4.4. Data analysis

All the information and values acquired from the questionnaires and morphological measurements were filled into Microsoft Excel (Microsoft Corporation 2018) spreadsheets and cleaned for further statistical evaluation.

To evaluate the ethnobotanical data and general characteristics of the sampled trees, basic descriptive statistics (i.e., means, medians, and modes) as well as One-Way ANOVA followed by post-hoc Tuckey's Test were performed in Microsoft Excel, using the Real Statistics Resource Pack software (Release 7.6) (Zaiontz 2021). The resulting values were processed into graphs and tables to clarify the outcomes.

The morphological data obtained from the fruits and seeds were used to assess diversity within and among the sampled populations of *R. heudelotii using* R Statistical Software (R Core Team 2021). Principal component analysis (PCA) was performed to determine diversity between the individual trees and the whole populations based on finding out correlations between fruit and seed characteristics of each specimen. The specific R packages used to carry out the PCA were: FactoMineR (Lê et al. 2008), factoextra (Kassambara 2016), ggplot2 (Wickham 2009), and ade4 (Bougeard & Dray 2018). Successively, K-Means clustering method was used to uncover similarities between the populations by grouping the similar individuals into clusters. The specific R packages used to carry out the K-Means clustering were: tidyverse (Wickham et al. 2019), cluster (Maechler et al. 2022), factoextra (Kassambara 2016), NbClust (Charrad et al. 2014), and tidyr (Wickham et al. 2023). Student's t-test was performed on the outcomes from the previous calculations to assess whether the results were significantly statistically different.

# 5. Results

#### 5.1. Morphological characteristics

The first objective of the present study was to measure and compare morphological characteristics (length, width, and weight) of fruits and seeds of *R*. *heudelotii* collected from 2 different regions (Centre and South) and 4 different sites (Ondeck, Alen, Mviili Mengale, and Tya' Assono) to describe possible, statistically significant differences between the populations. In case of fruits, the number of seeds per fruit was measured as well.

The average fruit was 4.47 cm long, 3.10 cm wide, weighted 32.93 g, and contained 1.82 seeds. The shortest and narrowest fruits were found in Ondeck (the Centre region) with an average length and width of 4.09 and 2.95 cm, respectively, while the longest and widest fruits were found in Tya' Assono (the South region) with an average length and width of 4.64 and 3.18 cm, respectively. When statistically compared (Table 3), the average length of fruit from Ondeck proved to be significantly different from the averages found in the other 3 sites among which there was no significant difference. In case of width, the averages from Alen and Mviili Mengale did not differ significantly from each other as well as in comparison with averages measured in Ondeck and Tya' Assono. However, the average fruit width did differ significantly between Ondeck and Tya' Assono. On average, the lightest fruits were found in Ondeck, while the heaviest were from Tya' Assono with average weight of 29.63 and 34.78 g, respectively. There was no statically significant difference between the average weight of fruit from any of the sites. Similarly, there were no statistically significant differences between the sites when the average number of seeds per fruit was compared. Most fruits contained 2 seeds.

Site	Length (cm) ± SD	Width (cm) ± SD	Weight (g) ± SD	Nr seeds/fruit
Ondeck	$4.09\pm0.35^a$	$2.95\pm0.20^a$	$29.63 \pm 6.42^{a}$	$1.89 \pm 0.19^{a}$
Alen	$4.58\pm0.33^{b}$	$3.14\pm0.18^{ab}$	$33.65\pm8.06^a$	$1.81\pm0.23^a$
Mviili Mengale	$4.58\pm0.28^{b}$	$3.14\pm0.19^{ab}$	$33.65\pm5.50^a$	$1.73\pm0.20^{a}$
Tya' Assono	$4.64\pm0.41^{b}$	$3.18\pm0.16^b$	$34.78\pm8.29^a$	$1.87\pm0.20^{a}$
Average	$\textbf{4.47} \pm \textbf{0.34}$	$3.10 \pm 0.18$	$32.93 \pm 7.07$	$1.82 \pm 0.21$

**Table 3.** Average values of the measured fruit morphological characteristics from the 4 geographical sites.

Data in the same column followed by the same letter were not significantly different from each other, according to Tukey's HSD test ( $p \ge 0.05$ )

The average seed was 1.41 cm long, 1.01 cm wide, and weighted 1.95 g. The shortest and thinnest seeds came from Alen with an average length and width of 1.37 and 0.96 cm, respectively. The longest seeds were found in both Ondeck and Mviili Mengale with the same average length of 1.42 cm. The widest seeds were found in Mviili Mengale with an average width of 1.04 cm. Between the 4 sites (Table 4), no statistically significant difference was found among the measured length and width averages. The lightest seeds were found in Ondeck with an average weight of 1.66 g, while the heaviest seeds came from Tya' Assono, weighting 2.09 g on average. Statistically, no significant difference was found between seed weight averages from Mviili Mengale and the other sites. However, the statistical difference of average seed weight measured in Ondeck was significant in comparison to averages from Alen and Tya' Assono, between which there was no significant difference.

