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Regeneration of threatened indigenous fruit species in Uganda, the case of *Parinari curatellifolia*

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Abstract

Indigenous wild fruits and, or underutilised plant species are of high importance for nutrition, beverages, and pharmaceuticals values. They provide invaluable benefits to the entire biosphere: including but not limited to climate regulation in terms of rainfall cycle, oxygen supply and carbon sequestration, and provide fodder to both wild and domestic animal herds. The Mobola plum tree, *Parinari curatellifolia* Benth. is one such under-utilised species that serves as a key source of household income particularly in rural areas in Uganda. It has versatile usages ranging from medicinal herbs with large food dietary values to the intensive sale of charcoal and wood fuel in urban centres, and construction materials for human settlement. The study objective was to investigate *P. curatellifolia*'s seed germination to improve its conservation and sustainable utilisation in Uganda. The current research study focused on two major areas of *P. curatellifolia* regeneration: (1) regeneration in nature, (2) regeneration under laboratory conditions of (a) seed (sexual) regeneration and (b) cuttings (vegetative) regeneration and (c) direct sowing. Previously published results also confirmed difficulties in germinating *P. curatellifolia* seeds. Hence the need to undertake this research study aimed at understanding best ways to regenerate the species both in nature and laboratory-based conditions. However germination results were not obtained during the study time. Therefore efforts towards species' regeneration in Uganda, require every exertion and support to succeed. To complement nature conservation research, plant genetic resources and biodiversity management. That supports other indicative mechanisms such as the Global Plan of Action to ensure food security, availability and sustainable utilisation of our natural resources.

Key words: *Parinari curatellifolia*, Mobola, threatened indigenous species, under-utilized fruit tree, regeneration, biodiversity.

Content

Introduction

The quest for food is a universal imperative which supports a diversity of life on earth. Its insufficiency and shortage threaten viability of life. This prompts humanity to conserve and sustainably utilise a spectrum of resources such as; domesticated crops, wild food plants; indigenous fruits and under-utilised species. Towards the aim of sustainably food sources, Akinnifesi et al. (2007) advocates for long term domestication strategies, including; selection of priority species, germplasm collection, genetic improvement, propagation systems, field management, harvesting and post-harvest technologies, economic analysis and market research. On a global level, conservation and utilisation of indigenous fruit species for food security and income generation have become increasingly important issues. Efforts are currently being made in the Sub Saharan region to actively engage local people in the domestication and commercialisation of indigenous fruits. It is considered advantageous to understand the values of plant genetic diversity and resources. Enhancing awareness serves to advance the goal of conservation and provision of new knowledge to the scientific community and farmers. Themes have become focused on alternative food harvest methods from wild fruit trees to support sustainability and security of food resources. These considerations are of particular relevance for the alleviation of poverty in the sub Saharan region. Akinnifesi et al. (2007), points out that research on indigenous fruits and nuts has accumulated considerably in sub Saharan Africa, and their role is being recognised in the domain of poverty alleviation.

Global crop diversity

The vast majority of today's food and fibre crops were once wild plants of ancient origin. These have been domesticated since the Stone Ages, and have undergone several progressive selections and crop improvements through human technologies (i.e. breeding, grafting, vegetative propagation) to adjust their taste, nutritional (chemical and mineral) contents, shape, size, and colour, among other values. Today, valuable genetic resources are at risk of depletion from human population growth, politics, development, urbanisation and industrialisation. Rescuing and collecting our crop diversity is the way to guarantee future food supplies as global challenges related to climate change increase. The world needs the endangered species that are indigenous, non-domesticated and under-utilised because they support the generation of new food varieties which can withstand droughts, pests, diseases, and global warming.

In developed countries, most homes have a variety of foods at each meal, a fact that is largely due to efforts made somewhere in conservation of crop diversity. All indications suggest that such efforts to rescue and collect the world's least-known species are vital for the entire natural resource base. In response to the growing needs of conserving and sustainably using the World's plant genetic resources, the Global Crop Diversity Trust has been established to provide financial aid in this important quest.

Domestication and commercialisation of indigenous fruits species

As people around the world wrestle with food and financial crises, another high-impact crisis goes less noticed; internationally, indigenous fruit species and forests in general are rapidly receding due to logging and expansion of land for cultivation and settlement. Valuable forest species and wild fruit trees are at risk of overexploitation and extinction. Akinnifesi et al. (2007) claim that relatively little research or attention has been given to the development of indigenous trees as crops with potential for wider cultivation. He further stresses that domestication of indigenous fruit trees in a farmer- driven; market- led process has created a viable agroforestry initiative in tropical regions of the world (Akinnifesi et al. 2006).

Role of propagation in domestication

Propagation of plants is a deliberate, controlled reproduction of plants using seeds and spores to secure food supply. It can be done through seed or vegetative regeneration methods for domestication (adaptation). Akinnifesi et al. (2008:a) reports that propagation is a key factor for successful cultivation and sustainable management of indigenous fruit trees (IFTs). It aids ecological restoration of ecosystems and conservation of endangered species. Tchoundjeu et al. (2004) examines vegetative propagation as used to multiply, test and select superior germplasm from the wild. It involves the asexual regeneration of new plants typically from meristematic parts of a 'mother plant' or stock-plant, and the resulting plants are genetic copies of that plant and form a clone. When a clone is derived from a genetically superior plant, it can be multiplied as a cultivated 'variety' ('cultivar'). Ray and Brown (1995) in Ming'omba et al. (2008) suggests that ecological restoration assists the recovery of an ecosystem that has been degraded, damaged or destroyed. Ming'omba et al. (2008) argues that propagation plays a crucial role in the reintroduction of such endangered species, and claim that macro- and micro – propagation techniques could be useful for mass propagation of species for restoration.

Indigenous fruit trees/ non- domesticated and underutilized species

The vast group of indigenous fruit trees and non-domesticated and/or under-utilized plant species offer vital ingredients for nutrition, pharmaceuticals and beverages. They also provide herbal medicines and the wood can be used as fuel and timber. Many wild fruit species contribute to climate regulation by affecting rainfall cycle and carbon sequestration. They are also a major source of fodder to both wild and domestic animal herds. Several authors (Katende et al. 1995, 1999; Akinnifesi et al. 2004:a; Nyoka and Rukuni 2000) have demonstrated that a high proportion of rural households in developing countries, benefit significantly from use of indigenous wild fruit species, depending on the extent of their consumption patterns. The dependence variation on indigenous wild fruits (Grosskinsky and Gullick 2000; Kwesiga et al. 2000; Rae in Bowes 1999; Maghembe 1995) is discussed with focus on the major beneficiaries, the majority of which are women and children. Additionally, women in most rural areas market indigenous wild fruits and use returns from sales to access other goods and services. Hence these wild plant species are utilised and incorporated into domestic agriculture in away that bolster household food security, income, health and the poverty alleviation. Akinnifesi et al. (2007) maintains choosing the 'Big Five' priority indigenous fruit trees in sub Saharan Africa. The Mobola Plum (*Parinari curatellifolia)* is on forefront ranked number two amongst the big five. He reports that a systematic priority setting was made in different regions across Africa (West, East and Southern Africa) where the indigenous fruit species exist and the exercised involved local communities in a participatory manner.

Biological, taxonomical and nomenclature information

One such important under-utilised species, the Mobola palm tree, *Parinari curatellifolia* (Planch. ex Benth) belongs to *Chrysobalanaceae* family. The *Chrysobalanaceae* family consists of several other genera including *Licania, Parinari, Exellodendron, Maranthes, Couepia, Hirtella, Acioa and Neocarya* (Prance Ghillean T 1989). Coradin et al. (1985) reports more than 32 species within the genus *Parinari*. In the World Agroforestry Centre (ICRAF) AgroForestryTree Database on species information, www.worldagroforestry.org, extensive details on *P. curatellifolia* are provided (i.e. taxonomy, synonyms, and vernacular or common names).

Taxonomy: current study species (P. curatellifolia)
Authority: Planch. ex Benth.
Family: *Chrysobalanaceae.*

Synonyms (s)
Parinari chapelieri Baill.
Parinari curatellifolia spp. Mobola R. Graham
Parinari gardineri Hemsl.
Parinarium curatellifolium Pland.

Vernacular/ common names
English: fever tree, hissing tree, mbola plum, mobola plum

Luganda: munazi
Swahili: mbura
Afrikaans: grysappel

P. curatellifolia bears an ever green spreading canopy, and ranges in size from small to large gaining tree heights of up to 20 m, with 20 cm large panicle-formed inflorescences bearing white and rose-like flowers (Mathurin 1997). The species is utilised for charcoal and wood fuel, construction materials for human settlement, and medicinal herbs as well as food dietary purposes. The species is used for a wide range of purposes: roots are used in the treatment of tooth decay, ear infection and malaria, the bark is used against fever, bronchitis and malaria, the species extracts, can treat wounds, boils, stomach troubles; diarrhoea, food poisoning and fevers, (Oladimeji et al. 2006). The pulp and seed are eaten and also used for making drinks or beverages, and the hard wood is heavy and resistant to insects, and is used in construction and for making mortars and dugouts (Nyoka and Rukuni 2000; Arbonnier 2000; Von Maydell (1983) in Mathurin (1997).

Ecological, eco-geographical and phenological distribution

Katende et al. (1995, 1999) alleges that in Uganda the species is found in open woodland, wooded grassland, and savannah grassland and often on rocky sites, in areas with a mean annual rainfall of 400 - 2300 mm, a mean temperature of 10 - 30° C and altitudes of 1100 - 1900 m. Generally *P. curatellifolia* can still be traced in the northern and western parts of Uganda. Noticeably, West Nile, Karamoja, and south western (SW) boardering Tanzania at the periphery or shores of Lake Victoria and grows in association with woodland. It is fire-resistant, making it a prominent feature of fire-maintained wooded grassland. It is typical of areas with a high water table and poor drainage. Sanogo et al (2006), reports that Local people tend to protect it due to its potentiality; however forest fires and land scarcity threaten its existence.