Site	Length (cm) ± SD	Width (cm) ± SD	Weight (g) ± SD
Ondeck	$1.42 \pm 0.10^{a}$	$1.02 \pm 0.06^{a}$	$1.66 \pm 0.30^{a}$
Alen	$1.37\pm0.14^a$	$0.96\pm0.06^a$	$2.05\pm0.33^{b}$
Mviili Mengale	$1.42\pm0.13^a$	$1.04\pm0.08^a$	$2.01\pm0.23^{ab}$
Tya' Assono	$1.41 \pm 0.14^{a}$	$1.03 \pm 0.09^{a}$	$2.09\pm0.38^{b}$
Average	$1.41 \pm 0.13$	$1.01 \pm 0.07$	$1.95 \pm 0.31$

**Table 4.** Average values of the measured seed morphological characteristics from the 4 geographical sites.

Data in the same column followed by the same letter were not significantly different from each other, according to Tukey's HSD test ( $p \ge 0.05$ )

In addition to fruit and seed morphological measurements, several habit characteristics of individual trees were taken in each site as well: height, circumference, DBH, and crown diameters (Table 5).

In general, the average measured specimen of *R. heudelotii* was 22.97 m tall with a trunk circumference of 248.59 cm, diameter at breast height (DBH) of 79.12 m, and a crown measuring 11.20-11.83 m in diameter. Overall, the greatest specimens in size grew in Ondeck with an average tree reaching 24.60 m in height, with a circumference of 282.74 cm, and a DBH of 89.99 cm, while the smallest trees were found in Tya' Assono averaging at 20.58 m in height, with a trunk circumference and having a DBH of 211.24 and 67.23, respectively. There were no statistically significant differences between the average height, circumference, and DBH measurements obtained in Alen and Mviili Mengale, nor were there any significant differences between average values from these 2 sites when compared to the averages of the 2 remaining ones. The average tree height, trunk circumference, and DBH in Ondeck did, however, significantly differ in comparison with Tya' Assono alone. In case of crown diameters, there were no statistically significant differences between any of the 4 sites.

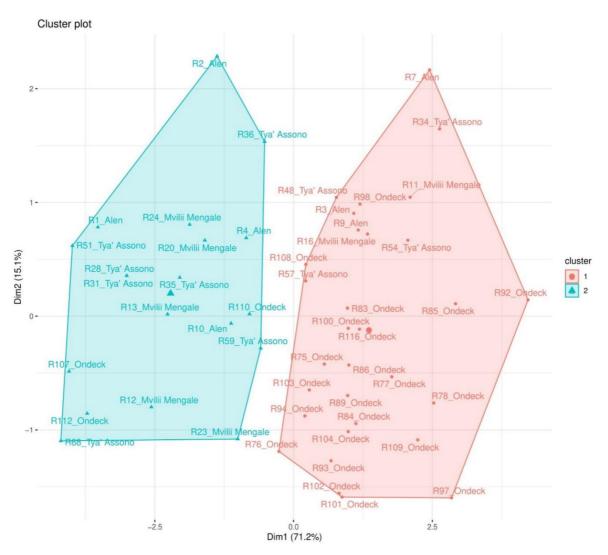
Site	Height (m) ± SD	Circumference (cm) ± SD	DBH (cm) ± SD
Ondeck	$24.60\pm5.01^a$	$282.74 \pm 103.21^{a}$	$89.99 \pm 32.85^{a}$
Alen	$23.90\pm2.64^{ab}$	$252.50 \pm 53.12^{ab}$	$80.36 \pm 16.91^{ab}$
Mviili Mengale	$22.79\pm4.90^{ab}$	$247.86 \pm 61.01^{ab}$	$78.89 \pm 19.42^{ab}$
Tya' Assono	$20.58\pm8.55^b$	$211.24 \pm 119.18^{b}$	$67.23 \pm 37.93^{b}$
Average	$\textbf{22.97} \pm \textbf{5.28}$	$248.59 \pm 84.13$	79.12 ± 26.78

**Table 5.** Average values of the measured tree habit characteristics from the 4geographical sites.

Data in the same column followed by the same letter were not significantly different from each other, according to Tukey's HSD test ( $p \ge 0.05$ )

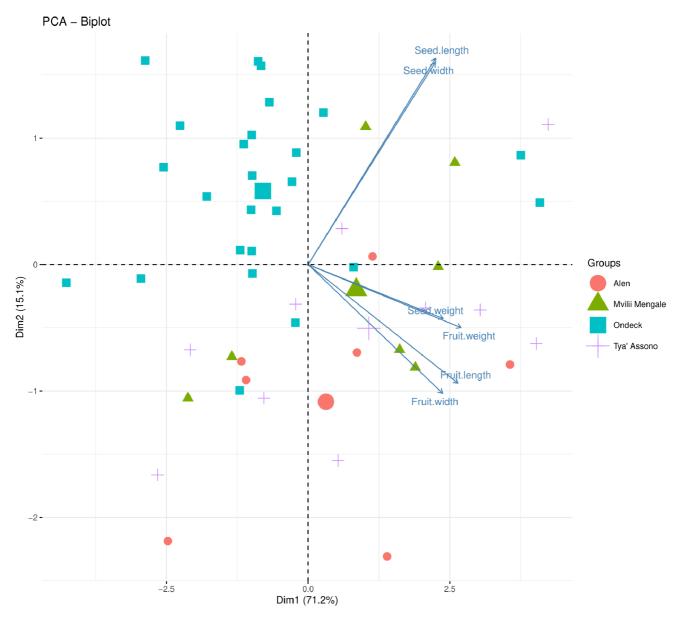
# 5.2. Intraspecific diversity based on fruit and seed morphology

To find possible population structure between the *R. heudelotii* trees sampled in 2 geographically distinct regions, K-means analysis was used as the method minimizes variation within the population and maximizes variation between the populations. The analysis showed 2 distinct clusters (Figure 23) among the sampled trees based on the fruit and seed morphological data.



**Figure 23.** A cluster plot showing the well-defined population clusters of *R. heudelotii* in the 2 geographical regions as shown by K-Means analysis.

Despite the data showing a slight overlap, the 2 clusters seem to point out that there was a statistically significant difference between the populations of *R. heudelotii* from the Centre (sites: Ondeck) and South (sites: Alen, Mviili Mengale, Tya' Assono) region, as the PCA biplot graph represents (Figure 24).



**Figure 24.** The principal component analysis (PCA) shows the relationship of each individual tree to each variable. In the biplot graph, the arrows explain the variation by their length and position in relation to one of the axes.

Thus, it can be assumed that while there was a high variability between the individual trees within the population there was also a well-defined population structure in between the regions (p<0.001 for all variables).

#### **5.3.** Ethnobotanical survey

#### 5.3.1. Socio-economic status

The total number of responding farmers in the survey was 66, 31 of them were from Centre region and 35 came from the South region. The groups of respondents in each region were of mixed sex in different ratios (Figure 25). (The median age of the respondents was 45 years in both regions, as well as the median value of 2 adult members per household. The median values for number of children per household were 5 and 4 in the Centre and South region, respectively.



Figure 25. The sex ratio of the questioned farmers in each divison.

Most of the respondents were married (Centre: 71%; South: 94%), educated (Centre: 55% primary and 42% secondary education; South: 31% primary and 63% secondary education), and the main means of occupation was farming (Centre: 97%; South: 94%). There was an apparent divide between monthly incomes of the households (Table 6).

Centre	South
6.45	0.00
38.71	34.29
22.58	20.00
9.68	11.43
22.58	34.29
	6.45 38.71 22.58 9.68

**Table 6.** The % of households with their respective monthly incomes (Central AfricanCFA franc) in the 2 regions.

#### 5.3.2. Agronomy

The median number of *R heudelotii* trees per farm found in the Centre and South region was 5 and 4, respectively. In the Centre region, fruiting of the trees and the subsequent harvest occurred once a year, while in the South region it was twice a year. The majority of the respondents (87%) from the Centre region used trees growing in the forest, in the South region the farmers used trees growing in more diverse settings, but trees found in fallow plots were the most prevalent option (Table 7). In both regions, almost all the trees were essentially wild as 100% farmers in Centre region and 97% in the South region used trees which were growing either as a part of the natural vegetation or retained after the forest had been felled.

**Table 7.** The % of *R. heudelotii* trees growing in different land-use settings in each region.

Land-use system	Centre	South
Forest	87.10	20.00
Fallow	0.00	48.57
FS	12.90	28.57
Homegarden	0.00	2.86

In the Centre region, the main cash crops were oil palm (35%) and cocoa (19%), 45% of farmers did not have any main cash crop. In the South region, cocoa was the most important cash crop being grown by 69% of the questioned farmers, followed by oil palm (20%), and banana (3%), while the rest of the farmers grew none. In case of the main food crops, most respondents answered they did not have any (90% and 89% in the Centre and South region, respectively). However, cassava and plantain were major food crops for a minority of respondents from both regions. In the South region, groundnut and banana were grown by some farmers as well.

#### 5.3.3. Use

All the questioned farmers used the kernels of *R. heudelotii* as food in both regions, although the utilization of the species was found to be slightly more diverse in the South region (Table 8).

Used tree part	Centre	South
Kernels	100.00	100.00
Bark	0.00	54.29
Leaves	0.00	2.86
Kernels		
Food	100.00	100.00
Games	0.00	5.71
Bark		
None	100.00	45.71
Medicinal	0.00	54.29
Leaves		
None	100.00	97.14
Medicinal	0.00	2.86

**Table 8.** The % of farmers utilizing kernels or other plant parts of *R*. *heudelotii* for different purposes in each region.

#### **5.3.4.** Harvest

In both regions, the harvest was performed mainly by women (Figure 26), although men did participate as well, especially in the South region where the harvest was performed solely by men in 11% cases and by members of both sexes in 34% cases.



Figure 26. The sex ratio of the njansang collectors in each region.

In the Centre region, the main harvest period lasted from September to November (65%) and the second one lasted from June to August (48%), the minority (3%) of respondents also harvested the kernels from December to February. The majority (86%) of njansang kernels collectors in the South region performed the harvest from June to August, with some choosing other times of the year: September to November (23%), and December-February (17%).

The median amount of the njansang kernels which one farmer harvested in a year was 50 kg in the Centre region, and 100 kg in the South region.

The storage period differed greatly from 3 months or less to more than 9 months, it seemed that in the Centre region the farmers preferred rather shorter storage times as majority of them stored the kernels for about 3-6 months, while in the South region 66% of the respondents stored njansang for more than 9 months (Figure 27).