Katende et al. (1995, 1999) reports that in some parts of Uganda *P. curatellifolia* begins to flower from July, and fruits in August – December. Collection of ripe fruits is done around December to January. Not all *P. curatellifolia* trees bear fertilized fruits every year. The fruits can be harvest when they turn yellowish and have a high content of vitamin C and are eaten fresh, cooked as porridge or made into beer (Katende et al. 1999).

Multipurpose and nutritional values

Excitingly, other study findings indicate that edible fruits harvested from indigenous trees provide vitamins and essential minerals for the proper maintenance of human health (Jardin 1967; Williamson 1975; Campbell 1987; Kalenga et al. 1988, 1994; Packham 1993). The Mobola plum tree (*P.*

curatellifolia) is rich in proteins, fats, carbohydrates and minerals as such; iron, magnesium, sodium, potassium (Kalenga et al. 1994). Sanogo et al. (2006), reports that the species wood contains a high quantity of silica crystals and is strongly fire resistant. The timber is very durable, hard and heavy (720 kg/m^3) often used for woodwork, mortars, canoes and mine timber. It is a potential for medicine to treat ailments: in Uganda, Katende et al (1995, 1999) reports that roots are used in the treatment of toothaches or decays, fractures, ear infections, snake bites and malaria. The tree bark extracts are often used to tan or dye baskets and also to cure; bronchitis, wounds boils, and stomach upsets; diarrhoea, and food poisoning (Kwesiga et al. 2000; Oladimeji et al. 2006). According to Katende et al. (1999) the fruits or seeds can be dried and used as a reserve food. Nuts can also be eaten alone or mixed with vegetables. Oil extracts from *P. curatellifolia* seeds contain about 40 percent oil (70 percent in kernels).

However, because the species is utilised so intensively, there is now a clear risk of genetic and/or species diversity loss, if no immediate mitigation measures are taken to avert the situation. This is likely to amply loss of biodiversity and its associated management challenges as presented by Gaston et al. (2004). The species require fast regeneration to surpass its anthropogenic demands and secure its sustainable utilisation. Furthermore, Akinnifesi et al. (2004b) reported that lack of the species planting materials has frequently been cited by farmers as the major constraint to sustainably grow the indigenous fruit tree species.

Study problem statement

The challenge is to regenerate and swiftly increase availability of the species as it appears to be over exploited. There is scarce literature on how the species reproduces in Uganda. Preliminary attempts to regenerate the species at Plant Genetic Resources Centre (PGRC), Entebbe Botanic Gardens, have been unsuccessful. The seed coat is very hard requiring special seed treatments to enable germination and/or access to the embryo. Previously published results have confirmed the difficulties in regenerating the species from seeds. Mathurin *(*1997) attempted a desiccation and germination experiment but failed to germinate any seeds, possibly due to collection of immature seeds or of dormancy problems. Dried seeds though can retain viability for over two years and the species seed can take over six (6) months to germinate according to other authors (Katende et al. 1995, 1999).

Seed dormancy is also another aspect to consider while dealing with *P. curatellifolia* seed germination. The species could be embodied with organic seed dormancy complexities such as; endogenous and exogenous or a combination of the two dormancies. Seed dormancy is such a state when seeds are quiescent or impaired to germinate due to a range of aspects not limited to environment or ecological factors only.

Nikolaeva (1977) in Baskin (2002) simplified the type of organic seed dormancy in the following way:

- Endogenous dormancy: physiological inhibiting mechanisms (PIM) of germination, under developed embryos (morphological dormancy) and PIM of germination and under developed embryo (morpho-physiological dormancy)
- Exogenous dormancy: seed (fruit) coats impermeable to water (physical dormancy), germination inhibition (chemical dormancy) and woody structures that often restrict growth (mechanical dormancy).

Why the interest in Parinari curatellifolia?

This project aimed at studying *P. curatellifolia* seed germination to understand its regeneration complexities and serve as a showcase of many threatened indigenous species in Uganda. Despite its over-utilization and often extensive destruction there is little attention from neither the scientific nor the local community to regenerate the species. This poses a major threat of its decline, if there are no quick mitigation measures to reverse the negative trend. In Uganda hardly any scientific work has been devoted to the regeneration of *P. curatellifolia* species. Therefore, this study was established to explore opportunities of increasing the availability of *P. curatellifolia*, thus avoiding scarcity of our valuable indigenous fruit species. Indigenous species are gradually disappearing, and vanishing from the field before being collected for safety at the genebanks or botanic gardens such as Entebbe Botanic Gardens. This interweave the need to carry on research studies aimed at understanding the species seed behaviour, both in nature and laboratory-based conditions to regenerate, increase and, or multiply threatened indigenous species.

Regeneration is a crucial activity in gene-bank management. It is costly in terms of time and resources. It also invokes risks of genetic integrity, and contamination due to mechanical mixing, out-crossing or possibly genetic drift. However, in the present work the following principle factors were kept in mind while attempting to regenerate *P. curatellifolia*;

- maintain special and apt species conditions to break dormancy and stimulate germination,
- regenerate accessions before viability drops to low levels, and
- ensure optimum number of plant sample sizes to produce sufficient quality seeds.

Project study objectives

The overall objective of the present work was to investigate the seed germination *P. curatellifolia* to improve its conservation and sustainable utilisation in Uganda. The specific aims were (1) to inventory growth sites; (2) assemble data on seed physiology and germination behaviour; (3) develop seed germination methods appropriate for the species; (4) identify factors favourable for seed germination in nature and under laboratory conditions, and (5) document indigenous local knowledge on how the species is used and regenerated.

The study hypothesis underscored the fact that *P. curatellifolia* seeds were difficult to germinate and the crucial need to develop apt regeneration protocols. The study focused on (a) trying to improve *P. curatellifolia* seed germination by using root hormones, (b) use of pepsin solution to simulate the gastric environment of frugivores to elicit seed germination, and (c) direct sowing into sawdust pots. These attempts were intended to impart, and cultivate, knowledge of effective regeneration methods to increase the species availability in Entebbe Botanic Gardens, thereby reversing the negative trend of disappearing trees.

Materials for the current investigation included seeds and cuttings (scions) for regeneration. Different regeneration methods were attempted such as pre-treatment procedures, *in vitro* regeneration (sexual or vegetative) on growth media, or direct sowing in pots filled with sawdust. The field work included studies of growth sites and in person interviews with local people.

Materials and methods

Study site

The study was conducted in Rakai district (-0.72E' to 31.400N') south west (SW) in Uganda during the months of August to December 2008. The district covers an area of 4989km^2 with a population exceeding 470 000 (UBOS 2002). It is generally characterised by a few semi deciduous forest patches and more of grass savannah vegetation type (Buyinza and Mukasa 2007). Most of the people occupy privately owned land in this rural countryside.

Site eco-geographical conditions

Rakai district, Lwanda sub county SW Uganda (**see Appendix 1**), experiences the tropical equatorial type of climate; generally rainy with two dry spells (December – February and June – August) annually. According to Buyinza and Mukasa (2007) argues that the mean temperature ranges (15 – 30^0 C), mean annual rainfall range between 400 and 2300mm and an altitude between 1100 and 1900 m above sea level. Buyinza and Mukasa (2007) state that rainfall totals decrease to 750 – 875 mm per year as one moves away from Lake Victoria shores towards west. The topography at the study site was characterised by dry stony, rocky and sand soils as shown in Figure 1where P. curatellifolia was found commonly growing. Buyinza and Mukasa (2007) further reports that Rakai district's soil texture ranges from red laterite, sandy loam to loams in different areas.

Site selection criteria

The selection of sites relied mainly on existing secondary data records from reliable sources such as library archives, publications and specimens at the Harbarium centre, in Makerere University and at Entebbe Botanic Gardens in Uganda. In addition, random field surveys were conducted among four districts: - Mpigi, Masaka, Rakai and Mbarara districts – to identify potential sites for *P. curatellifolia.* The total survey area comprised c. 1693km^2. Rakai district, Lwanda sub county, was the only place where *P. curatellifolia* was still in existence, however, but trees showed signs of massive human harvest for charcoal processing, fire wood collection, tree debarking for medicinal purposes and construction materials (poles).

The identified sites (A, B, C, D) on the map (figure 1) where also randomly selected during the collection of field study materials while interviewing local informants.

Noteworthy, sites (B and C) had interesting unique study features as later explained in this report. The four sites on Lwanda sub county where P.

curatellifolia was found did not differ very much but were similar in terms of topography (terrain, landscape and soils), climate (temperature and rainfall amounts) or vegetation.