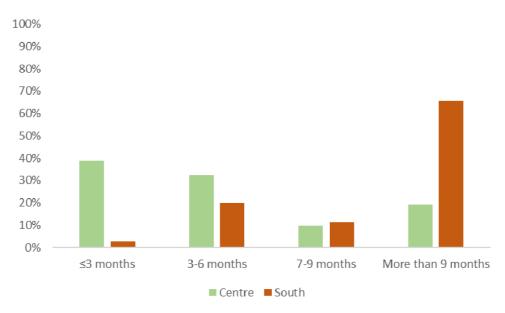


Figure 27. The kernel storage times in each region.

#### **5.3.5. Economy**

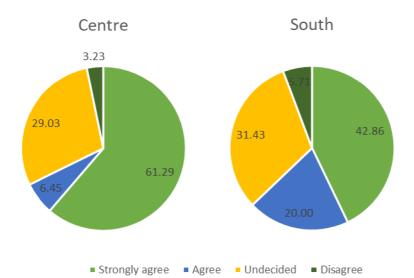
While in the Centre region the harvested njansang was rather sold on the market than used directly by the households (about 94% of the farmers use half or less of the harvest for themselves), in the South region more than half (51%) of the respondents said they used 75-100% for household consumption. Most farmers (87%) from the Centre region sold the kernels at home, while in the South region the selling locations were more variable: 11% at home, 23% at the local market, and 20% in the city. In the Centre region, the women were more involved in the selling of the produce (87%) and in 6% of cases both genders were involved, while in the South region it was 37% women, 9% men, and 3% both. The money gained through this activity was used in many ways, benefiting the local communities (Table 9).

Purpose of money	Centre	South
Food	83.87	48.57
Healthcare	19.35	45.71
School fees	74.19	34.29
Savings	38.71	8.57
House construction	3.23	0.00
Agricultural inputs	0.00	2.86

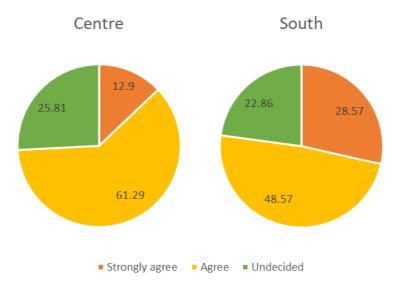
**Table 9.** The % of money gained from selling *R. heudelotii* kernels used for differentpurposes in each region.

#### 5.3.6. Sustainability

The farmers were also questioned about their opinions touching the sustainability of njansang production. Majority of the respondents from both regions agreed that sustainable management of *R. heudelotii* was needed (Figure 28), however there was a predominant opinion that the species should be left free to be exploited as well (Figure 29).

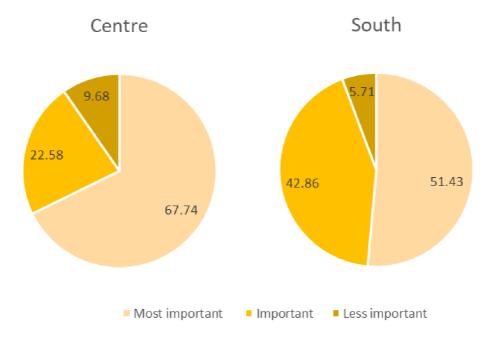


**Figure 28.** The % of farmers with different opinion on the need of sustainable management of *R. heudelotii* in each region.

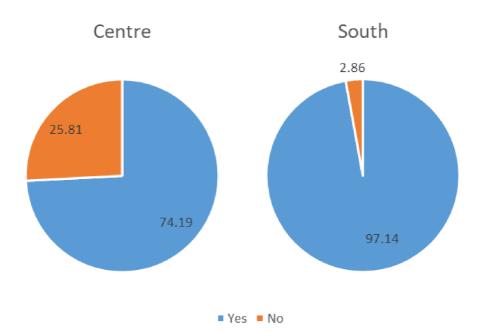


**Figure 29.** The % of farmers with different opinion on the free exploitability of *R. heudelotii* in each region.

In both regions, most farmers valued the tree as important to them (Figure 30) and were in favour of having more *R. heudelotii* trees in the future (Figure 31).



**Figure 30.** The % of farmers with different opinion on the importance of *R*. *heudelotii* in each region.



**Figure 31.** The % of farmers in favour of having more *R. heudelotii* trees in the future in each region.

## 6. Discussion

#### 6.1. Morphological characteristics

The first aim of this study was to measure and compare morphological characteristics (length, width, and weight) of fruits and seeds of *R. heudelotii* from different regions and sites. The obtained values were statistically compared using One-Way ANOVA and the post-hoc Tukey's Test to find out potential variability between the populations from 4 different sites.

#### 6.1.1. Fruits

In terms of both length and width, only the fruits collected in Ondeck were significantly different from those collected in the other sites. This could be explained either by genetic factors, environmental conditions, or a combination of both. The Ondeck population was in a different region (Centre), isolated from the 3 populations found in the South region among which the fruit measurements did not differ. In trees, genetic variability and environmental factors were proved to influence each other, creating significant and unique changes in fruit development, as a study on Juglans regia L. by Sarikhani Khorami et al. (2014) showed, thus serving as a possible explanation of our observations. In case of *R. heudelotii*, the influence of elevation had been considered an important factor affecting the morphological characteristics of this species' fruits (Fondoun et al. 1999), but the hypothesis remained unproven. Although statistically insignificant, the present results suggest that increase in elevation causes decrease in fruit length and width as the trees with generally smallest fruits were in Ondeck, the Centre region, with average altitude of 709 metres above sea level, while the trees in the South region grew at the average altitude of 517 metres above sea level, which is not consistent with results of (Mpeck et al. 2003), thus it could be argued that these trends in fruit size might be influenced by other, more prominent factors, than altitude. For example, a study on Garcinia kola Heckel carried out by Maňourová et al. (2023) did not observe significant differences between the phenotypic traits and genotypes of the studied trees, suggesting strong correlation between morphological traits and the underlying genetic composition.

Perhaps surprisingly, there was no statistically significant difference in terms of weight of the fruits among the populations, but similarly to the length and width, the results seem to show a slight decrease in weight of the fruits with increasing elevation. The fruits from all 4 populations were found to mostly contain 2 seeds per fruit which is consistent with the description characteristics of the *R. heudelotii* subspecies *africanum* which should occur in the studied area (Onefeli 2021).Since Mpeck et al. (2003) did not observe strong correlation between fruit and kernel traits in *R. heudelotii*, fruit measurements do not seem to be reliable indicators of good productivity in the species.

#### 6.1.2. Seeds

Interestingly, there were no significant differences in seed length and width between the sites. However, there was a significant difference in seed weight between Ondeck and the other sites. Since the seeds found in Ondeck were the lightest, it could be suggested that this characteristic was affected by similar forces as the fruit length and width, although such an argument seems highly controversial as the seed length and width together with the fruit weight do not appear to be affected enough to show statistical difference. The study carried out by Boko-Haya et al. (2022) explored the effects of geographical provenance on morphological characteristic in *R. heudelotii* and discovered a strong correlation between the length, width, and weight of seeds and kernels of the species. Thus, the data obtained on the seed characteristics in the present research could serve as a source of valuable information in search for superior specimens suitable for domestication.

# 6.2. Intraspecific diversity based on fruit and seed morphology

The data obtained via measurement of fruit and seed morphological characteristics was used to evaluate intraspecific diversity and population structure of *R. heudelotii*. Since the results suggested that the populations from the 3 sites in Vallée-du-Ntem, a department of the South region, were not significantly different from each other, while the population from Ondeck, the only study site located in a department of Nyong-et-Mfoumou in the Centre region, did differ significantly in several aspects, it could be said that 2 distinct populations were observed in this study.

The K-means analysis supported the suggestion of geography playing an important feature in diversification of a species (Mosca et al. 2012), in this case *R*. *heudelotii*, as the analysis identified 2 distinct groups of specimens in each distinct geographical location. The results of principal component analysis showed that while there was a high variability between individuals within each population, there was also a well-defined structure between the 2 populations. This finding is in harmony with the result of a study by Waruhiu et al. (2004) focusing on morphological and genetic diversity of *Dacryodes edulis* (G.Don), which is a tree species with similar ecology, and range as *R. heudelotii*. The obtained knowledge could be useful in identifying superior specimens to be used in the ongoing efforts to domesticate the species. Because the present results suggest that the species is highly morphologically variable both within and between its populations, there might be a good chance to discover individual trees with outstanding qualities in each population and use them for controlled breeding to develop cultivars suitable to different locations with specific conditions or to satisfy the unique needs of each farming community using *R. heudelotii* for slightly different purposes.

Probably the greatest limitation of this study, and the domestication of the species in general, is the uncertainty of which of the observed characteristics in each individual tree and population were more likely, and to what degree, shaped by external environmental forces, a manifestation of a stable genetic makeup, or rather a mixture of both, and possibly more, factors. Therefore, a thorough study on the genetics of the species and practical experiments focused on propagation of selected specimens with long-term observations of the results and testing the stability of each desired trait seem of utmost importance for future success with any efforts to domesticate *R. heudelotii*.

#### 6.3. Ethnobotanical survey

The last main aim of the present thesis was to gather ethnobotanical knowledge about *Ricinodendron heudelotii* among the local farmers in 2 different divisions found in 2 different regions: Nyong-et-Mfoumou (the Centre region) and Vallée-du-Ntem (the South region). Then, the obtained data was compared to find similarities and differences between the 2 areas in terms of socio-economic status of the farmer households, agronomical practices, use of the species, harvest, economic importance, and ideas about managing the species in a sustainable manner.

#### 6.3.1. Socio-economic characteristics

Although the median age of the respondents and number of adults in each household were both 45 years and 2 persons in both regions, the sex ratio of respondents did differ between the regions. In the Centre region, more than 80% of the questioned individuals were women, while in the South region it was almost exactly the opposite with 80% of respondents being men. It remains unknown whether the difference was a coincidence or if it was caused by the possibility that people of different sex/gender traditionally participate in different social roles under distinct cultural settings (Akinola 2018). Since gender roles seem to have strong influence on life in rural communities in West Africa (Balgah et al. 2019) and bias based on gender roles was documented in job interviews (Latu et al. 2015), it can be assumed that gender of people involved in other types of interviews, such as the survey in the present study, can be seen as a significant factor possibly influencing the answers of the responding individuals, thus influencing the results of the study.