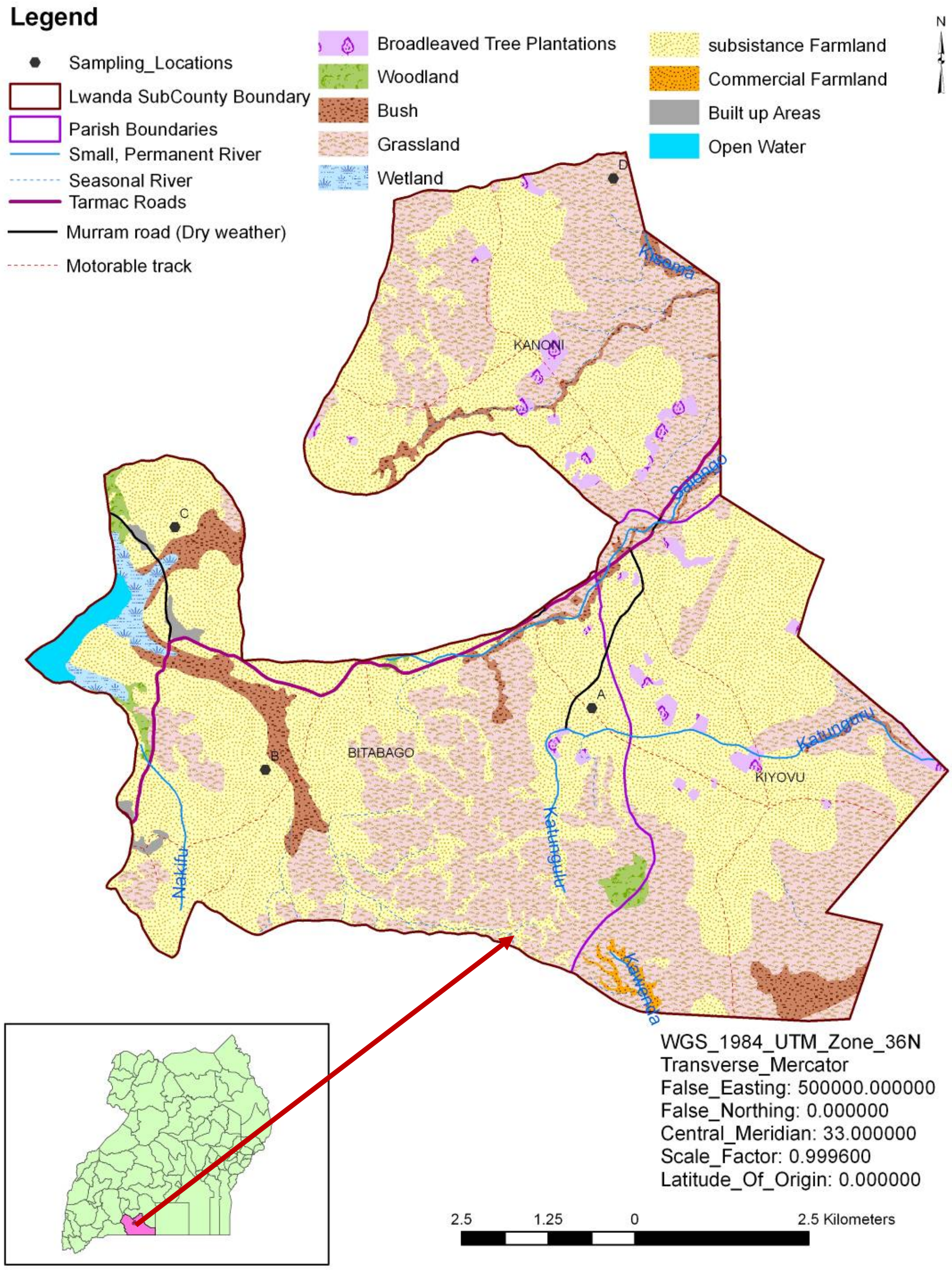

Figure.1. Illustrating the Map of the Study area in Rakai district

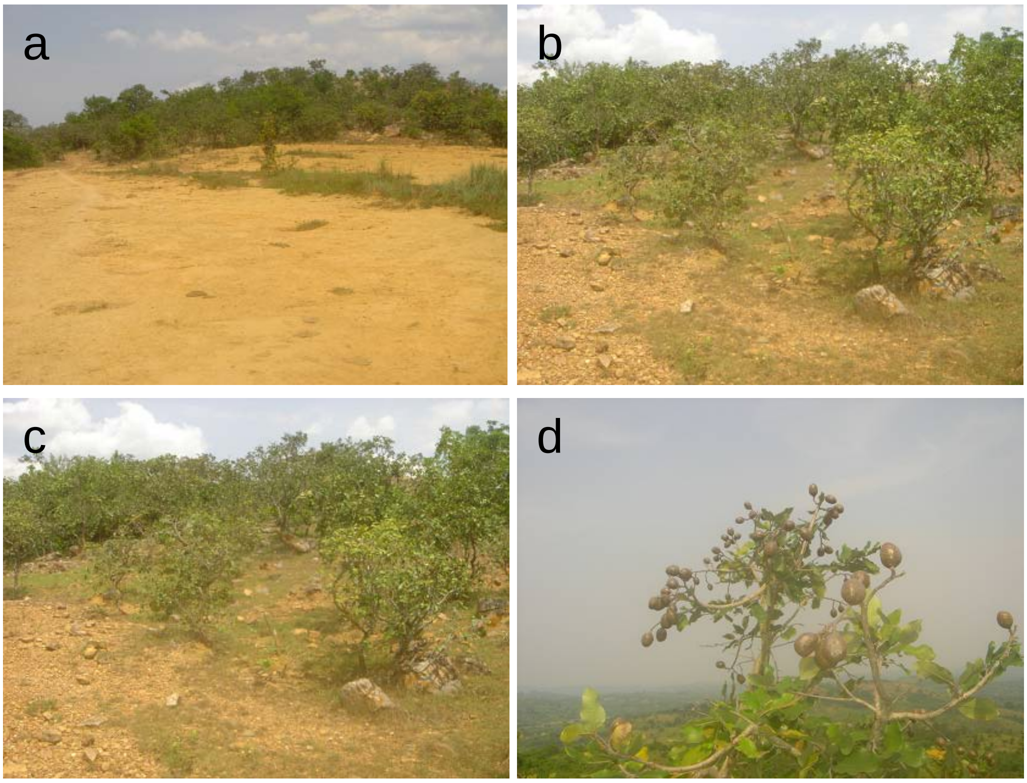

Figure 2 a-d. Landscape and topographic features at study site A.
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Baseline inventory and observation

The baseline survey consisted of both primary and secondary data captured through quantitative and open-ended, semi-structured questions focusing on the species' eco- geographical and biological characteristics (natural regeneration). Narrative answers were processed verbatim. In-person interviews were held with eleven respondents, both men and women, and mostly household heads. Some were more acquainted than others with the species natural regeneration. Information gathering during interviews also included social parameters: people's consent to age, education, marriage statuses, daily life, and ethnicity as well as unstructured conversations to assemble complete cultural inventory on the species' propagation and usage. Examination and evaluation of the species' natural regeneration, and recording of the characteristics of surrounding environment in the field, was made through careful observation.

Collection of study specimens

Collection of plant specimens was executed late in December. As most of the fruits at the time of collection were unripe, in contrast to previous reports (Katende et al. 1995, 1999 and Sanogo et al. 2006), focus was initially put on collecting scions. Ten scions were picked from branches and suckers of each individual tree. At each site A, B, C, D on the map (**Appendix I**) was represented by 10 trees selected randomly to provide 400 specimens. Immediately after collecting and labelling the scions according to their individual trees and sites, they were kept intact in coolers which contained ice bricks, and nitrogen gas to deter withering and elongate at 5 – 10°C following the procedures developed by Pritchard HW (2004) procedures.

At site B (Bitabago area), two trees were found bearing ripe fruits. A total 140 ripe fruits were collected and kept separately in cotton cloth bags, correlating to their respective sources for further study. The collected ripe fruits were taken to the tissue culture laboratory for treatment and seed germination tests.

The process of field data capture involved coding the geographical coordinates using the Global Position System (PGS) device, observations, and interview responses. Photographs were taken of study features in context of the species' behaviour in its wild ecosystems, topography and how it regenerates under natural conditions.

Regeneration study

Seeds

Seed pre-treatment procedures were followed in accordance with those developed by Katende et al. (1999) and Sanogo et al. (2006). The collected scions and fruits were brushed and washed with tap water to remove the ripe fleshy fruit (pulp) from the species hard seed coats. Cleansed seeds were later exposed open sunlight for the purpose of drying. The seeds were then immersed in boiling water 100^0 C for 10-15 minutes and later soaked in cold water to cool for one day.

Three treatments were pursued for this study: (a) use of root hormones and with out root hormone and (b) use of pepsin solution to mimic the bio-agents gastric digestion system and (c) direct sowing of seeds in sawdust pots.

Seeds were then sterilised in 96 percent ethanol for 10 minutes, rinsed or put into 1 percent silver nitrate with an extra five drops of Tween 20 for 15 minutes. This was conducted under a laminar air flow hood. The seeds were then rinsed with autoclaved water in accord with procedures developed by Oladimeji Audu and Amupitan (2006), on three separate occasions. Due to the nature of *P. curatellifolia* seeds, nicking or abrading with a handsaw was necessary to break through such hard seed coats, for access to the embryos.

The process as described above was repeated with the second selection of *P. curatellifolia* seeds but with application of stricter sterilisation measures throughout the entire process. Explicitly, seeds were sterilised with 96 percent ethanol, transferred to 1 percent Silver nitrate with 10 drops of Tween 20 and 15 percent Jik for 45 minutes. They were later rinsed into sterilised water three times between (5 – 15) minutes, before embryo extraction using handsaws for mechanical scarification. This involved autoclaving and, or sterilising test tubes, jars, water, hoods, and instruments at every stage of germination procedure. Moreover, every extracted embryo was sterilised with 70 percent ethanol and also rinsed in cold autoclaved water, then inoculated on media for germination. Each of the tools used were also sterilised frequently after every embryo extraction to adequately avoid any mould infection.

Pepsin assay

The pepsin assay was carried out to explore the possibilities of frugivores influencing the germination of the species. Many tropical wild fruit species are often fed on by frugivores as they drop seeds in their faeces after which seed dormancy is broken down allowing for the seeds to germinate. An experiment was therefore conducted to simulate gastric fluid mimicking the conditions of a frugivore's digestion and to find out, if this process would have any influence on seed germination. To this effect, the study used previously published *in vitro* pepsin digestion protocols (Thomas et al. 2004).

Seeds were incubated at 60 ˚C for one day and allowed to cool down for 24 hours. The pepsin assay buffer containing (0.03 ml NaCl at pH 1.2, and HCl) was heated up to 37 ˚C for 10 minutes to activate enzyme activity before introducing the incubated seeds. For enzyme activation, the incubated seeds were immersed into the buffer, heated to 37 ˚C, over a period of four hours to mimic the frugivores' digestion system. An intermediate seed transfer was arranged to a termination buffer (0.2 ml Na_2CO_3) to neutralise the hydrochloric acid for two hours. Subsequently, the same seeds were rinsed into sterilised water for further neutralisation of any hydrochloric acid before seed extraction. The extracted embryos were then inoculated on Embryogenic Cultural Media (ECM), divided into two replicates for darkness and light and transferred to the growth room for further observation of germination. Incubated and non-incubated seeds were both sown directly into sawdust pots for germination following Sanogo et al (2006) procedures and currently await germination.

Cultivation on growth media

Procedures for *in vitro* growing in (table 1) followed those of Murashige and Skoog (1962), McKendrick (2000), Oladimeji Audu and Amupitan (2006) and Stangeland et al. (2007). All media used was autoclaved following Oladimeji Audu and Amupitan (2006) procedures. The second ECM was introduced to enhance the germination process, since the seeds had stayed for long (over three months) at the initial stage. Gibberellic acid was used to boost embryos treated with the naphthalene acetic acid (NAA) rooting hormone, and activated charcoal was also applied to stimulate the entire media for all germination treatments.