The majority of the farmers in both regions were married and educated, which could be a beginning for improving the living standards in the future (Sahn & Younger 2007), although the sizeable divide between monthly incomes of the poorest and richest households and the 'middleclass' being a minority could be a troubling sign (Ravallion 2014). Since the household composition, income, and farming being the main occupation for more than 90% of the respondents did not differ largely between both regions, it could be suggested that the inequalities in between the individual households were more household-specific than region-specific. However unfortunate, significant inequality among households seems to be a long-term issue in the rural communities of Cameroon (Makoudjou et al. 2017).

#### 6.3.2. Agronomy

From an agronomical viewpoint, the most noticeable differences between the 2 regions were the median number of *R. heudelotii* trees per farm (5 in the Centre region and 4 in the South region) and the fact that, probably due to differences in environmental factors, mainly the amount of rainfall (Mapongmetsem et al. 2002), the fruiting and harvest in the Centre region occurred once a year, while it was twice in the other region. This finding supports the idea of environmental conditions having a strong effect on the

biology of the species (George & Ault 2014) and could also explain the variability in cultural habits of the locals (Devereux et al. 2013).

It is interesting that most (87%) farmers in the Centre region were harvesting trees growing in the forest, while in the South region, the respondents used trees from variable land-use systems with almost half of the crop being sourced from the fallows, followed by agroforestry systems, and then forests. A possible reason for such a different approach in management of the species could be that people in in the South region find agroforestry systems with *R. heudelotii* more useful compared to the other region, as it is stated in the study carried out by Mbosso et al. (2015b) in Cameroon (including both regions involved in this study) that use of such land management practices depends on how much it benefits the farmers, thus the locals from in the South region would form a more 'intimate' relationship with the trees, resulting in the trees being present in a more 'cultural landscape.

Despite the differences in terms of land-use systems in which the trees occurred and environmental conditions, essentially all farmers used wild-grown trees either found directly in their natural environment or left in place after the forest was cleared for a different purpose, this is in harmony with the work of Ayuk et al. (1999) which focused on uses and management of the species in Cameroon, and could be expected, since the domestication of *R. heudelotii* is still in its early phase, possibly due to the slowly changing social and cultural believes of the locals and communication issues with the extension agents (Njiei & Asongu 2021) and the general obstacles in propagation of the species, e.g. low germination rate without the proper treatment and sowing substrate (Kouame et al. 2012). Only in the South region, less than 3% of respondents said their trees were planted on purpose, although seemingly insignificant, this implies that there are at least some attempts on cultivation of the species in the area which might become more popular if accessible and easy-to-follow methods of propagation and cultivation of *R. heudelotii* were developed, especially using trees bred or cloned from specimens with superb characteristics.

Since farmers in both regions grew cocoa as a valuable cash-crop and growing trees in cocoa plantations is a common practice (Ndah et al. 2023), it could be suggested that higher implementation of *R. heudelotii* as a shade tree in cocoa farming could result

in increased popularity of the species, making its benefits more accessible while saving land by using the plots in a multipurpose manner (Shidiki et al. 2020).

#### 6.3.3. Use

All the questioned farmers used the kernels of *R. heudelotii* as food, which was expected and in accordance with works of Leakey (1999) or Facheux et al. (2007). Since in in the South region, unlike in the Centre region, other parts of the tree are used, such as bark or leaves for medicinal purposes it could be said that the relationship of locals towards *R. heudelotii* is more traditional (Wanche Kojom et al. 2022) and the species is seen as a truly multipurpose one.

#### 6.3.4. Harvest

The data concerning harvest seem to be in harmony with the claims of authors doing research on *R. heudelotii* who state that women are the main actors in collecting the kernels (Ndumbe et al. 2019; Tieminie et al. 2023), this is almost certainly true in the Centre region where 87% respondents said that women were the sole harvesters. Even though in the South region most kernel collectors were also women (54%), the number of answers stating that both genders (34%) or exclusively men (11%) participated in the harvest was higher. The difference could be caused by different gender roles or cultural practices in each region, although it could also be connected to the level of importance of the species which might vary regionally(Mbosso et al. 2015) consider-mechanization of the post-harvest processing of the kernels as an important factor possibly contributing to rise in interest of the local men to participate in this traditionally 'feminine' activity.

Despite the trees in the Centre region fruiting just once a year, the farmers usually collect the kernels in a rather broad period with most doing so from September till November (65%) or from June to August (48%) and the rest (3%) choosing the period from December till February. While in the South region, where, according to the farmers, the trees fruit twice a year and most of the farmers perform 2 harvests a year, 86% of the respondents harvested the kernels from June to August, with minority of them doing so from September to November (23%) or from December to February (17%). Ako (2016) claims that the *R. heudelotii* trees flower at the beginning of the rainy season and fruiting occurs later, during the rainy season, mostly from August till December, thus the

harvest periods could differ between distinct geographical regions and slightly vary each year. The fact that Djeugap et al. (2014) collected mature seeds under the parent trees in Central Cameroon in June also seems consistent with the present data as a substantial number of farmers favoured June as a start of the harvest period, especially in the South region.

In the Centre region, the median yearly harvest was 50 kg per household and the most preferred storage times were less than 3 months or 3-6 months, while in in the South region the median yearly harvest was 100 kg per household and the most usual storage time was more than 9 months with minority storing the kernels for less. These differences could exist simply because of the different fruiting patterns in each region, or they could also be culture-specific, depend on the use, or economic importance of the crop in each specific region and community. Ndumbe et al. (2019) mention a connection between the storage time length and the fluctuations of the price of the kernels on the local marked, motivating the farmers to store the kernels to have more control over price fluctuations.

#### **6.3.5. Economy**

The most noticeable difference in the role of *R. heudelotii* kernels in between the 2 regions was that in the Centre, most farmers sold at least half of their harvest, with vast majority of them doing so from home, while in the South, half of the farmers stated they used at least 75% of their harvest for household consumption and their usual selling places were more diverse, including local markets and cities.

In both case women were involved in the selling process, although in the Centre region their involvement was greater than it the other region, where the roles were slightly more mixed, perhaps due to the same reasons influencing involvement in the harvest based on gender-roles.

The number of kernels sold or kept for household consumption could also be linked to the overall amount of the crop harvested in each region. In both cases, the money gained through selling the kernels was used to benefit the communities in terms of satisfying basic needs (food, healthcare) or to increase education and financial security. Thus, it can be said that *R. heudelotii* and its kernels indeed have an important role as a valuable NTFP in the region.

#### 6.3.6. Sustainability

Majority of the questioned farmers in both regions agreed that sustainable management of R. *heudelotii* was needed, while also keeping free exploitation of the species allowed. Such statements, together with the fact that the overwhelming majority of the respondents regarded the species as important and expressed the desire for more R. *heudelotii* trees being present in the area in the future, could be perceived as a good foundation for promoting further research and domestication efforts of this species.

As the natural forests and biodiversity of Cameroon seem to be at risk of decline (Tchoumbou et al. 2020), a proper and namely sustainable management of the species both in the wild and cultural landscape should be developed. It is considered essential to implement such management practices which would allow for sustainable yet accessible use of the species to benefit the local communities and biodiversity as much as possible.

## 7. Conclusion

The main aim of this research was to assess variability of *R. heudelotii* based on morphological characteristics of fruits and seeds from populations found in different geographical regions of Cameroon. The results showed that the morphological traits did differ significantly in several cases when the dimensions and weight of fruits and seeds from 4 geographical sites were statistically compared. The greatest difference was found between the populations in Ondeck (Nyong-et-Mfoumou division, Centre region) and Tya' Assono (Vallée-du-Ntem division, South region) which were topographically the most different from each other. The principal component analysis based on the morphological characteristics also lead to a conclusion that the populations from the Centre and South regions as a whole were significantly different from each other with a well-defined structure, while having high levels of variability in between individual trees with the populations.

Another objective of the study was to assess local ethnobotanical knowledge concerning management and importance of the species. It was found that *R. heudelotii* was an important source of food and income for local farmers. It was also a popular tree used in agroforestry systems, especially in the South region, where it was used for medicinal purposes as well. There was also a significant level of interest in both studied regions in sustainable management of the species and obtaining more *R. heudelotii* trees in the future, to satisfy the needs of the farmers.

In conclusion, it can be said that there is a great potential for improvement of the species in cultural and commercial settings, since the huge morphological diversity suggest great genetic variability which could be useful in breeding programmes. The local farmers seem to favour such efforts, although any progress so far seems to be hindered by the lack of proper knowledge of the species' genetics and cheap reliable propagation or breeding methods. A large-scale study focusing on the genetic makeup of *R. heudelotii* populations across Cameroon and implementation of local ethnobotanical knowledge in management practices of the species is suggested.

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# Appendices

## **Appendix A – Questionnaire**



Introduction: This questionnaire was developed for the purposes of data collection under a project entitled: "Morphological and genetic characterization of Ricinodendron heudelotii (Baill.) Heckel in natural and domesticated populations in Cameroon". All the activities are related to collect information about household management and silvicultural practices of the targeted specie. The collected information will remain anonymous. The mentioned activities are related to the University Grant Competition (UGC) student grant and implemented under project Improvement in Quality of the Internal Grant Scheme at Czech University of Life Sciences, CZU, reg. no. CZ.02.2.69/0.0/0.0/19\_073/0016944. Thank you

No	Description	code
1	Date of the interview:	
2	State/region/province	
3	Name of village (locality)	
4	GPS Latitude range N Longitude range E Elevation range (m)	
5	Number of household members Adults Children	
6	Sex: a- Male b- female	
7	How old are you?years	
8	Education: a- No formal education b-Primary school c-secondary school d-university degree	
9	Length of formal education?years	
10	Marital status: a- Single b-Married c-Divorced d-Widow (er)	
11	Main source of income: a- Farmer b-Civil servant c-Business d- Handcraft e-other Monthly income a-less than 20,000 xfa b-21,000-40,000 xfa c-41,000-60,000 xfa d- more than 60, - less than 14,000 xfa b- 15,000-24,000 xfa c-25,000-40,000 xfa d-above 40.00	