First extracted embryos were inoculated in the MS medium without benzylaminopurine (BAP) rooting medium. Embryos in test tubes were then put on the rooting media, divided into two replicates for darkness and light and finally transferred to the growth room. They were checked after seven days in case of germination and no change were noticed at that time, so they were left for one month.

Table 1. Composition of the artificial media used in seed germination tests

First Media	Quantities	Second Media	Quantities
Macronutrients	1.0 ml	Micro elements (Gp A)	1.25 ml
Micronutrients	1.0 ml	Macro element (Gp B)	2.5 ml
MS Vitamins	5 ml	Vitamins (Gp C)	
		Myo-inositol	
Iron	10 ml	Thiamine HCl	2.5 ml
		Supplementary (Gp E)	
Ascorbic Acid	5 ml	Glutamine L	
		Activated Charcoal	1.25 g /l
		Sucrose	5.0 g/l
		Agar	1 g/l
Supplementary (Gp F)		Supplementary (Gp F)	
Sucrose	3.0 g/l	IAA	3.0 mg/l
Gerlite/ phytagel	2.4 g/l	Glutamine L	
		Sucrose	7.5 g/l
pH	5.9	2.4. D, BA, Casein Hydrolysate	
		Gel rite	0 .3125 g/l
		Giberellic Acid	5.0 mg/l
		pH	6.0

Standard MS = (Murashige and Skoog (1962) medium with gibberellic Acid (GA) and indole-3-acetic acid (IAA), pH 6.0

Scions

Collected materials were taken to Entebbe Botanic Gardens (EBG) nursery centre to be planted in sawdust after dipping into Seradix (2) powder rooting hormone. Treated material was transferred to either the green house or tissue culture laboratory conditions for further observation.

Scions were washed in running water mixed with liquid soap Jirecon and later rinsed with sterilised water three times. Fungal detergent 30 percent Bennalite was used for 30 minutes to counteract possible infections. Scions were first rinsed five times with Jik (15 percent) and five drops of Tween 20 for 10 – 15 minutes in the laminar air flow hood, and later with sterilised water for three times. Afterwards, they were dipped into the NAA solution for 10 minutes and then transferred to the media containing BAP in jars. The experiment was divided into two replicates for light and darkness and transferred to the growth room. Temperature ranged between 26-30° C.

Informant study

In addition to field observations for the purpose of determining the species phenological stages and life cycle (flowering, fruiting and harvesting season) the knowledge of local people was also collected and assessed.

Interview process and coded answers

Interviews were conducted using the study questionnaire (**Appendix II**). A household interview in-person was also undertaken to direct unbiased information and avoid delays in posting filled questionnaires. The in-person interviews were focussed at generating information on favourable factors for germinating seeds in nature. All interviews with local people were carried out in Rakai district, South Uganda.

While selecting informants, emphasis was put on stewardship (custodianship) and acquaintance with the species. Particularly criteria of the selection process involved as follows:

- Age: ranged from 18 to 60+ years of the interviewee but most important was the respondent's logical responses, judged and assessed by the researcher guided by the questionnaire.
- Stewardship (custodianship): local people known to be citizens of the study area and deemed knowledgeable on the community's geographical and social political information and its neighbours. The target responses where those who having lived in the area fro more than 10 years and knowledgeable of the species' potential growth sites, favourable eco- geographical and regeneration conditions were deemed important.
- Acquaintance: in familiarising with the species for instance understanding its phenological stages and usages (how and when it growth, flowers, fruits, and harvest time and associated usages and purposes) in the mind of interviewee and his/ her neighbours. Information obtained from respondents was revalidated at recurrent visits. The interviewee offered their local knowledge in a manner they felt and acknowledged as interactive, about the species' behaviour in nature and its propagation and, or regeneration methods.

Data analysis

Data was captured during the interviews and coded according to the questionnaire for descriptive statistical analysis. The analysis of both field and laboratory data was initially intended to involve Chi^2 tests and/or other nonparametric statistical tests. However, due to, firstly, the relatively limited number of informants interviewed in the field study and, secondly, to the unforeseen obstacles encountered in the laboratory experiments, a conclusive statistical analysis did not seem meaningful. Therefore, data are only descriptively presented in the following Results section.

Results

Regeneration in nature

During the 2008 season, *P. curatellifolia* fruit development and harvest delayed. This resulted into limited number of ripe fruits collected late December 2008. In turn this impeded subsequent and sufficient experimentation in the laboratory, limiting the number of replicates that could be carried out.

Regeneration in nature (in-situ)

In the surveyed study sites, the species was often found reproducing through vegetative propagules (orthotropic growth), assuming the upright or normal tree growth from parental trees' root suckers as shown (figure 2).

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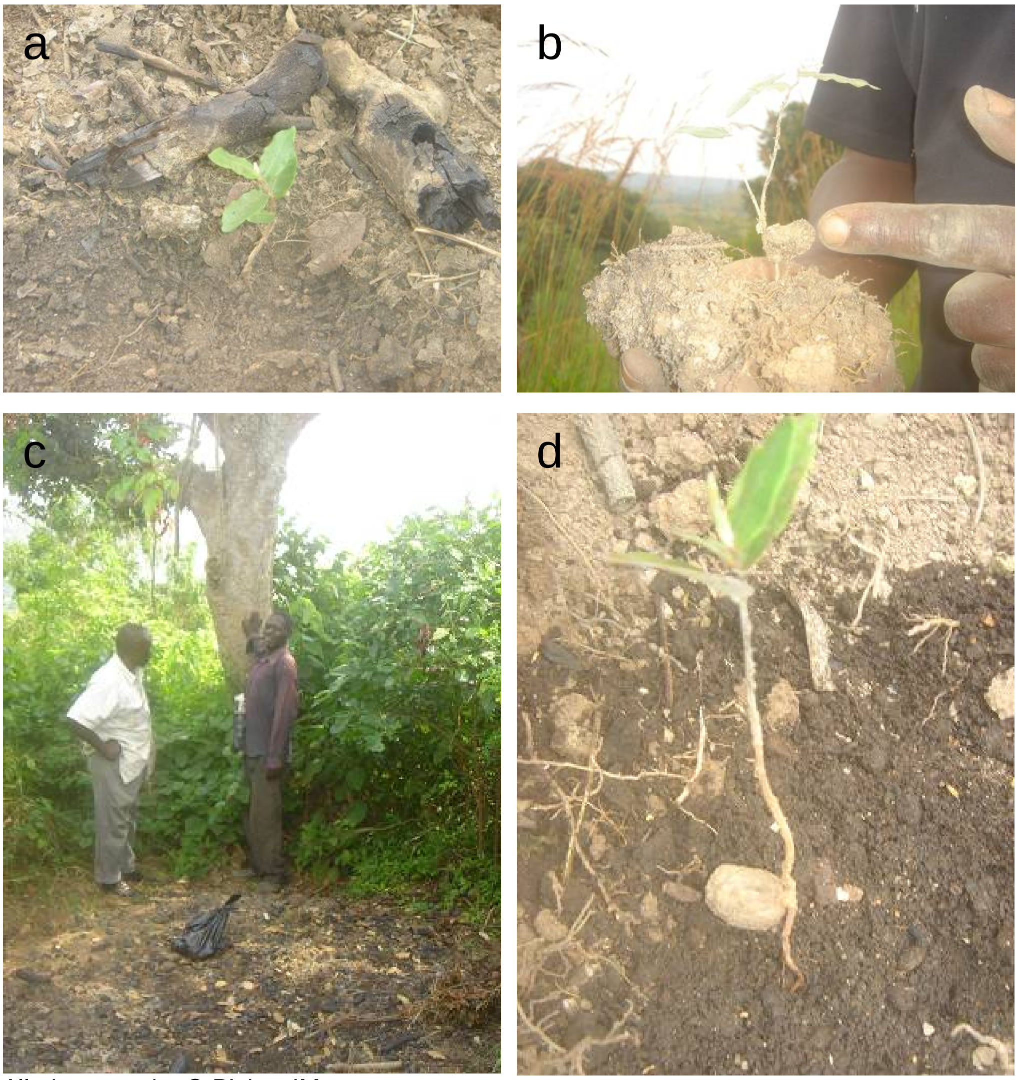
Figure 3 a-d. Detailed illustration of vegetative reproduction and growth (orthotropic growth) in site A.

Interesting field study findings

During the collecting of study specimens (ripe fruits and scions) at site B, Bitabago area, *P. curatellifolia* seedlings were found beneath a tree's shade. The surrounding environment demonstrated clearly a previous charcoal processing activity. This may suggest that to germinate seeds may require fire or heating to break the seed dormancy. Unfortunately, however, the limited time assigned for the project did not allow for any experimentation involving fire or high temperatures.



All photographs © RichardM

Figure 4 a-d. *P. curatellifolia* seedlings were found in a charcoal field made underneath its tree shade at site B, Bitabago area.

Regeneration in the laboratory (ex-situ)

Most of the fruits that were collected from the field contained embryos and endosperms that were filled up, looking viable for germination. The experiments involving sexual regeneration (seed germination) attempts, however, did not yield positive results even after six months. The tables below present summaries of the ongoing regeneration experiments and figures shows a snap shot of seed pre-treatment.

Phase I

There were no results achieved due to severe mould infection as shown in (table 1), with the exception of four embryos that were rescued and re-sterilised in 70 percent ethanol and again put on the rooting media. The four rescued embryos where nevertheless re-infected by mould infection and later discarded.

Table 2. Results from first regeneration trials which were later affected by mould infections

	Treatment (s)	Darkness	Light	Survival rate	Germination
Embryos pressed in tubes	NAA	12	7	0	0
	Control	8	12	0	0
Scions pressed in jars	NAA	32	40	0	0
	Control	32	44	0	0

Phase II

The second experiment was closed on 17 February 2009, and all the treated embryos were still alive and at initial stage (table 2).

Table 3. Results of the second treatment following the first treated embryos after mould infection.