#### Section 2: Ecology and typology

12	Do you distinguish different types/varieties of Djangsang tree? Yes / No	
13	Which part of the tree do you use (multiple choice is allowed)?	
	a-barks b-leaves c-roots d-kernels e- fruit	
14	13.1-What do you do with barks (multiple choice is allowed)?	
	a-food b-medicinal c-firewood d-construction material e-other	
	13.2-What do you do with leaves (multiple choice is allowed)?	
	a-food b-medicinal c-other ()	
	13.3-What do you do with roots (multiple choice is allowed)?	
	a-food b-medicinal c-other ()	
	13.4-What do you do with kernels (multiple choice is allowed)?	
	a-food b-medicinal c-other ()	
	13.5-What do you do with fruits (multiple choice is allowed)?	
	a-food b-medicinal c-other ()	

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15	Place where the tree is grown, and seeds are collected:	
	a- forest b- savannah c-home garden e- oil d- cocoa agroforestry systems	
	palm f- other	
16	What is the current situation of the tree?	
	a- trees are from wild populations?	
	b- trees are planted?	
	c- trees were retained during clearing?	
	d- other (specify)	
17	Who are the collectors?	
	a-men b-women c-both	
18	How many Djangsang trees do you harvest annually (number of tree)?	
19	What is the approximate quantity of Djangsang fruit you collected annually (in Kgs)	
20	19.1-Main cash crops on the present farm	
	a- cocoa b-coffee c-cotton d-bananas e-rubber f-oil palm	
	19.2- Main food crops on the land	
	a-plantains b-cassava c-corn d-millet e-sugarcane	
21	Associated tree	
	species a-	
	b-	
	c-	

#### Section 3: Commercialization

22	What are your post-harvest processing techniques? (collect, store, and deshell?) a-	
	b-	
	c-	
23	Do you know how to process nuts into oil? (Yes / No)	
24	Do you have operational units for processing? (Yes / No)	
25	Which technique are you using to process the	
	nuts into oil? a-	
	b-	
	C-	
26	25.1- What is the quantity harvested per season?	
	a- 5 bags of 50 kgs	
	b- 10 bags of 50 kgs	
	C- 15 bags of 50 kgs	
	d- >50kgs	
	26.2- From the quantity harvested what part is for household consumption: (	%)
27	Do you sell the nuts in raw form? (Yes / No)	
28	For how much do you sell the seeds?	
	Bucket 5 Kgs (); Bucket 10 Kgs (); Bucket 15 Kgs (); Bags of 50 kgs (	)
29	For how much you sell 1 Kg oil?	
	1 Kg () 10 Kgs ()	
	5 Kgs () 20 Kgs ()	
30	What is quantity of oil you sold out in the last year (Kg)?	
31	Where do you sell the products?	
	a-home (while fruits are still on	
	the tree) b-local market	
	c-cities (Yaoundé, Douala etc.)	
	What is the category of actors involved in the commercialization/trade of Djangsang produc	ts?
	a-producers b-wholesalers c-middlemen d-consumers	
32		1

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33	Who is mainly involved in the commercialization /trade of Djangsang products on the market? a- men b-women c- both d-children What are the	
	costs you are paying for the purpose services?	
	a- Transportation (from the forest to home for collection) []	
	b-Taxes []	
34	c- Transportation from home to market (if you are selling in the market) []	
35	What is the purpose of the money gained from sales? (uses)	
	a-feeding b-school fees c-health care d-savings agricultural e-buying	
	inputs f- others (specify)	

### Section 4: Management of the tree

36	What is the source of the planting material? (How did you get the tree?) (Multiple choide)
	a- wild populations d-donated from NGO b- planted material e-
	farmers' cooperative c- retained wild trees during clearing f-
	other
37	How many times does the fruiting take place?
0,	a-1 time/year b-2 times/year c-3 times/year
20	· · · ·
38	How many times do you harvest?
	a-1 time/year b-2 times/year c-3 times/year
39	What is the best time for harvesting? (months calendar)
40	For how long do you store the seeds?
	a- <3 months b- 3 - 6 months c- 7 - 9 months d-> 9 months
41	41.1- Djangsang tree population in the area need sustainable management.
	a- Strongly disagree b- Disagree c- Undecided d- agree e- strongly agree
	41.2- Djangsang tree population in the area do not need sustainable management
	a- Strongly disagree b- Disagree c- Undecided d- agree e- strongly agree
	41.3- Free exploitation of Djangsang should be allowed in the area
	a- Strongly disagree b- Disagree c- Undecided d- agree e- strongly agree
	41.4- Djangsang tree populations need controlled exploitation of the resource
	41.4- Djangsang tree populations need controlled exploitation of the resource
42	41.4- Djangsang tree populations need controlled exploitation of the resource
	41.4- Djangsang tree populations need controlled exploitation of the resource         a- Strongly disagree       b- Disagree       c- Undecided       d- agree       e- strongly agree         How can you define the importance of Djangsang for your household?         a- most important c-undecide e-not important b- important d-less important
42 43	41.4- Djangsang tree populations need controlled exploitation of the resource         a- Strongly disagree       b- Disagree       c- Undecided       d- agree       e- strongly agree         How can you define the importance of Djangsang for your household?         a- most important c-undecide e-not important b- important d-less important         Do the trees require pruning activities? Or protection? Yes or No?
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