Treatment (s)	Darkness	Light	Survival rate	Germination
NAA	6	6	x	0
Pepsin assay	6	8	x	0
Control	8	12	x	0

X = still surviving

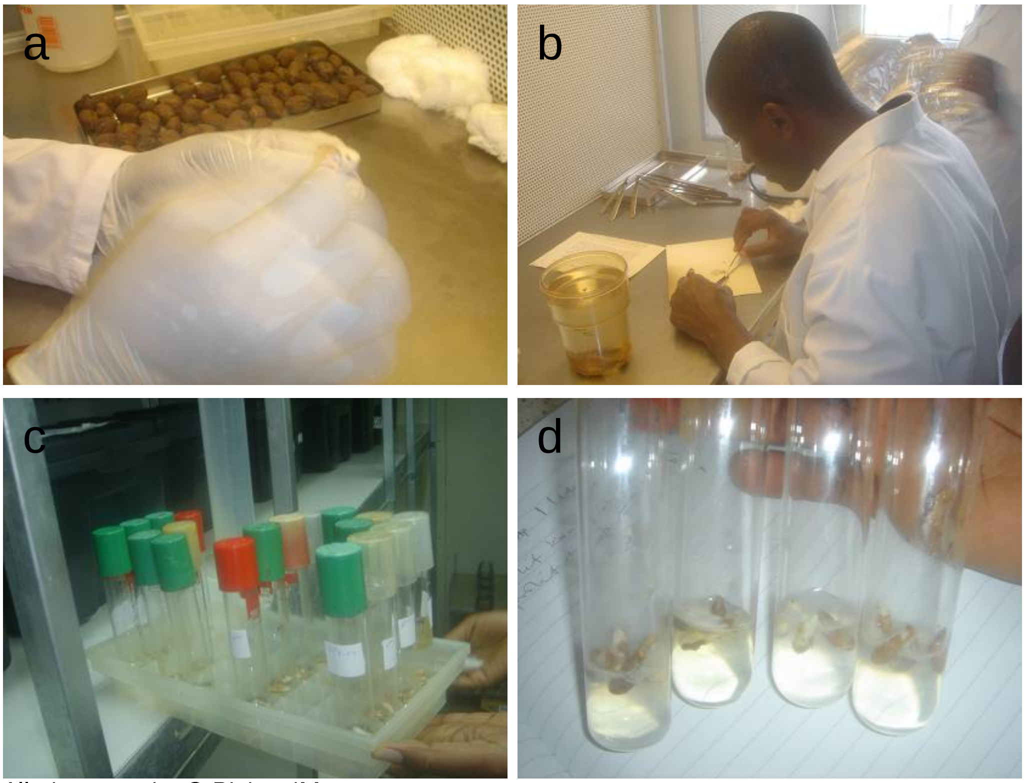


Figure 5 a-d. First set of *P. curatellifolia* seeds prepared for germination tests and later attacked by mould infections.

Phase III

In this experiment the setup was similar to the previous one. The seeds (embryos) moved to new medium survived but had not germinated by the time of the last reading on 16th March 2009. The final conclusions were made on 28th April 2009; by this time all seeds had expanded as a sign of survival and physiological activity (see figure 8) but without showing clear signs of germination such as root development. Table 4 (below) shows the status of the experiments and treatments at the end of the experiment.

Table 4. Results from embryo treatment using upgraded media and gibberellic acid.

Treatment (s)	Darkness	Light	Survival rate	Germination
Gibberellic acid	6	6	x	0
Pepsin assay	6	8	x	0
Control	8	12	x	0

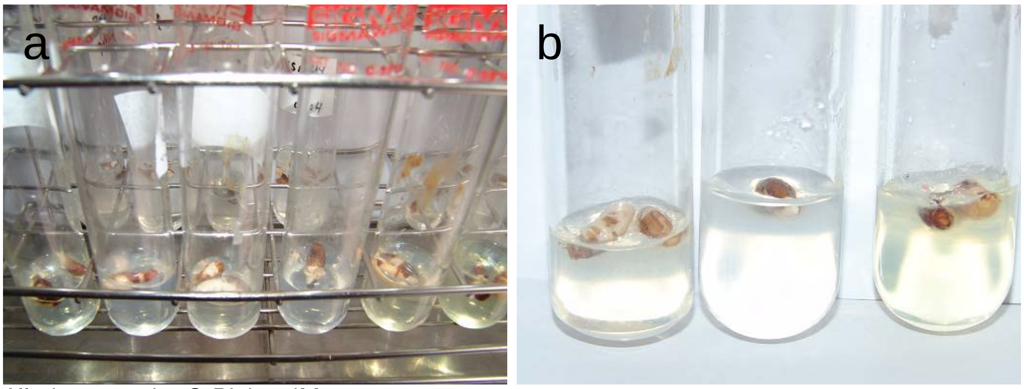


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Figure 6 a-b. Close-up of P. curatellifolia seed germination trials on upgraded media and use of gibberellic acid and pepsin assay solution at Kawanda tissue culture laboratory centre.

Vegetative (cuttings or scions) regeneration

Attempts were also made to regenerate the species vegetatively placing scions on both media with or without rooting hormone under laboratory conditions. However, due to mould infections (figure 8) all transferred scions on media trials were attacked and died. As the scions that had been planted in sawdust pots also dried off, all attempts to regenerate *P. curatellifolia* vegetatively were unsuccessful.

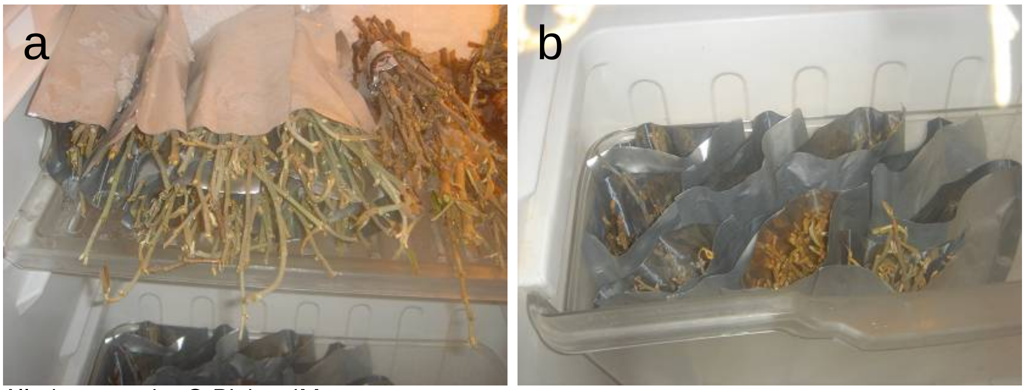

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Figure 7 a-b. Cuttings (scions) stored in coolers and transported from the field to the laboratory for regeneration trials/tests.

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Figure 8 a-b. Scions stored in fridges at the laboratory before regeneration trials.

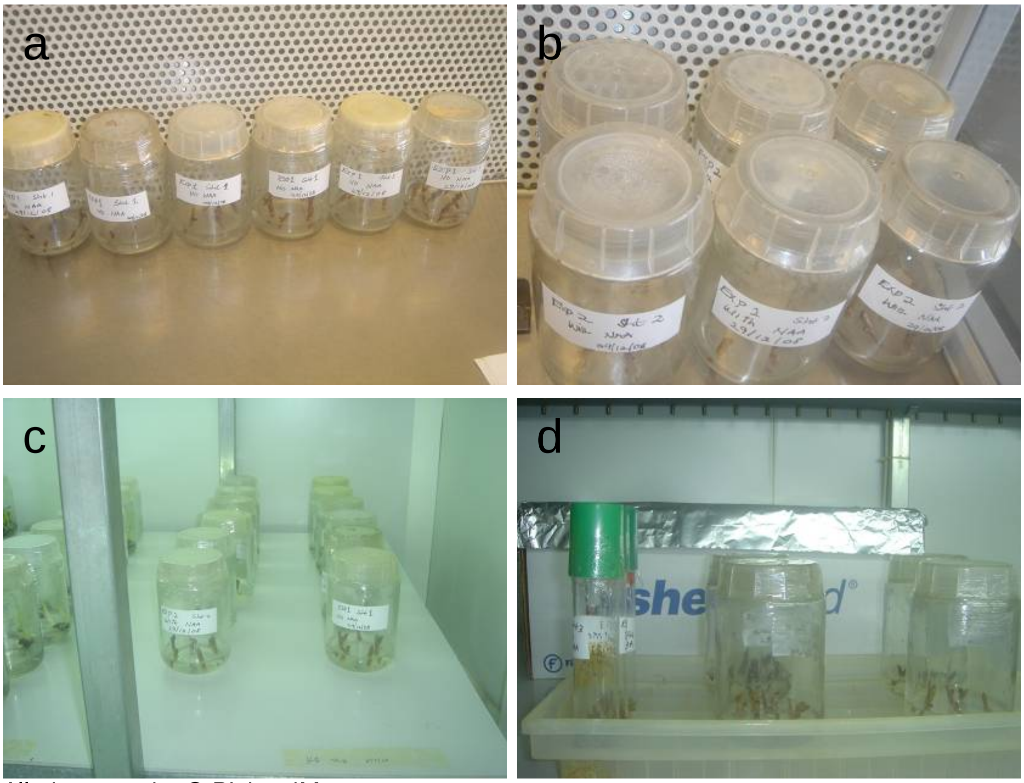

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Figure 9 a-d. Scions tested with and without NAA rooting hormone in laboratory.

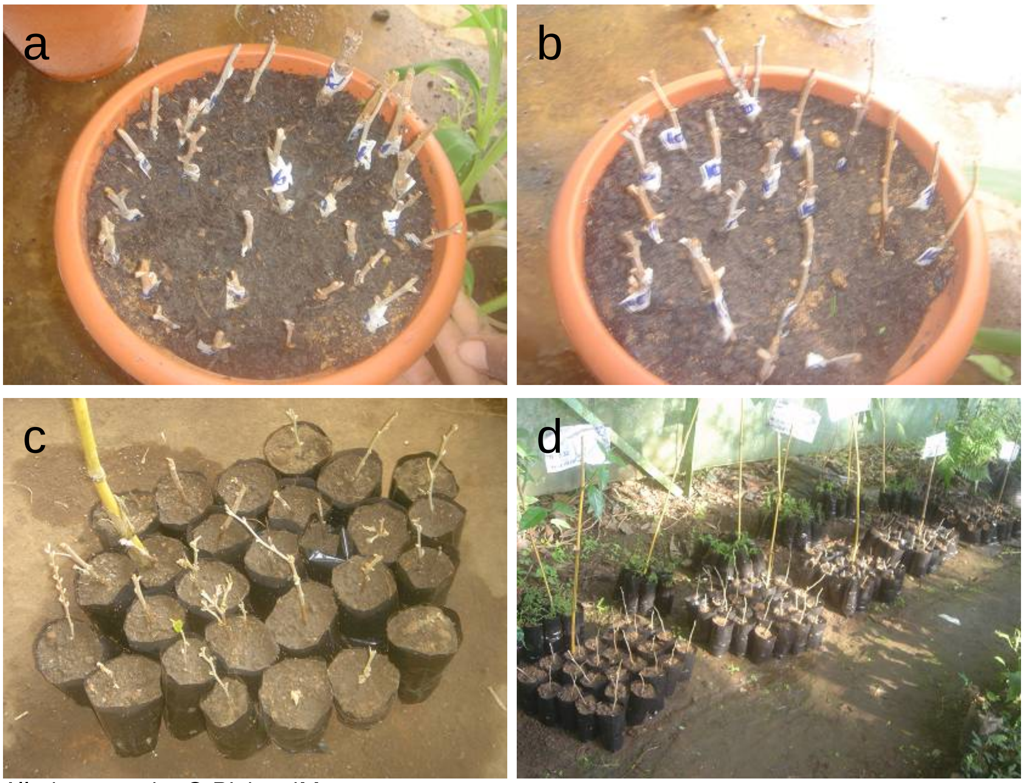

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Figure 10 a-d. Scions sowed in sawdust pots placed in green house tree nursery centre at Entebbe Botanic Gardens.

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Figure 11 a-b. Mould infections to the first set of seeds and scion tested for germination under laboratory conditions.

Field interview responses and observations

P. curatellifolia grows naturally on its own without human intervention. In the surveyed areas it was found to grow on hills with dry soils and rocky surfaces. As previously reported, no observations were made of this species growing in valleys or water catchments areas.

Informant responses

The eleven informants interviewed consisted of both men and women, and mostly household heads. Some were more familiar with the species; their knowledge pertaining to its natural regeneration, use values and phenological development (**see** table 5 and figure 13). Of the eleven informants interviewed, the men seemed to have more contact with *P. curatellifolia* conceivably due to their activities among charcoal processing and rearing of animal herds (cattle and sheep) in the wild, as well as hunting.

Table 5. Informants' response on *P. curatellifolia* regeneration in nature, and its use values.

Gender; M= Male, F= Female	M	M	F	M	F	F	M	F	M	M	M
Propagation											
Seed						X	X				X
Vegetative	X	X	X	X	X	X	X	X	X	X	X
Fruit Harvest time											
November											
December		X			X		X		X		
January	X	X	X	X	X	X	X	X	X	X	X
February	X		X	X	X	X	X	X	X	X	X
March							X				
Topography (soil types)											
Dry soils	X	X	X	X		X	X		X	X	X
Stony, or rocky	X	X	X	X	X	X	X	X		v	
Coarse	X					X					
Type of Vegetation											
Savannah grassland	X			X		X		X	X		
Shrubs			X		X		X			X	X
Thickets			X		X		X				X
Bush		X			X						
Local knowledge (use values)											
Fruits	X	X	X	X	X	X	X	X	X	X	X
Medicine	X		X		X		X				
Oil							X				
Fire wood					X	X	X	X	X	X	X
Charcoal		X		X	X	X	X	X	X	X	
Fodder (feeds)						X	X	X		X	X
Pools					X		X	X	X	X	

Species use values

Generally, informants consented that *P. curatellifolia* is a multipurpose tree and resourceful for their livelihood. They confirmed the many uses including, but not limited to, charcoal processing, fire wood collection, food from ripe edible fruits, animal fodder (feed) and medicinal herbs. These human demands were observed to have exerted massive debarking (figure 10) and undue tree cut down (felling) for charcoal processing and provision of construction materials. Ten out of eleven people interviewed unanimously agreed that the species is multipurpose and a major resource in their area. They said it is a drought and fire resistant tree which can survive under harsh (dry) conditions. Five of the local informants ascertained that ripe fruits can be a potential for processing juice and other flavoursome beverages.

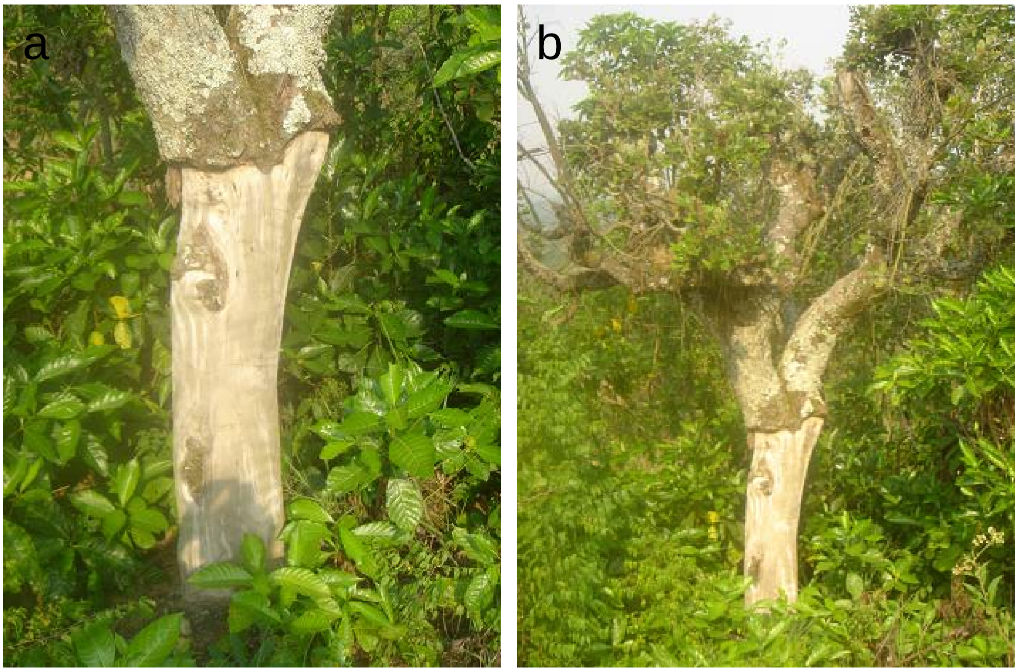

Figure 12. Debarking of *P. curatellifolia* tree at site D, Kanoni area to harvest medicinal herbs. Such trees would inevitably die from water deficit.
All photographs © RichardM

Species natural regeneration

Three respondents out of eleven said that the species can germinate from seed, but it is very rare to find in nature. They also said that it is difficult to know the exact time the seeds need to germinate, or grow into mature trees. One respondent said that the fruit harvesting season depends on previous rainy seasons. During dry seasons the species' trees take longer periods to flower, resulting in delayed fruit harvesting periods. A majority of the respondents maintained that they often saw suckers emerging from exposed roots on or near the soil surface. Two elderly men interviewed said that those trees grown from seeds are normally taller than those which grow from root suckers. Normally *P. curatellifolia* trees sprouting from root suckers grow in shrub-like shapes and are rarely found to grow upright.

Figure 13. Some of the respondents interviewed during the field research study in Rakai district, Lwanda sub country SW Uganda.
All photographs © RichardM

Own field observations

The species was found sparsely populated in Rakai district, South West Uganda. There were many sites found for charcoal processing at the time of specimen collection. The informants had scarce information on the species seed propagation. According to the respondents, the species regenerates commonly through root suckers. The phenological stages involving flowering to fruiting is estimated for about two to three months (July – October) while flowering to harvest falls between (December – February) according to the field study findings. The area inventoried was characterized as dry with seasonal water ponds where herdsmen bring their animal herds to get water.

Discussion

P. curatellifolia in the field

The literature review on *P. curatellifolia* distribution in Uganda made prior to the field survey suggested the inventory to be made in central and south western (Mpigi, Masaka, Mabarara and Rakai). Rakai district, however, was only site where *P. curatellifolia* was found to be still in existence, although with signs of over-utilisation. Coincidently, this demonstrate similar challenges mentioned in Tabuti et al. (2003) report on over- utilized medicinal plants in Bulamogi in Uganda and Akinnifesi et al. (2007) report on domestication and commercialization of indigenous fruits for food security in Zomba, Malawi.

Obviously, the findings in this project on *P. curatellifolia* can not adequately be representative enough for the entire country due to the limited available scientific literature on *P. curatellifolia* in Uganda as well as study time to inventory other areas of the country. However, the study underlines the urgent need to make a general and validated survey on the status of *P. curatellifolia* in the country. The assessment study will enhance conservation and management planning efforts. Thereby to regenerate the species' and address the severe threats of charcoal process, fire wood collection and tree debarking for herbal medicinal use.

Based on field observations, P. curatellifolia grows on rough topography with stony, rocky, laterite dry soils fond atop hills. The eco-geographic conditions in Rakai district were generally characterised by laterite, dry, rocky coarse soils which are conducive for *P. curatellifolia's* growth. The species was not found growing in water catchments such as valleys, river banks, wetland or swamps in Rakai district, contrary to Katende et al. (1995, 1999) suggests that *P. curatellifolia* grows near riverbanks. However this could also depend on the variations within P. curatellifolia species or species eco- geographical preferences in the different ecosystems.

Observations noted that in nature the species commonly regenerates through vegetation propagation (root suckers), though rarely fond regenerating sexually (seed) and often grows into upright trees and shrubs. However, *P. curatellifolia* seedlings were also found sprouting underneath a parental tree at site B, (Batabago) area Lwanda sub country in Rakai district. The seedlings were emerging from a previously abandoned charcoal field and the surrounding environment indicated signs of previous bush fires which might have occurred a year earlier. Possible linkages between seed germination and fire are discussed further in chapter 4.3 Stratification techniques. This proves that *P. curatellifolia* seeds can germinate in nature under certain environmental conditions. The challenge now remains for the scientific community to understand the mechanisms and develop regeneration protocols accordingly.

Many *P. curatellifolia* trees were found to be fire resistant with signs of several previous bush bushfires. Possibly, these fires were lit or set by local residents and herdsmen mainly to stimulate growth of new savannah grass to graze their animals. The area is gradually and, or increasing gaining settlers who deforest trees for agriculture expansion and settlement. Charcoal business is envisaged as the source of household income by residents. Several literatures (Katende et al. 1995, 1999; Gomez 1988; Kayanja and Byarugaba 2001; Tabuti 2007) demonstrate related conflicts between charcoal/ firewood production and conservation of biological resources.

Kayanja and Byarugaba **(**2001) demonstrate that fuel wood is the main source of energy in particularly rural and among the poor.

- "Extraction and supply of fuel wood is an important source of income and employment.
- Charcoal and fire wood are used in urban households, and in many institutions and commerce (restaurants, hotels and bakeries).
- Wood is widely used in many industrial processes: brick and tile making, lime production, tea and tobacco curing, and the baking and food processing industries".

To safeguard extinction of threatened species, biodiversity losses and environmental services, there is need to front use of effective participatory approaches in conservation and management practices including local community and farmers' engagement.

The in-person interviews generated useful information but mostly relating to the use values of *P. curatellifolia.* All interviewees agreed that the species' optimal harvest time falls between January and February. They said the species often regenerates via root suckers and it's a multipurpose wild plant to the entire local community. Thereon consented that the ripe fruits serve as food and its outer fleshy part tastes sweet and nutritious. Interviewees disclosed to the researcher that younger people and herdsmen, enjoy eating these ripe fruits while raring domestic animals in the wilderness.

All eleven interviewee consented that they grew up eating the species' ripe fruits. The ripe fruits were thus a source of food to people and the potential to process the ripe fruits into marketable products such as juice, alcohol, wine and other beverages was also stressed. None of the interviewee ever saw anyone cultivating *P. curatellifolia* trees on-farm in their local area. One interviewee related the species to their daily life saying that, fruit harvesting begins from December – February which coincides with grasshopper season one of the popular and delicious insects eaten by people in Uganda. During the species' flowering season (August- October) plenty of blossoms attract many bees which can be a potential for introducing bee hive activities. This could provide an alternative household income and divert people's attention from cutting down *P. curatellifolia* tree to process charcoal for sale. To conclude, therefore, *P. curatellifolia* was identified as a resourceful tree species to the local community in many ways, confirming previous reports (Katende et al. 1995, 1999; Kalenga et al. 1994; Mathurin (1997; Nyoka and Rukuni 2000;Sanogo et al. 2006; Akinnifesi et al. 2002, 2004, 2006, 2007; Lejju et al. 2001).

Seven interviewees said charcoal made out of *P. curatellifolia* lasts longer due to its hardwood and therefore gives higher income. One of the interviewee disclosed that the cost of *P. curatellifolia* charcoal sack range between (15000 - 20000/= Uganda Shillings (USH) compared to charcoal from other tree species costing between (4000 – 10000/=USH). This relates to the increasing prices of firewood in urban areas where a piece of wood or pole costs (1000 – 2000/=USH) and fuel from car petrol stations a litter of gasoline costs (3500 – 5000/=USH) per litter. Present foreign exchange rate into USD (1$ = 2000 USH).

Other interviewees deliberated on its use of medicine which is a major threat to the species' survival. *P. curatellifolia* tree debarking is gaining ground since local people believe that its herbal medicine treats sorts of ailment and purported some HIV/AIDS symptoms such as diarrhoea, cough, fever, and wounds among others. Interviewees said that herbal medicines are prepared in different forms; powered leaves, bulk and, or pounded tree bark and root particles, decoctions, saps, boiled liquid concentrations, and ash. The recipe doses also vary according to the person giving prescription to a particular ailment.

Tabuti et al. (2003) reports that medicinal plants are mainly collected from the wild, experience unsustainable intensities of use and patterns of harvesting. Particularly the vulnerable are the wood or slow growing trees. Local environmentalists and farmers in Europe have begun to realize that, many species are declining as a result of 'wild harvest' for medicinal purposes. Many wild herbs used for pharmaceutical purposes are being overexploited to the verge of extinction in SE Europe. "Rarer plants such as yellow gentian have become virtually extinct and even common plants have become difficult to find" (see. http://www.transrural.org/balkans-herbtrade.htm). Therefore as time goes by without counteracting efforts to restore, re afforest and regenerate materials of threatened indigenous wild species, we are bound to face difficulties to sustain our lives in turns of food, and deny ourselves to enjoy environment/ ecosystem services

With respect to its regeneration, only three interviewees agreed to have been *P. curatellifolia* seeds germinating in the field. One informant furthermore claimed that "seedlings bear upright tree trunks unlike those regenerated from root suckers". I found the response interesting and true because seedlings penetrate deep into the soil with their tap roots which give them a stable position to sway and also strive for resources (light, water, and soil nutrients) without relying on their parental plants.

P. curatellifolia in the lab

Regrettably, no germination results were obtained from all the attempted experiments within the six months of study time. There are several possible explanations for this: that the seed were too immature to germinate, that damages may have been inflicted on seeds during mechanical scarification by use of hand saws to break the hard seed coats or due to seed dormancy complexities. Only ripe fruits were collected and care was taken during scarification to lessen seed damage. The third, and perhaps most likely explanation, that *P. curatellifolia* seeds show strong intrinsic dormancy was not studied in detail during the given project time, but will be discussed in the following.

There are other reports in the literature that *P. curatellifolia* displays poor germination tendencies. Mathurin et al. (1997), for example, also reported difficulties in germinating *P. curatellifolia* seeds (tables 6 and 7) while conducting seed desiccation experiments of indigenous fruit species (*Kigelia africana* (Lam.) Benth, *Lophira lanceolata* Mathurin et al. (1997), *Parinari curatellifolia* Planch. ex Benth, and *Zanthoxylum zanthoxyloides* (Lam.) in Burkina Faso. Regardless of varying moisture contents of the seed, no positive germination results were obtained for *P. curatellifolia*. Mathurin et al. (1997), suggested reasons for this included the possibility of using immature seeds or seed dormancy factors.

Table 6. Intrinsic characteristics of fruits and seeds of four fruit species native to

Burkina Faso.

	K. africana	*L. lanceolata*	*P. curatellifolia*	*Z. zanthoxyloïdes*
Collection	23/08/2000	20/03/2001	5/11/ 2001	13/12/2000
Fruit harvested (kg)	236.125	95.3	44.21	27.65
Seed prepared (kg)	3.721	51.7	6.49	6.19
Initial MC (%)	26.98	21.30	17.49	13.85
Germination (%)	71	49	0	2

Source: Mathurin et al. *(*1997).

Table 7. Percent germination (G%) after drying seeds of four fruit species to different moisture contents (MC%).

K. africana		*L. lanceolata*		*P. curatellifolia*		*Z. zanthoxyloïdes*	
MC (%)	G (%)	MC (%)	G (%)	MC (%)	G (%)	MC (%)	G (%)
6.63	72	3	0	2.18	0	3	2
8.95	78	6	4	3.63	0	6	0
11.9	66	9	2	7.11	0	9	3
14.8	72	12	13	16.3	0	14	2
20	74	15	22				
25	68	20	47				

Source: Mathurin et al. *(*1997).

Frugivores are fruit eaters such as animals, reptiles and birds. It has been suggested by several authors (for instance; Barnea 1991, Fredrickson 1997, Jones and Landon 2002) that digestive (gastric) system of frugivores may enhance seed germination, thus overcoming seed physical dormancy in some species. Trucker (1964) and Van de Pijl (1972) in Barnea (1991) also argue that the process of frugivores to consume seed pulps and excrete unharmed seeds is advantageous. Baskin and Baskin (2000) suggest that animal feeding activities may result in holes being cut in impermeable seed coats, thus breaking seeds mechanically. They argue that although the digestive system of animals could be effective in breaking physical seed dormancy, environmental factors eventually result in the loss of physical dormancy. In conclusion, however, the acid gastric fluid probably contributes in breaking down physical dormancy.

It was against this background that the pepsin trial was conducted to mimic the digestive system of fruit eaters on *P. curatellifolia* seeds. Despite subjecting the seeds to a pepsin solution, however, the treatment had no effect on germination. This does not rule out the possibility that *P. curatellifolia* seeds could be affected by gastric enzymes, but more experimentation is needed.

Seed dormancy is a major factor in many plant species (Baskin and Baskin 1998, 2001). Dormancy occurs as a result of deficiency or lack of sufficient water, oxygen, light and, or exposure to temperatures above or below those suitable for plant growth. However, other reasons why seeds do not germinate can be due to intrinsic seed properties. According to Nikolaeva (1969, 1977 in Baskin and Baskin 1998) there are two general types of organic seed dormancy: endogenous and exogenous. In endogenous dormancy some characteristics of the embryo prevents germination whereas in exogenous dormancy characteristics of the seed structures, including endosperm and/or perisperm, seed coats, or fruit walls covering the embryo prevents germination.

Hartmann et al. (2002), on the other hand, use a slightly different terminology for seed and bud dormancy. E*co-, para-* and *endo*-dormancy refer to dormancy factors related to the environment (eco), to physical or biochemical signals originating externally to the affected structure (para), or physiological factors inside the affected structure (endo). Regardless of which, there are reasons to believe that *P. curatellifolia* seeds express several types of dormancy. Sanogo et al. (2006) argue that the seeds show severe dormancy and difficulties in germinating due to seed being a hard stone embedded in a reddish thick fibrous pulp or seed coat (c. 3 mm thick). Even when the seed coat had been excised, as in this study, the free embryo did not germinate within the experimental time of almost six months. It is possible; however, that prolonged experimental time could have yielded positive results.

Baskin and Baskin (2001) stress that to understand seed germination ecology it is important to investigate the characteristics of the germination season. Some species have long germination seasons, and based on the findings in this study *P. curatellifolia* appears to fit this category. As previously stated, however, due to the limited research study time not all aspects of seed germination ecology and physiology were pursued in depth to understand the species' seed dormancy. Sanogo et al. (2006) recommends that sowing *P. curatellifolia* seeds for germination should be given ample time since the species has a poor and prolonged germination process, and Katende et al. (1995, 1999) claim that dried seeds can retain viability for over two years but that seeds can take over six months to germinate.

The ecological advantages of seed dormancy relates to the seed bank in nature where not all seeds of plant species germinate annually or in the same year. Naturally there are plant species with such endowed insurance that flowering and fruiting does not occur regularly. This could be for purposes of safeguarding for any unforeseen environmental disasters. Hartmann et al. (2002) argue that some plant seeds remain dormant in a seed bank for decades which is advantageous to ecological adaptation. In order to understand why seeds of *P. curatellifolia* did not germinate within the study time a thorough investigation should be conducted to analyse and understand the physiological control of dormancy factors. Such a study could very well be extended also to other important members of the family *Chrysobalanaceae*.

P. curatellifolia seed germination received more focus as the main theme of this study, though scions were also collected for vegetative propagation. However, scions were either severely attacked by mould infections or dried off from their sawdust pots and none survived. The reasons why all scions dried off prior to propagation can only be speculated such as inappropriate storage conditions and long periods (3 days) after cutting them off their parental plants. The long distances covered while transporting study specimens (scions) from the field to the laboratory for germination tests could also have resulted into physiological stress. It is also important to adequately identify the suitable parts of *P. curatellifolia* for vegetative propagation considering other studies that recommend the use of root cuttings rather than or stems, twigs or grafting (Akinnifesi et al. 2006). Mhango and Akinnifesi (2001) in Akinnifesi et al. (2007) suggest that grafting is the most efficient way to rapidly achieve improvements in indigenous fruit trees, but claim that this is not successful for *P. curatellifolia* and *Strychnos* species. To conclude, the national genebank at Entebbe Botanical Gardens should also embark on more research activities to ensure successful vegetative regeneration of *P. curatellifolia* in Uganda.

Seed stratification as a means of improving germination

Stratification is a technique by which seeds with dormancy need to go through a duration of cold or warm-temperature treatment. In cold stratification, seeds are placed in a moist pre-chilling media after harvest to mimic their "expected" natural conditions in order to improve germination. Warm stratification is carried out at temperatures between 20 and 30 °C (http://www.ces.ncsu.edu/depts/hort/hil/hil-8704.html). Many trees with hard seeds coats (physical dormancy) often have this kind of seed dormancy and gardeners regularly use this technique. The delayed germination of *P. curatellifolia* seeds could perhaps be linked to the need for a period of stratification.

Baskin (1998, 2001) suggests dry heat as effective in breaking physical dormancy in seeds of a number of tropical species (*Acacia longifolia, Acacia saligna, Rhus javanica, Tephrosia appolina, Pueraria phaseoloides, Sida rhombifolia, Sida sipinosa and Sida veronicifolia)*. The same author argues that the most common method for giving impermeable seeds a dry heat treatment is to place them in an oven at the specified temperature. However, care should be taken to balance the combination of temperature and duration of treatment that results in high germination percentages, but not death, of the embryo. Baskin (1998) further states that "there is a decrease in the length of time required to break physical dormancy with an increase in temperature".

During the field collecting of study specimens, one unique *P. curatellifolia* tree, with seedlings underneath its shade, was found at site B in Bitabago area, SW Uganda. These seedlings were proven to have germinated from the seeds. However, the surroundings had previous signs of bush fire, which could have taken place approximately a year earlier or so. On the basis of this observation, fire should be considered as putative factor for breaking seed dormancy in *P. curatellifolia*. Fire has been ascertained as useful in regenerating other species in their natural ecosystems (Torunn et al. (2007); Hartmann and Kester's (2002); Baskin (1998, 2001; Johnson and Miyanishi (2001) and Walker (1987).

In nature, fires form an integral part of many natural forest ecosystems and they are reported significant in forestry management and succession of new grass or plants species (Keeley 1991). Future research investigations should therefore look closer into the use of fire or high temperatures as a means to elicit *P. curatellifolia* seed germination.

Meanwhile, the national genebank at Entebbe Botanic Gardens should continue to foster alternative regeneration (vegetative) methods to increase the species availability to sustain its utilisation in Uganda with the aim of preventing *P. curatellifolia* diversity loss, genetic erosion or even extinction. The challenge here is also to adequately understand the species' proper regeneration needs and develop methods accordingly (Tabuti 2007; Lejju et al. 2001; Kayanja and Byarugaba 2001).

Conclusion and recommendations

The project has demonstrated the difficulties in propagating *P. curatellifolia* from seed which provides good reasons to suggest alternative propagation methods to safeguard conservation and increase availability of the species in Uganda. This study has also set a precedent for future research in conservation of threatened indigenous fruit species in Uganda. It communicates a message to conservationists and biodiversity managers that threatened wild indigenous fruits and/or under-utilized species can be vital for sustainable food production in our lives.

The study attempted to understand and develop appropriate regeneration methods for the Mobola plum (*P. curatellifolia*). However, germination results were not obtained during its study time, probably because of seed dormancy that could based on both external and internal factors. Or in the words of Keeley (1991): "The conditions necessary for eliminating dormancy may not be conducive to the germination process itself. Therefore a state of dormancy is not always static but some seeds may go through cycles of dormancy and non-dormancy, often induced by environmental conditions. Likewise the conditions suitable for germination may change with time or with changes in the environment. Finally, the requirements for germination or for overcoming dormancy are not necessarily identical for all seeds from the same parent; meaning that there may be genetic or somatic differences".

P. curatellifolia has never been a cultivated crop in Uganda; it grows wildly in its natural ecosystems where it is massively harvested by local people. Efforts geared towards its regeneration should be accorded every exertion and support to succeed. Therefore, an awareness campaign is needed in Uganda to improve its long-term survival. This should include dissemination of low-cost regeneration techniques that local communities could easily apply in an effort of 'participatory regeneration'.

The documentation of peoples' local knowledge is identified as a key to enhance community engagement in conservation and management of wild indigenous fruit species in Uganda. A combination of an integrated natural resource management approach;

- close dialogue with the local communities (stakeholders),
- awareness-raising through educational programmes, and,
- control of anthropogenic pressure on threatened indigenous fruit species, is all envisaged as needed to enhance biodiversity management in Uganda.

Such an approach would complement the role of research and conservation management that both are essential components of the Food Agricultural Organisation (FAO) - Global Plan of Action, including; in-situ conservation and development, ex-situ conservation, utilization of plant genetic resources and building of institutions and capacity.

Finally, the following aspects could be considered for future research or activities:

- regeneration of *P. curatellifolia* using vegetative propagation methods
- development of stratification treatment to improve seed germination
- fire or heating as a means to elicit germination
- study on diversity of *P. curatellifolia* in Uganda to understand variation in regeneration behaviour
- study on chemo-therapeutic compounds in the species in relation to local peoples' knowledge and use, and
- the involvement of local communities into participatory conservation and management of threatened indigenous fruit species to ensure their sustainable utilisation.

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Appendix I

Questionnaire for Research Field Study

Study specie: *Parinari curatellifolia*

Research Lead person (s): ……………………………………..

1. Respondents' No**.**

2. Sex: Male -1 Female- 2 Ethnic:

3. Age: < 18, 18 – 27, 28 – 37, 38 – 47, 48 – 57, 58 >

4. Marital Status: Single – 1, Married – 2, Widowed- 3, Divorced- 4

5. Education: Primary- 1, Secondary- 2, College- 3, University- 4

Eco-geographical studies:

6. Area/ Location/ Site:

District Sub county Parish Village/LC

………………… ………………………..

a) Altitude b) Latitude c) Longitudinal

Climate zone: i) Temperature: ii) rainfall amount:

7. Genus Common name Propagation Procedure

………………………….. ……………………………………..

8. Phenological stage: Species' Harvesting season:

…………………………… ………………………………..

Soil type (s): Vegetation type:

…………………………… …………………………………

9. a) Numbers of sites sampled: …………… b) Number of trees sampled:……

10. Materials collected and their sizes:

a) Shoot tips: (Fill in the attached table for different cut sizes, including branchlets,)

Parinari curatellifolia's tender shoots/ younger twigs and, or mature seeds.

Site A (Popn 1).	**1 - 5**	**5 - 10**	**10 - 15**	**15- 20**
Tree (No. 1)				
Tree (No. 2)				
Tree (No. 3)				
Tree (No. 4)				
Tree (No. 5)				
Tree (No. 6)				
Tree (No. 7)				
Tree (No. 8)				
Tree (No. 9)				
Tree (No. 10)				
Site C (Popn 3).				
Tree (No. 1)				
Tree (No. 2)				
Tree (No. 3)				
Tree (No. 4)				
Tree (No. 5)				
Tree (No. 6)				
Tree (No. 7)				
Tree (No. 8)				
Tree (No. 9)				
Tree (No. 10)				

Site B (Popn 2).	**1 - 5**	**5 - 10**	**10 - 15**	**15- 20**
Tree (No. 1)				
Tree (No. 2)				
Tree (No. 3)				
Tree (No. 4)				
Tree (No. 5)				
Tree (No. 6)				
Tree (No. 7)				
Tree (No. 8)				
Tree (No. 9)				
Tree (No. 10)				
Site D (Popn 4)				
Tree (No. 1)				
Tree (No. 2)				
Tree (No. 3)				
Tree (No. 4)				
Tree (No. 5)				
Tree (No. 6)				
Tree (No. 7)				
Tree (No. 8)				
Tree (No. 9)				
Tree (No. 10)				

Wild/ Local management of the species:

11. How does *P.curatellifolia* grow in its natural environment?
a) Seed germination b) Vegetative propagation

12. If it's by seed germination, how long does it take for the seeds to germinate?

13. Do farmers grow *P.curatellifolia* in their home gardens?
Yes No

14. How do they or you grow the species?

Species' use values:

15. What is the community's attached values to the species?

16. Any other remarks: