M A K E R E R E **U N I V E R S I T Y**

PHENOTYPIC, CHEMICAL AND MOLECULAR CHARACTERISATION OF SHEA TREE (*Vitellaria paradoxa* subspecies *nilotica*) ETHNO-VARIETIES IN UGANDA



BY

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JULY 2012

DECLARATION

This thesis is my original work and has not been submitted for any award from any other university.

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DEDICATION

This thesis is dedicated to:

My mother: Mrs. Irene Naikesa Gwali (RIP)

My father: Mr. Stephen Gwali (RIP)

And, my brothers: Mr. Jonathan Kintu (RIP) and Mr. Gideon Anthony Kintu

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LIST OF ABBREVIATIONS

AMOVA:	Analysis of Molecular Variance
ANOVA:	Analysis of Variance
ASE:	Automatic Solvent Extraction
CIRAD:	Centre de Coopération Internationale en Recherche Agronomique pour le Développement
CV:	Coefficient of Variation
DBH:	Diameter at Breast Height
DNA:	Deoxyribonucleic Acid
HCA:	Hierarchical Cluster Analysis
IDP:	Internally Displaced Persons
INNOVKAR:	Innovative Tools and Techniques for Sustainable Use of the Shea Tree in Sudano-Sahelian zone
INRA:	Institut National de la Recherche Agronomique
MANOVA:	Multivariate Analysis of Variance
NaFORRI:	National Forestry Resources Research Institute
NARO:	National Agriculture Research Organisation
NBSAP:	National Biodiversity Strategy and Action Plan
NEMA:	National Environment Management Authority
NIRS:	Near Infrared Spectroscopy
PCA:	Principal Component Analysis
PCR:	Polymerase Chain Reaction
PIC:	Polymorphic Information Content
PRA:	Participatory Rural Appraisal
RAPD:	Randomly Amplified Polymorphic DNA
SSRs:	Single Sequence Repeats
UBOS:	Uganda Bureau of Statistics
UMR:	Unité de Mixte de Recherche
UNCCD:	United Nations Convention to Combat Desertification

SUMMARY

The shea tree (*Vitellaria paradoxa* C. Gaertn.) is an indigenous tree species that is widely distributed in the Sudano-Sahelian belt of Africa. In Uganda, it grows naturally in the savannah parklands of northern and eastern parts of the country. It is widely known for its oil which is produced from its kernels and used in cooking, cosmetics, traditional medicine and cultural ceremonies. Due to its socioeconomic and cultural importance, a folk classification has evolved among the local communities in the shea tree parklands of Uganda. Folk classifications have been extremely useful in many crop improvement programmes. In Uganda, folk classification recognises the presence of shea tree ethno-varieties on the basis of fruit and nut differences. However, these ethno-varieties are faced with extinction due to extraction pressures (for the provision of charcoal, poles and firewood) and neglect due to lack of a shea tree improvement programme. This study was conducted in three farming systems (northern, Teso and West Nile) of the shea tree belt of Uganda to document the folk classification and characterise local ethno-varieties as a basis for conservation and future improvement/breeding programmes. The objectives were 1) to document folk classification and management of shea tree ethno-varieties in Uganda based on farmers' criteria; 2) examine the morphological variation within and among the different shea tree ethno-varieties; 3) analyse the fat content and fatty acid composition within and among these ethno-varieties; and 4) assess the genetic diversity and molecular variation within and among the different shea tree ethno-varieties. Folk classification was investigated using participatory rural appraisal (PRA) tools, morphological variation was assessed using morphometric measurements while chemical analysis was conducted by Near Infrared Spectroscopy (NIRS) and conventional wet chemistry (soxtec petroleum – ether and gas chromatography). Nuclear simple sequence repeats (SSRs) were used to determine the molecular variation among the ethno-varieties. The results show 36 different uses of shea trees and their products, 24 of them based on shea oil, making it the most frequently used product. The local communities manage shea trees through practices such as weeding, pruning and on-farm retention. Traditional conservation practices are influenced by belief systems involving taboos and rituals. A total of 44 ethno-varieties based on single fruit and nut morphological and organoleptic traits were documented across the three farming systems in the shea parklands of Uganda. Quantitative morphometric data showed no clear aggregation that is congruent to folk classification indicating that the ethno-varieties cannot be adequately distinguished on the basis of morphological traits. Morphological traits such as pulp weight, stem diameter,

fruit weight and canopy diameter were highly variable between individual trees suggesting that the environment may influence morphology to a large extent. With regard to fat content, and fatty acid composition, all ethno-varieties were dominated by oleic and stearic acids. Other fatty acids include palmitic, vaccenic, linoleic, linolenic and arachidic acids. Pairwise comparisons did not find any significant variation in fatty acid composition between ethno-varieties. Shea tree ethno-varieties can therefore not be distinguished on the basis of fat content and fatty acid composition. In terms of molecular variation, most variation (86.90%) occurred within individual trees while 8.43% was found among individual trees within ethno-variety groupings and 4.67% was found among ethno-variety groupings. All shea tree ethno-variety groupings constitute a single out-crossing population with very low genetic differentiation and high gene flow. In conclusion, this study shows that the ethno-varieties as perceived by farmers in the shea parklands of Uganda are arbitrarily defined sub-groups of a single randomly mating population.

ORGANISATION OF THE THESIS

This thesis is structured into eight chapters which are a collection of published and/or unpublished papers. Except for **Chapter One**, the presentation of the subsequent chaptersfollows the style of the journals in which they are either published, submitted or in preparation for publication. **Chapter One** is written in the style of the 6th edition of the American Psychological Association of (APA) and gives a general background of the study species, *Vitellaria paradoxa* C. F. Gaertn. (the shea butter tree), and provides a detailed description of *Vitellaria paradoxa* C.F. Gaertn. subsp. *nilotica* (Kotschy) A.N. Henry & Chithra & N.C. Nair which occurs in the shea tree parklands of Uganda. The importance of this tree to the people of Uganda's shea tree parklands are given as well as its folk taxonomy. This eventually leads to the enunciation of the study problem followed by the aims and objectives, hypotheses, justification, significance and conceptual or theoretical framework of the study.

Chapter Two provides an assessment of the traditional management and conservation practices of shea trees in Uganda. Local uses of shea trees are given and categorized on the basis of shea tree products while differences in traditional conservation practices between the different ethnic groups and farming systems of the shea belt are outlined. The chapter recognizes the important role of rituals, taboos and other prohibitions in the conservation of shea trees. A conservation framework that recognizes the importance of traditional practices as well as contemporary practices is developed in an attempt to put the traditional management in context.

Chapter Three explores the folk knowledge which is the basis for shea tree folk classification in Uganda. Folk nomenclature and levels of classification are assessed in conjunction with the classification criteria and traits. The relationship between folk classification knowledge and education levels, ethnicity, gender and age of respondents is explored. The socio-economic importance of shea oil is given and its lack of utilization in shea tree classification and ethno-variety nomenclature is given. A detailed analysis of the factors that influence the nomenclature of shea tree ethno-varieties is presented as well as comparisons with folk classification systems of related crops such as potatoes.

Phenotypic variation of shea tree ethno-varieties is assessed in **Chapter Four.** Using the morphological as well as organoleptic traits used in folk classification, the pattern of variation and its congruence with folk classification are assessed.

In **Chapter Five**, the importance of fat content as well as fatty acid composition in nutritional quality of shea nuts is recognized. A descriptive account of the fat content as well as fatty acid composition of shea oil from different ethno-varieties is presented. In addition, the variation of fat content as well as fatty acid composition is investigated. The importance of local site, climatic and tree-to-tree differences in fat content as well as fatty acid composition is expounded, based on the results obtained. Recommendations on the potential commercial value of shea oil from particular ethno-varieties are given.

Chapter Six investigates the genetic variation within and between shea tree ethno-varieties and compares this with folk classification in order to establish their congruence. The hypothesis under investigation here is that, while farmers report the presence of shea tree folk ethno-varieties, these are arbitrarily defined sub-groups of a single randomly mating population. The observable variation is mainly phenotypic, continuous and plastic as a cause of micro-environmental factors and hence does not account for significant difference between ethno-varieties.

In **Chapter Seven**, a general synthesis is presented, detailing the findings and implications of those findings in line with the objectives and major aspects of the study. The synthesis is followed by a series of conclusions and recommendations arising from the study findings (**Chapter Eight**).

CHAPTER ONE

INTRODUCTION

1.1 Background

Traditional agricultural systems based on farmers' knowledge have fostered the maintenance of genetic diversity over millennia. A diversity of crop species have been utilised in traditional agriculture in the form of local landraces or folk varieties. These have subsequently been the basis for the breeding of high yielding commercial crop varieties (Harlan, 1992). Although these folk varieties are extremely valuable genetic stocks, they are faced by the threat of extinction due to change of focus to the much touted high yielding varieties and genetically modified crops. Loss of these folk varieties will not only impact on valuable sources of genes for crop improvement programmes, but they will also impact on the resilience of the human populations through loss of traditional knowledge.

In Uganda, several trees are utilised to provide the fruit requirements of local populations. While many fruit trees such as citrus, mangoes, avocado, etc are domesticated and are cultivated in farms, many are actually wild or semi-domesticated. During periods of drought or famine, the wild or semi-domesticated fruit trees provide useful fall back options to the food and fruit requirements of the human populations. In the present study, the traditional knowledge of one such wild or semi-domesticated trees species, *Vitellaria paradoxa,* is examined using modern phenotypic and molecular analytical tools in an effort to improve our understanding of the useful variation in the species and set the ground for possible domestication.

1.2 The study species

1.2.1 Ecology

Vitellaria paradoxa C. F. Gaertn. (also widely known as the shea butter tree or simply shea tree in English and karité in French) is a small to medium-sized deciduous tree that grows up to 20 m in height (Hemsley, 1968). It has been noted to take on different tree forms (Boffa, 1995; Hall, Aebischer, Tomlison, Osei-Amaning, & Hindle, 1996; Ruyssen, 1957). There is wide variability in tree form, ranging from short twisted, multiple stems to tall, straight, un-branched boles. The shea tree has therefore been described variously as a small to medium sized deciduous tree, 10 – 15 m high; as a 3 m shrub – like tree; as a high canopy tree of 30

m. The diameter of mature tree crowns has been recorded to range between 30-60 cm, with figures as low as 7 cm and as high as 200 cm (Boffa, Yaméogo, Nikiéma, & Knudson, 1996; Fontaine, Lovett, Sanou, Maley, & Bouvet, 2004; Hemsley, 1968). Its stipulate leaves are usually in terminal rosettes. The flowers are axillary, fragrant and usually clustered at the end of the short shoots. The fruits contain a large solitary seed (sometimes the seeds can be 2 or 3) and the cotyledons contain abundant fat and oil.

Shea tree reproduction is marked by seasonality in flowering and fruiting. Bud production in subspecies *nilotica* occurs in December to January while flower production occurs from January to February. Initial fruit production occurs at 15 – 20 years (Okullo, 2004; Okullo, Hall, & Obua, 2004) while full production is reached at 40 – 50 years (Okullo, et al., 2004; Ruyssen, 1957). Fruit yield varies from one year to another and is cyclic in nature, being influenced by climatic, anthropogenic and genetic variability (Kelly, Gourlet-Fleury, & Bouvet, 2007; Maranz & Wiesman, 2003). Shea trees grow on different soil types, excluding alluvial and flood plains. It is tolerant to fires, its thick bark acting to protect the delicate inner tissues from fire damage.

1.2.2 Taxonomy and Botany

This species was first described under the name *Vitellaria paradoxa* in 1807 (von Gaertner, 1807). Later, a collection of this same species was described under the name *Butryospermum* (Kotschy, 1865). In 1942, Chevalier proposed eight varieties of the genus based on leaf and fruit characteristics (i.e. *cuneata, ferruginea, floccosa, mangifolia, nilotica, parvifolia, poissoni and serotina*) (Chevalier, 1943). While there had been a proposal to conserve the name *Butryospermum* instead of *Vitellaria* (Hepper, 1962), the correct name for the species, which is currently considered as a mono-specific genus of the angiosperm family Sapotaceae, is *Vitellaria* (Hall, et al., 1996; Hall & Hindle, 1995; Hemsley, 1968). It is currently divided into two subspecies (*V. paradoxa* subspecies *paradoxa* and *V. paradoxa* subsp. *nilotica*. Attempts to elevate subspecies *paradoxa* to specific status have been proposed (Andrews, 1952), especially based on material from Cameroon (Pennington, 1991), but this has not met with agreement from the general taxonomic community.

1.2.3 Geographical distribution

The geographical distribution of *V. paradoxa* is estimated to be 1 million km^2 i.e. between western Senegal and eastern Uganda (Hall, et al., 1996; Salle, Boussim, Raynal-Roques, &

Brunck, 1991). Despite its wide swathe of territory, the ranges of the two subspecies do not overlap. Subspecies *paradoxa* occurs only in West Africa while subspecies *nilotica* occurs only in Uganda, Ethiopia, the Democratic Republic of Congo and the Sudan. The genus therefore occurs in a wide swathe of territory with differing ecological conditions.

Within Uganda, the sub-species *nilotica* covers the whole range of the savanna drylands above 1° of latitude. The southern-most distribution is in Pallisa district in eastern Uganda while to the west, it occurs in Nakasongola and Masindi districts across into the Democratic Republic of Congo (DRC). To the north, its occurrence is contiguous with its southern Sudan distribution. The species therefore occurs in eastern, mid-western and northern Uganda. The highest density of *V. paradoxa* subsp. *nilotica* is reported to be in present day Otuke district (Masters & Puga, 1994).

1.2.4 Uses

Although its fruits are the most important commercially, the flowers, leaves and roots of *V. paradoxa* are also used for various purposes. Shea fruits whose fleshy and sweet epicarp is rich in ascorbic acid, sugars, iron and calcium, are eaten when ripe and therefore serve as an important dietary supplement (Maranz, Kpikpi, Wiesman, De Saint Sauveur, & Chapagain, 2004). The stem, flowers, leaves, bark and roots are used for socio-cultural purposes, such as rain-making, funerals, weddings (Carette, Malotaux, van Leeuwen, & Tolkamp, 2009; Ferris, Collinson, Wanda, Jagwe, & Wright, 2004; Goreja, 2004). The wood from shea trees is prized in the manufacture of traditional hand tools, such as hoe handles, mortars and pestles, and building poles (Hall, et al., 1996).

The most valuable product from shea trees is its oil which is extracted from the kernels and is traditionally used in cooking (Lamien, Sidibé, & Bayala, 1996a, 1996b). Shea oil is used in massaging pregnant women and children and also works well as a stretch mark remover and sunscreen (Marchand, 1988; Moharram, Ray, Ozbas, Juliani, & Simon, 2006). The oil is increasingly becoming popular for cosmetic and skin care products (Abbiw, 1990) due to its high content of non-glyceride constituents (Alander, 2004). These non-glyceride compounds that include α-amyrin, β-amyrin, lupeol and butryospermol (Itoh, Tamura, & Matsumoto, 1975; Itoh, Tamura, & Matsutmoto, 1974; Itoh, Uetsuki, Tamura, & Matsumoto, 1980) exhibit anti-inflammatory, anti-tumor, chemo-preventive and anti-mycobacterial activities (Akihisa, Kojima, Kikuchi, et al., 2010). Due to its composition, shea oil can be used as an

unguent for the skin and as a nasal decongestant (Tella, 1979). It can also be used as a sedative or anodyne for the treatment of sprains and dislocations and for the relief of minor aches and pains. In addition, the basic flow properties of shea oil, such as density, viscosity, cloud and pour points, of its biodiesel and its blends have been found to have a linear density-temperature relationship similar to diesel fuel (Enweremadu & Alamu, 2010; Enweremadu, Rutto, & Oladeji, 2011), putting shea oil as a potential candidate for future sources of biodiesel

Other important and traditional uses include the water-proofing of beehives, doors and walls, illumination (as a fuel in lamps and as candles), and for the manufacture of soap (Adomako, 1985; Marchand, 1988). Recent studies have shown that shea tree bark produces a dye extract which is yellowish – orange in hue and occupies over 65% of the yellow – red wavelength band (Wanyama, Kiremire, Ogwok, & Murumu, 2010, 2011) thereby exhibiting good colour absorption on cotton fabrics with good fastness to light, including a remarkable resilience to washing and rubbing.

1.3 Shea tree characterisation

1.3.1 Folk taxonomy

Rural indigenous people usually possess systems of classifying and identifying plant and animal species in their community (Johnson-Gottesfeld & Hargus, 1998). This involves intimate understanding of the desirable traits, an attribute that is usually available in traditional (folk) knowledge. Farmers therefore use various folk taxonomy descriptors such as introduction and use histories, use-related traits and morphological attributes to classify, name and group their crop varieties (Mekbib, 2006, 2007). A comparative study by (Jinxiu, Hongmao, Huabin, & Lei, 2004) in Xishuangbanna, China, local people were able to identify more plant 1016 and 1062 plant species in two separate studies compared to 951 and 1007 species identified by very experienced field botanists. The local people also took much less time to arrive at differences between species compared to the field botanists. The local people were however quicker and more accurate at identifying species that they cultivated or that had a long history of interfusion with their sedentary life styles (Jinxiu, et al., 2004). The importance of folk taxonomy is more aptly documented in the domestication of *Colocasia esculenta* (Xu, Yang, Pu, Ayad, & Eyzaguirre, 2001), *Manihot esculenta* (Sambatti, Martins, & Ando, 2001) and *Oryza sativa* (Appa Rao, Bounphanousay, Schiller, Alcantara, & Jackson, 2002).

In Africa, small scale farmers are known to breed their local crop varieties on the basis of local knowledge and taxonomy, selecting and naming their varieties (Haugerud & Collinson, 1990; Lamola, 1992). It is known that farmers use local names to describe variation in fruit yield, fruit colour and size, pulp taste and quantity and other dendrometric characters of shea trees. *Vitellaria* has been inter-fused with African parkland savanna peoples for a long time (Mauny, 1953; Neumann, Kahlheber, & Uebel, 1998; Park, 2000) and there is evidence indicating the existence of a number of local morphotypes differing in habit, vigor, size, quality of the fruits, pulp taste and oil yield. These criteria can be of immense value to crop breeders. Except for Maranz and Weisman (2003) and Maranz et al. (2004a, b), documentation of shea tree folk classification is scanty. Local communities in the shea parklands have therefore developed their own folk taxonomy for differentiating between local varieties (de Saint Sauveur, 1999).

1.3.2 Morphological variation

Apart from indigenous (farmer) shea tree preferences, the earliest characterisation of the shea tree based on leaf and fruit morphology was by Chevalier (1943) who distinguished eight varieties (i.e. *cuneata*, *ferruginea*, *floccosa*, *mangifolia*, *nilotica*, *parvifolia*, *poissoni* and *serotina*). Ruysen (1957) utilised tree shapes and sizes, fruits, nuts and leaves to report *mangifolium* as a subspecies and indicated that this had two varieties (*viridis* and *rubifolia*). Since then, many attempts have been made to characterise *V. paradoxa* using morphological, biochemical and molecular traits. Morphological characterisation based on fruit, leaf and tree characters has been reported in Cameroon (Diarrassouba, N'Guessan, Koffi, & Sangaré, 2007), in Mali (Sanou et al., 2006) and in Ghana (Lovett & Haq, 2000a). In addition, fruit yield prediction models based on dendrometric and fruiting variables have been developed and variation in these parameters between upland and lowland shea tree populations in Burkina Faso (Lamien, Tigabu, Guinko, & Oden, 2007). All these studies show that there is very high intra-specific morphological variation among shea trees.

1.3.3 Chemical variation

Although shea oil is known to contain several compounds, such as β-carotene (a source of vitamin A), vitamin F (Kar & Mital, 1981; Wiesman, Maranz, Bianchi, & Bisgaard, 2003) and tocopherols (or vitamin E) (Alander, 2004; Maranz, Wiesman, & Garti, 2003; Moharram, et al., 2006), the main focus in chemical characterisation of shea tree has mainly been on the

variation of lipid composition. Several authors have described the lipid content of shea trees across its range. This is because shea oil provides an important source of lipids, especially for the rural poor in the dry sahel zone of Africa. Fat content and fatty acid composition are known to be very variable across the shea tree range (Akihisa, Kojima, Katoh, et al., 2010; Badifu, 1989; Davrieux, Allal, Piombo, et al., 2010; Glew et al., 1997; Maranz, Wiesman, Bisgaard, & Bianchi, 2004).

Shea oil is mainly composed of palmitic, stearic, oleic, linoleic, and arachidic acids. Stearic and oleic acids are the two main fatty acids, whose relative proportion defines the two subspecies of shea trees (Hall et al. 1996). In subspecies *paradoxa*, stearic acid is the dominant fatty acid while oleic acid is the most dominant in subspecies *nilotica*. Fatty acid composition varies significantly since the nutritional value and shelf life of shea nuts and oil depends on it (Beuchat & Worthington, 1978). Fat content and fatty acid composition therefore provide a good opportunity to characterize shea trees with desirable traits for possible production linked to particular markets.

1.3.4 Molecular variation

Characterisation utilising molecular methods indicates that there is high genetic partitioning within and between shea tree populations (Bouvet, Fontaine, Sanou, & Cardi, 2004; Bouvet, Kelly, Sanou, & Allal, 2008; Sanou, Lovett, & Bouvet, 2005). Fontaine and others (2004) utilised RAPD and chloroplast microsatellite markers to quantify the genetic variation of *V. paradoxa* across its geographic distribution and found that genetic distances between populations were correlated to geographic distances. Kelly *et al.*, (2004) utilised microsatellite markers to study temporal genetic structure of *V. paradoxa* in southern Mali, showing that spatial genetic structure was more pronounced in fallow than in forest where adults showed no spatial structuring. Microsatellite markers have proved more useful in resolving genetic differentiation in *V. paradoxa* and specific primers have therefore been developed by Cardi et al. (2005) and Allal et al. (2008).

1.4 Statement of Problem

Characterisation of *Vitellaria paradoxa* across its range shows a considerable degree of phenotypic variability (Diarrassouba, et al., 2007; Lovett & Haq, 2000b; Sanou, et al., 2006). This variation has given rise to the classification by farmers of several intergrading forms or varieties, based on tree dendrometry, fruit/nut colour, fruit/nut size and habitat. The different

shea varieties known to farmers have not been documented and tested for various attributes. Understanding the folk taxonomy and phenotypic variability of a species are important steps in the process of selection of plant species for improvement.

However, the recognition of many different forms (ethno-varieties) purely on the basis of local selection criteria cannot produce an adequate understanding of the range of variation within the species. Folk taxonomy and phenotypic observations can only describe a small portion of the underlying genetic diversity present in a species. Nevertheless, it is important to document the folk taxonomy or classification systems of useful and yet undomesticated species as a first step in the selection process. Farmers are reported to utilise this folk classification to actively select and preserve superior shea (Lovett & Haq, 2000b) and other priority trees on farm (Assogbadjo et al., 2008). However, there is only scanty documentation of shea tree folk classification in Uganda. There are no studies in Uganda to-date that have quantified phenotypic variation of shea trees across their range. Although genetic diversity studies have been undertaken for shea trees across the whole range and have included Ugandan populations, the criteria for sampling did not include folk classification and phenotypic variation. Although the reproductive biology and flowering and fruiting of subspecies *nilotica* in Uganda were studied by Okullo (2004), additional studies are needed to consider folk classification, phenotypic and genetic variability of the subspecies *nilotica*. A sound knowledge of the relationships between folk taxonomy, phenotypic variation and the underlying genetic variability is useful in developing efficient selection and domestication/breeding strategies for shea trees in Uganda.

1.5 Aims and objectives

The aim of this study was to characterize shea tree ethno-varieties and better understand the integrity of folk classification as a basis for future improvement/breeding programmes in Uganda. The specific objectives of this study were:-

1. To document the folk classification and management of shea tree ethno-varieties in Uganda based on farmers' criteria
2. To examine the morphological variation within and among the different shea tree ethno-varieties in Uganda
3. To analyse the fat content and fatty acid composition of shea oil from different shea tree ethno-varieties in Uganda

4. To assess the genetic diversity and molecular variation within and among the different shea tree ethno-varieties in Uganda

1.6 Hypothesis and Research Questions

This study was premised on the hypothesis that there is no significant difference in morphology, chemical composition of shea oil and genetic variation within and among the shea tree ethno-varieties as perceived by farmers in Uganda. These ethno-varieties are arbitrarily defined subgroups of a single large random mating genetic population of shea trees in Uganda. To test this hypothesis, the following questions were explored:

1. What management and conservation practices do farmers carry out on shea trees?
2. What criteria do farmers use to identify differences in shea tree varieties?
3. Are there significant differences in chemical composition of shea nuts from different shea tree ethno-varieties?
4. Do the different shea tree ethno-varieties exhibit significant genetic variation?

1.7 Justification of the study

Vitellaria paradoxa contributes to subsistence production and income generation for rural people in an area estimated to be 40% of Uganda. Clarification of varietal distinctions is important in pointing to useful varieties for improved nutrition and in suggesting optimal sampling strategies for breeding and long-term monitoring or conservation of this species. Locally superior varieties can be selected to boost the commercial and traditional uses of the species. This work also contributes to the attainment of the objectives of the Global Strategy for Plant Conservation.

1.8 Significance of the study

This study has provided baseline data useful for optimising sampling schemes and for developing conservation strategies for the management of this or similar species. This study has made a direct contribution to Uganda's National Biodiversity Strategy and Action Plan (NBSAP) which lays a strong focus on the conservation of the country's dwindling plant resources, especially in threatened ecosystems. it has also contributed to the aims of the United Nations Convention to Combat Desertification (UNCCD) which Uganda signed in 1994 and ratified in 1997. Besides contributing to the attainment of the objectives of the Global Strategy for Plant Conservation, it has also contributed to the attainment of the UN Millennium Development Goals.

1.9 Conceptual/Theoretical Framework

The genetic diversity of shea trees is maintained by farmers due to the traditional-cum-socio-economic benefits accruing from this species. Traditional uses therefore offer a very good entry point for shea tree conservation and improvement programmes. The development of strategies for conservation, pre-breeding and eventual domestication is, however, influenced by many factors, such as farmers' perspectives, traditional and cultural practices, phenotypic as well as chemical and genetic variation. Farmers' perspectives are usually clearly embodied in the folk classifications which arise out of the recognition of and need to maintain crop diversity.

This thesis investigates the folk classification of shea trees in Uganda and its congruence with genetic, chemical and morphological variation. The starting point of the investigation is that farmers recognize the apparent variation in shea trees and have developed a system of folk classification to describe different "ethno-varieties". The complex nature of shea tree variation in the perspective of farmers is given due consideration and compared against scientific observation of variation at the morphological, chemical and genetic levels. To evaluate the ethno-varieties from farmers' classifications, it is useful to utilize different methodologies and tools while focusing on the three levels of assessment (viz. morphological, chemical and genetic variation).

Folk classification can provide a good understanding of crop variation and farmers' perspectives of genetic diversity. It is therefore important to understand the fine grained classification system of farmers' crop diversity, including how they distinguish and name their varieties. In addition, this fine grained classification of shea trees has generally received little attention, but is potentially valuable as it can be used to represent the inherent variation. Folk classification, coupled with the local socio-cultural and biophysical conditions can be integrated into shea tree pre-breeding and/or formal domestication/breeding programmes. However, few data are available for comparing the shea tree ethno-varieties and their inherent morphological, chemical and genetic variation.

It is therefore important to assess shea tree folk classification and systematically compare this with morphological, chemical and genetic variation. In addition, it is important to consider effects of farmer selection practices on the phenotypic, chemical and genetic diversity. Traits

utilized in folk classifications should ideally be rigorously tested against scientifically proven tools and methods. Microsatellite markers, for example, have proven to be very useful tools for variety identification and breeding programmes. Microsatellites have been efficiently utilized in structuring genetic diversity in shea trees (Allal et al., 2011; Allal, Vaillant, Sanou, Kelly, & Bouvet, 2008; Bouvet, et al., 2008; Cardi, Vaillant, Sanou, Kelly, & Bouvet, 2005; Fontaine, et al., 2004; Kelly, Hardy, & Bouvet, 2004; Logossa, Camus-Kulandaivelu, Allal, et al., 2011) and other crop species (Gupta & Varshney, 2000; Lemes, Gribel, Proctor, & Grattapaglia, 2003). They can be utilized together with morphological markers to provide a very versatile tool for identification of shea tree ethno-varietiessince it is known that shea nut fat content varies with specific site conditions (Akihisa, et al., 2010; Maranz, et al., 2003), it is worthwhile to test whether shea oil quality varies with shea tree ethno-variety.

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CHAPTER TWO

TRADITIONAL MANAGEMENT AND CONSERVATION OF SHEA TREES IN UGANDA[1]

Abstract

Traditional practices are universally recognised as a basis for conservation of biodiversity. However, such practices are often not included in natural resource conservation policies. This study assessed local conservation practices of shea trees (*Vitellaria paradoxa*) within different farming systems in Uganda and developed conservation guidelines for the species. The assessment involved 300 respondents, 15 focus groups and 41 key informants. Content analysis was used to identify the most important management and conservation practices. Local uses were categorised on the basis of shea tree products while differences in conservation practices were analysed using the Friedman test. The results show that eight shea tree products are used for 36 different purposes. Respondents' age significantly influenced their knowledge about the shea tree. Traditional conservation practices include on-farm retention during cultivation and the use of folklore (mainly taboos), customs and rituals. Traditional management practices include weeding, bush burning, pollarding and pruning. Based on the current management and traditional conservation practices, a framework for the conservation of shea trees is proposed for integration into conservation policy decisions.

Keywords: Shea tree, *Vitellaria paradoxa*, Conservation, Taboos, Folklore, Traditional knowledge

2.1 Introduction

The importance of traditional practices as a basis for conservation of biodiversity is gradually gaining universal recognition (Berkes et al. 2000; Colding and Folke 2001; Kajembe et al. 2003; Mgumia and Oba 2003; Moller et al. 2004; Saj et al. 2006). Since many of the traditional conservation practices serve similar functions to those provided by formal and contemporary conservation (Colding and Folke 2001), traditional practices can therefore be

[1] This chapter has been published by Springer publications as "Gwali, S., Okullo, J. B. L., Eilu, G., Nakabonge, G., Nyeko, P., & Vuzi, P. (2012). Traditional management and conservation of shea trees (*Vitellaria paradoxa* subspecies *nilotica*) in Uganda. *Environment, Development and Sustainability*. 14(3): 347-363. doi: 10.1007/s10668-011-9329-1"

invaluable in ensuring successful utilisation of natural resources (Alieu 2010). Indeed, the literature abounds of participatory community conservation practices involving indigenous communities in such diverse places as Africa (Adams and Hulme 2001; Alcorn 1993; Fargey 2009; Infielda 1988), Asia (Anthwala et al. 2010; Bhagwat et al. 2005; Chandrakanth et al. 2004; Chandrashekara and Sankar 1998) and America (Colding and Folke 1997, 2001).

In many cases, however, traditional conservation practices are not included in formal natural resource conservation. This is partly due to the narrow definitions of conservation (Colding et al. 2003) and failure to appreciate the 'immense conservation capital' in traditional systems. In most cases, traditional conservation consists of informal measures that are largely 'invisible' in conventional analyses (Colding and Folke 2001; Colding et al. 2003). An inherent feature of traditional conservation is the central role of cultural and traditional practices (Hongmao et al. 2002). Cultural practices include a set of social behaviours and responsibilities that are important to a community of people (Arnett 2007).

Transmission of cultural practices from generation to generation is usually through folklore in an oral manner (Mafu 2004). However, there is lack of clarity in understanding the relationships between cultural practices, traditional practices and folklore. According to Ben-Amos (1971) and Kuruk (1999), folklore includes all the un-codified practices of different communities and consists of legends, sayings, words, poetry, riddles, songs, dances, plays and all works that portray the culture of a community. Traditional practices on the other hand involve the adherence to and inheritance of such cultural practices between generations. These cultural and traditional practices influence the interactions between people and their environment in a complex and dynamic manner (Masalu et al. 2010).

The shea tree (*Vitellaria paradoxa* C. F. Gaertn.) is one of those African trees with a long history of traditional management and conservation (Hall et al. 1996; Neumann et al. 1998). It is a characteristic tree of the Sudano-Sahelian savannah vegetation, distributed over a 6,000 km long and 500 km wide belt stretching from Uganda to Senegal (Hall et al. 1996). Two subspecies of *V. paradoxa* are recognised: subspecies *paradoxa* (distributed exclusively in West Africa, extending from Cameroon to Senegal) and subspecies *nilotica* (distributed exclusively in eastern Africa), extending from Central African Republic to south-western Ethiopia in the east and Uganda in the south (Hall et al. 1996). This research focused exclusively on subspecies *nilotica* found in Uganda.

Shea trees serve a range of important ecological and livelihood roles including the provision of food, medicinal, economic and cultural needs (Boffa 1995, 1999; Hall et al. 1996; Maranz et al. 2004). Shea trees are most prominently valued for their nuts whose kernels are rich in oil (Hall et al. 1996; Lovett et al. 2000; Maranz et al. 2004; Saul et al. 2003). Apart from its use for cooking, shea oil[2] is also used as an illuminant, in soap making, as a hair and skin lotion, and as a medicine (Boffa 1999; Hall et al. 1996; Maranz et al. 2004). Shea butter can be used as a nasal decongestant (Tella 1979) and is a common component of commercial skin moisturisers (Kraft and Lynde 2005). Shea oil is used for the treatment of wounds (Abbiw 1990; Maranz et al. 2003) and to facilitate child birth (Moore 2008). The ripe mesocarp (fruit pulp) is full of sugars (Hall et al. 1996; Maranz et al. 2003, 2004; Okullo et al. 2010b) and is a delicacy, usually eaten by children and women who are mainly involved in fruit and nut collection (Boffa et al. 2000; Lovett et al. 2000; Maranz et al. 2003). Since shea trees are found in the hotter areas of the African savannah, they also provide shade for crops, farmers, herders and their animals (Boffa et al. 2000). In some communities, shea trees and oil are used for cultural rituals such as in traditional marriages and weddings (Ferris et al. 2004; Carette et al. 2009).

Due to their socio-cultural and economic importance, a form of traditional management and conservation of shea trees that involves local selection of superior land races has been practiced in Africa for a very long time, over 1,000 years (Neumann et al. 1998; Wicker 1998). In West Africa, archeobotanical evidence from Saouga village in northern Burkina Faso (Neumann et al. 1998) shows that in the last 1,000 years, millet was grown under agroforestry systems that included trees such as *Vitellaria paradoxa, Sclerocarya birrea* Hochst., *Adansonia digitata* L. and *Diospyros mespiliformis* Hochst. ex A.DC. Apart from the evidence of shea tree presence at Saouga, the authors (Neumann et al. ibid) also presented evidence of shea tree charcoal remains, indicating that selection of individual trees for burning was practiced. In eastern Africa, despite the lack of archeobotanical records that mention the ancient presence of shea trees, evidence for its historical presence can be gleaned from Egyptology (Allal 2010), especially the works of Wicker (1998). Historical records indicate important trade contacts between ancient Egypt and a more southerly kingdom called 'Punt' which was a primary supplier of luxury goods, including some of the 'seven sacred

[2] Shea oil is the same product (albeit with a different melting point) as ''shea butter'' that is extracted from the shea tree in West Africa and that is commonly referred to in the literature.

oils' used in beautification, worship and temple rituals (Wicker 1998). One of the seven sacred oils sought then by the ancient Egyptians from Punt is 'hknw-oil' which Wicker (1998) suggests would probably have been shea oil. Although the exact location of Punt is not known, there is agreement among most Egyptologists that this kingdom covered the area between the Lake Albert and 'Mountains of the Moon' in Uganda to the Red Sea areas of present day Eritrea and Ethiopia (Glenister 2008; Kitchen 1971; Smith 1962; Wicker 1998). This would imply that shea tree management in this area must have been in practice since the first mention of Punt in Ancient Egyptian records in the third millennium BC (Glenister 2008).

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Evidently therefore, traditional shea tree management has had a long history. Contemporary farmers spare particular trees with desirable characteristics including those used in traditional rituals and only cut those that are of undesirable form, usually the smallest in size or those that are unproductive (Boffa 1995, 1999; Lovett and Haq 2000). Apart from cutting, undesirable trees are sometimes ring barked to kill them (Lovett and Haq 2000). Shea trees have therefore been protected through participatory management involving a complex mix of traditions and customs.

The traditional conservation practices of shea trees in Uganda, however, remain inadequately documented (Alieu 2010); hence there is a general paucity of scholarly literature on this subject. Further, the traditional management and conservation of shea tree resources in Uganda is threatened by the breakdown of informal and self-imposed community restrictions (Okumu-Alya 2009). In this paper, we explore the hypothesis that local conservation of shea trees is strongly influenced by the traditional and cultural uses of shea tree products. We assess the traditional knowledge and management practices that are used in local conservation of shea trees. We specifically document the traditional knowledge, management practices and utilisation. We then propose guidelines for the conservation of shea trees in form of a market-based community conservation framework. The documentation of local conservation practices and development of conservation guidelines will contribute to conservation policy decision making and foster a linkage between contemporary conservation and the local communities whose livelihoods are largely met by shea tree products and services. The conservation framework will be used in the design of the shea conservation interventions.

2.2 Methods

2.2.1 Study area

Uganda is located in East Africa astride the Equator stretching from 4° 12'N, 1° 29'S to 29° 34'W, 35° 0'E with an area of 241,551 km2 (UBOS 2010). The country is divided into nine broad farming systems with fairly homogeneous farming practices and ecological conditions (Rusoke et al. 2000). This study was conducted in the Teso, Northern and West Nile farming systems which form the shea tree–dominated wooded savannah parklands of Uganda (Fig. 2.1). The combined population of the three farming systems is six million persons which is 25% of Uganda's population (UBOS 2010). The Northern farming system is the most densely populated (11%) while each of the West Nile and Teso farming systems has 7% of the

national population. The three farming systems are inhabited by 19 ethnic groups (UBOS 2006, 2010). The two largest ethnic groups are the Acholi and Iteso found in the northern and Teso farming systems, respectively. Other large ethnic groups include the Lango, Lugbara, Madi, Madi-Okollo[3], Kuku and Thur. The Kuku (found in the West Nile farming system) and the Thur (from the northern farming system) form the smallest ethnic groups each with less than 1% of the national population and are faced with extinction due to their diminishing numbers of 34,692 and 2,342 individuals, respectively (UBOS 2006, 2010).

The vegetation cover is composed of Sudanian undifferentiated woodlands and Guineo-Congolean mosaics (White 1983). The dominant vegetation community is *Vitellaria*/*Combetrum*/*Terminalia* woodland (Mwebaze 2010). The Teso farming system receives between 800 and 1,000 mm of bimodal rainfall annually (Ebanyat et al. 2010) while the northern and West Nile farming systems receive 800–2,000 mm of rainfall annually in a less markedly bimodal pattern (Mwebaze 2010; NEMA 1997). Most people in the area are agro-pastoralists and are heavily dependent on subsistence mixed annual cropping and livestock production for their livelihoods (Okorio et al. 2004; Oluka et al. 2002).

Management of tree resources in the study area is driven by customary land tenure. Land ownership is regulated by local customs through clan affiliation. Land and tree resources are owned by families or households by superintendence of clans through which rules on land tenure are enacted. Although most of the shea tree belt has experienced a large increase in human population, there are considerable areas under fallow in the northern farming system (Adebua et al. 2002). Fallow periods are variable and are based on human population size, with short fallows ranging between 1 and 5 years (Byakagaba et al. 2011). Long fallows may range between 5 and 10 or as long as 10–20 years (Okia et al. 2005; Byakagaba et al. 2011). Most shea trees in Uganda occur on cultivated land because of the increase in human population. Moreover, most of the shea belt of Uganda faced political and social instability (from 1986 to 2004). During this period, people were gathered in internally displaced persons (IDP) camps resulting in the decimation of shea trees targeted for charcoal, fuel wood and income (Okumu-Alya 2009).

[3] In this study, the Madi speaking people of Okollo County in Arua district (often referred to as Madi— Okollo) are considered a distinct ethnic group on account of the different speech characteristics in comparison to the Madi of Moyo district (often simply referred to as Madi) with whom they have similar origins (Boone & Watson, 1996). The Madi-Okollo have a lexical similarity of 87% with the Lugbara compared to 76% with the Madi of Moyo district (Boone & Watson, 1996).

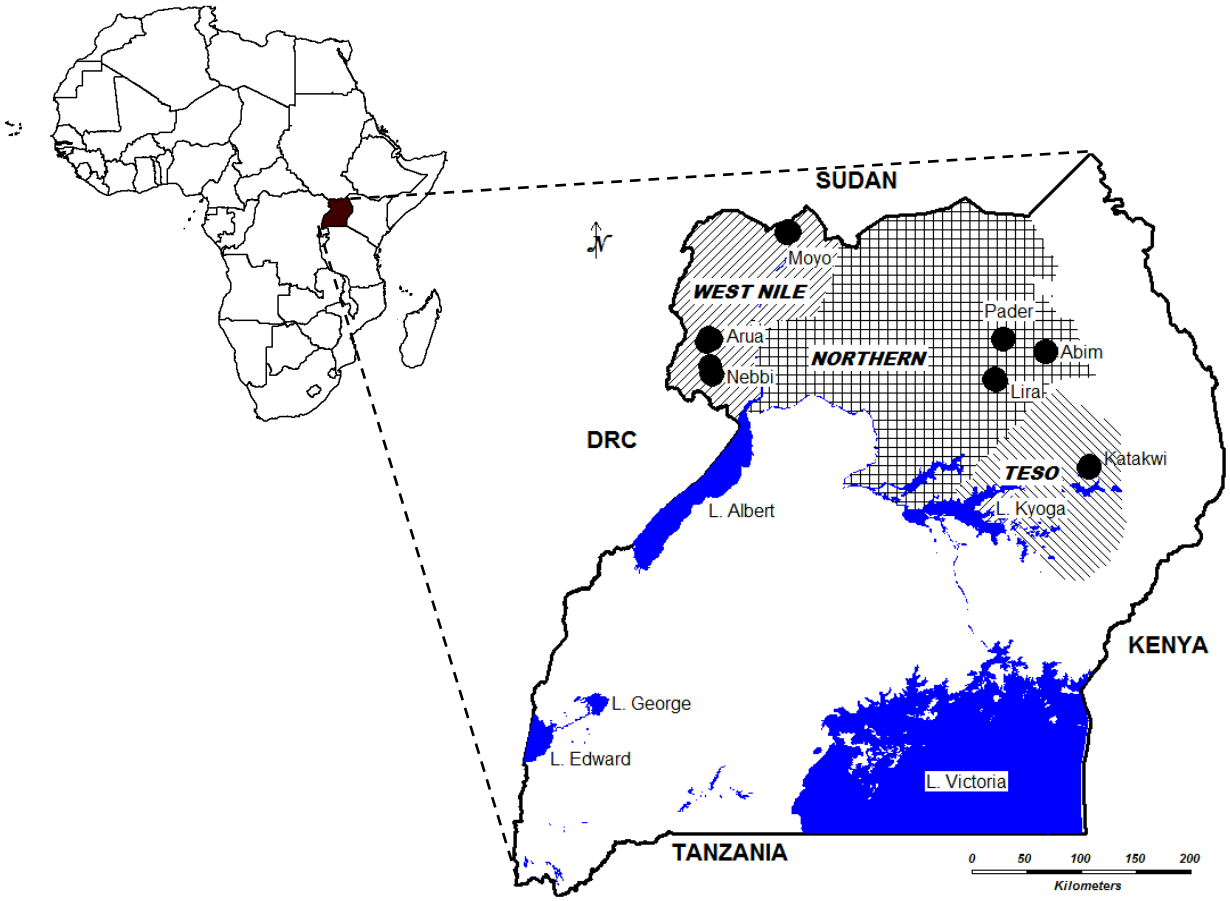


Figure 2.1. Location of study sites in the West Nile, northern and Teso farming systems of shea tree distribution in Uganda

2.2.2 Data collection

Data collection was conducted between April 2008 and March 2010 comprising of key informant interviews, focus group discussions and semi-structured interviews as detailed in the following three steps:

Step I. Selection of 41 key informants (13 from Teso, 18 from northern and 10 from West Nile) with the help of local civic leaders and community/opinion leaders. They were selected from sub-counties with relatively high shea tree population densities based on their knowledge of traditional management of shea trees. A pre-set questionnaire was also pre-tested through cognitive interviewing (Fisher and Geiselman 1992) of the key informants.

Step II. Data collection comprised 15 focus group discussions of 6–10 participants each (6 in Teso, 4 in Northern and 5 in West Nile). Participants in the discussions were randomly selected from the local community. The discussions provided an in-depth understanding of the traditional uses and management of shea trees and triangulated the information obtained from the key informants. While the discussions were guided by an agenda, the sequence of inquiry in each group was based on the nature of preceding discussions.

Step III. A total of 300 respondents were interviewed using a semi-structured questionnaire to document traditional knowledge and management, utilisation as well as conservation of shea trees. Variables investigated included traditional shea tree utilisation and management practices as well as folk classification knowledge. The structuring of folk classification within the farming systems and age categories was investigated. Traditional and cultural beliefs (proverbs, taboos, rituals and folklore) regarding shea trees and their uses were explored.

In order to capture the whole range of local management and conservation practices, eight representative ethnic groups consisting of the Acholi, Iteso, Kuku, Lango, Lugbara, Madi, Madi-Okollo and Thur were surveyed. With the exception of the Kuku and Thur ethnic groups, we selected ethnic groups that comprise over 1% of the national population.

2.2.3 Data analysis

Data were coded in Microsoft Excel and analysed using the Statistical Package for Social Scientists (SPSS) version 16.0 (2007 release, © IBM Corp., Chicago, IL, USA). Descriptive statistics were used to group the different local uses of shea trees and their products while differences in conservation practices across different ethnic groups and age groups in the three farming systems were analysed using the non-parametric Friedman test (Friedman 1937). The Friedman test is used to detect mean differences between several related samples (Ho 2006; Hinton et al. 2004). Qualitative content analysis was performed to identify the uses, traditional management and conservation practices. Content analysis is a form of scientific inquiry that helps researchers to explain common trends in survey data (GAO 1989). In content analysis, it is assumed that words and phrases that are frequently mentioned are relatively more important to the subject matter than those less frequently mentioned (Stemler 2001). Multiple response data for uses, taboos and proverbs relating to shea trees were grouped together using the multiple response command of SPSS. The descriptive words

provided for each use were categorised and counted through the multiple responses command, collapsed into larger categories to represent particular shea tree products and used to clarify the interpretation of common underlying conservation practices.

2.3 Results

A total of 36 uses of shea trees and eight product-related use categories were identified (Table 2.1). The use categories were shea oil, wood, whole tree use, fruit pulp, bark, latex, leaves and shea nuts. Twenty-four (67%) of the 36 uses involve shea oil, which is thus the most prevalently used shea tree product (Fig. 2.2). Shea nuts and fruit pulp were mentioned by over 50% of the respondents. The nuts were highly valued for commercial purposes but the oil was most valued for use in cooking. Only a small percentage (4%) of the respondents mentioned the ecological service roles of shea trees (shade, windbreaks, soil conservation and soil fertility).

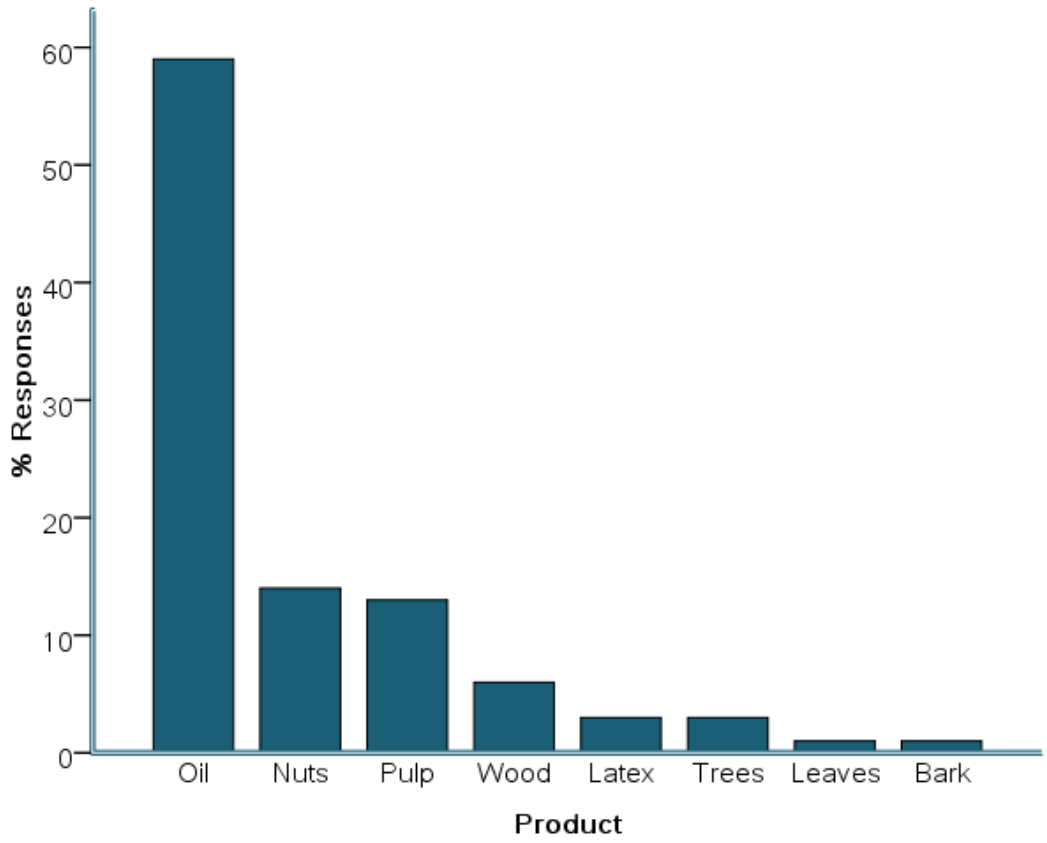


Figure 2.2 . Shea tree uses in the three farming systems determined by content analysis. Uses are based on per cent open-ended responses of respondents (n = 300).

Table 2.1. Shea tree products and their uses among the communities in the shea belt of Uganda

Category	Use	N	% of responses
Oil	Cooking	238	20.3
	Body/hair cream or lotion	230	19.7
	Treating wounds	52	4.4
	Facilitate child birth	36	3.1
	Bride price	26	2.2
	Lubricating tools	22	1.9
	Soda ash	20	1.7
	Insect repellent	12	1.0
	Smear traditional costumes	12	1.0
	Soap making	7	0.6
	Anointing	7	0.6
	Ingredient in food sacrifices	6	0.5
	Special gifts	5	0.4
	Making traditional body marks or decorations	5	0.4
	Fattening lean children	2	0.2
	Soften traditional napkins	2	0.2
	Charcoal from residues of charcoal production	1	0.1
	Smearing mourners during funerals	1	0.1
	Smearing warriors for bravery during war	1	0.1
	Chasing demons	1	0.1
	Preserving certain foods, e.g. termites	1	0.1
	Celebration of twins	1	0.1
	Smear widows/widowers during bereavement	1	0.1
	Smearing walls for decoration	1	0.1
Wood	Firewood	29	2.5
	Charcoal	14	1.2
	Domestic tools	14	1.2
	Poles	9	0.8
Tree	Shade	18	1.5
	Windbreaks	13	1.1
	Soil conservation	1	0.1
Pulp	Eaten when ripe	157	13.4
Bark	Decorating pots	12	1.0
Latex	Trapping small animals	36	3.1
Leaves	Soil manure	15	1.3
Nuts	Sale	162	13.8
	Total response	**1,170**	**100.0**

There was no significant difference in shea tree uses between ethnic groups across the three farming systems (Friedman χ^2 = 12.65, *df* = 7, p > 0.05). However, based on presence/absence of particular uses by the respondents, middle-aged respondents (aged 20–49

years, n = 216) mentioned more uses compared to the relatively younger ones (aged \20 years, n = 12) or the older (> 49 years, n = 72) respondents (Friedman χ^2 = 76.7, *df* = 7, p < 0.001).

The traditional knowledge of shea tree management practices, taboos and rituals is transmitted from one person to another orally through parents (56%), elders (45%) as well as peers and friends (27%). Some knowledge was obtained from personal experiences (6% of respondents) and folklore (8% of respondents). Several cultural rituals were prominently mentioned in the management of shea trees (Table 2.1). The highest percentage of respondents who mentioned ritual practices were recorded from the northern (49%) and West Nile (47%) farming systems compared to only 4% from the Teso farming system. There were significant differences between age groups (Friedman χ^2 = 28.36, p < 0.001) and farming systems (Friedman χ^2 = 8.71, p < 0.05). Up to 71% (n = 104) of the responses regarding taboos and rituals were from respondents aged between 20 and 49 years. Among ethnic groups, knowledge of taboos and rituals was not significantly different (Friedman $\chi^2$2 = 10.95, p = 0.09).

In general, 10 management practices were recorded (Fig. 2.3), most of them common to all three farming systems (Friedman χ^2 = 0.054, p = 0.973). Twenty-nine per cent of the respondents reported pruning as a frequent management practice. Over 70% of the respondents weeded their gardens as a management practice. This practice was prevalent in northern and West Nile farming systems. Selective sparing of shea trees was the third most prominent practice followed by burning of vegetation around shea trees (Fig. 2.3). Coppicing and pollarding (cutting of tree trunks and branches, respectively, to encourage re-sprouting), manuring, mulching and staking were reported by less than 10% of the respondents. Some of the management practices (e.g. mulching, staking and manuring) were not reported in all farming systems. Selection of trees for retention on farm was based on oil yield from the nuts (52% of the responses, n = 177) and sweet fruit pulp taste (46% of responses, n = 157). Other selection criteria included heavy fruiting (1%) and large-sized nuts (1%). A higher proportion of female respondents (over 64%, n = 124) than males (51%, n = 176) learned shea tree management practices from their parents. Most female as well as male respondents (85 and 72%, respectively) considered weeding around shea trees as the most important management practice. A similar proportion of males (63%) and females (62%) reported that selection of shea trees was mainly based on high oil content and sweet pulp. Over 83% of the women

were able to extol the importance of shea trees in song and folklore compared to only 54% of the men.

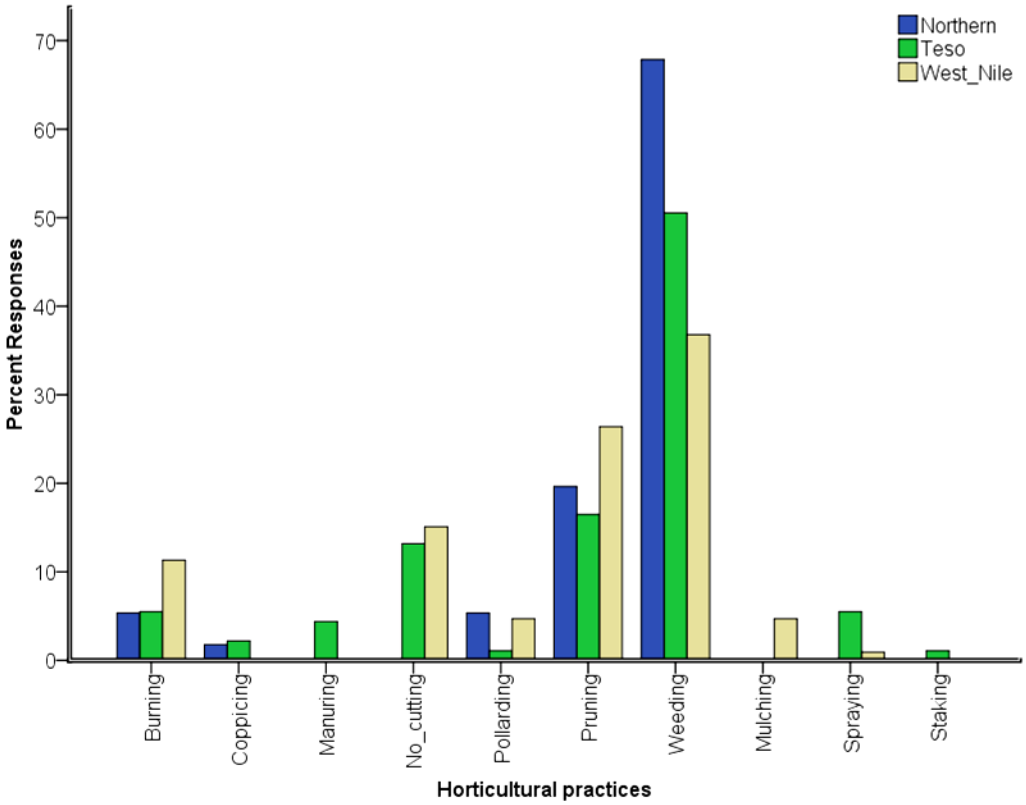


Figure 2.3. Shea tree management practices used in the shea belt of Uganda

2.4 Discussion

2.4.1 Uses of the shea tree

This study shows that the utilisation of the shea tree and its products in Uganda is not different from that reported elsewhere in Africa. While there are references to the medicinal and cultural uses of the shea tree, such as in the making of funeral beds, pregnancies, births and weddings (Goreja 2004), the use of shea oil in war rituals that is reported here has only previously been reported in Uganda (Sturges 2008).

The use of shea tree products for commercial purposes was not as prominent as it is in West Africa where shea butter is marketed internationally through organised groups under fair trade arrangements (François et al. 2009; Greig 2006). The sale of shea oil in Uganda is at subsistence level in the local market (Ferris et al. 2004). This is probably because of the informal nature of shea oil trade in Uganda where the market sector is entirely traditional and

commercial consumption levels are minimal (Ferris et al. 2004). The low commercialisation of shea tree products, especially shea oil, is probably due to the increase and availability of alternative oil for cooking, e.g. sunflower and palm oil as well as modern skin care products that are more vigorously advertised on the local market.

Use of the shea tree for firewood, charcoal, poles and domestic tools was hardly mentioned. This is in contrast to widespread cutting of shea trees in the area (Okumu-Alya 2009) and its use for charcoal or firewood as reported by Ferris et al. (2004). Shea tree wood is highly preferred over wood from most savannah trees for tool handles and poles mainly due to its durability and resistance to termites (Hemsley 1968; Okia et al. 2005). Respondents are probably afraid of mentioning such uses because it is illegal to cut shea trees (ALCODE 2007; Ferris et al. 2004). With regard to ecological service roles, a small percentage (4%) of respondents was able to mention the importance of shea trees in soil conservation, soil fertility, wind control and shade. Shea trees are known to play important roles in microclimate modification, nutrient cycling and soil fertility, especially in crop gardens of the parkland agroforestry systems (Bayala et al. 2006; Traore et al. 2002). The shallow root system of shea trees also stabilises soil structure against soil erosion and acts as a wind break (Hall et al. 1996). In recognition of these functions, harvesting of shea trees in Uganda is highly discouraged both traditionally (through taboos and other prohibitions) and legally (through community bye-laws; ALCODE 2007; Ferris et al. 2004). The enforcement of these community initiatives has been rather difficult. This is partly due to high economic and social depression resulting from the long and protracted period of political and social instability in northern Uganda between 1986 and 2004.

2.4.2 Traditional knowledge

The absence of ethnic differences in the use of shea trees and their products suggests a considerable degree of local knowledge and cultural homogeneity regarding the shea tree in Uganda. In addition, it could be due to cultural integration of the shea tree and its products into the lives of the different ethnic groups. The different ethnic groups are also largely dependent on similar subsistence means of survival, with the shea tree providing the single largest source of vegetable oil. The fact that the ethnic groups in Uganda's shea parklands live in a continuous shea belt may also explain the similarities in shea tree utilisation across farming systems.

In terms of gender, the lack of differences in responses from women and men is not surprising at all. Similar findings have been recorded in Ghana (Lovett and Haq 2000) and Burkina Faso (Elias 2010) where women's knowledge was found to be more detailed but not significantly different from that of men. Considering the different age categories, elderly people are generally expected to have more local knowledge about plant uses than the youth (Camejo-Rodrigues et al. 2003; Johnson 1992), but we found a higher diversity of uses reported by respondents aged between 20 and 49 years contrary to the widely known view. Even when local knowledge may be possessed by the elders, it is the younger people who work with shea trees everyday and are most likely to easily mention more uses of shea trees. The fewer responses from the elderly may be attributed to loss of memory with advanced age (Grady and Craik 2000) and the fact that some of the uses may be non-traditional. The 20–49 year olds should therefore be considered a major repository of knowledge on utilisation of shea trees. This implies that local knowledge of shea tree utilisation may not disappear soon because of the multiple uses of shea trees.

The relatively high diversity of uses of shea trees recorded in the northern and West Nile farming systems compared to the Teso farming system may be the result of differences in knowledge of taboos and cultural rituals between the farming systems. According to Colding and Folke (1997), natural resources that are often used are usually protected by indigenous communities through co-evolutionary processes that involve taboos and cultural rituals. Hence, ethnic groups in the northern and West Nile farming systems probably still retain more intimate knowledge and relationship with shea trees compared to those in the Teso farming system. Even if only a small percentage of the respondents reported personal experiences as a source of their knowledge (probably due to low appreciation for this as a source of knowledge), it is also generally accepted that frequent exposure to the natural environment is a major avenue for acquiring knowledge of traditional uses (Pilgrim 2006).

Regarding the traditional management practices, there were strong similarities with those that are practiced elsewhere in the shea belt of Africa (Boffa 1999; Boffa et al. 2000; Djossa et al. 2008; Hall et al. 1996; Lovett and Haq 2000; Maranz et al. 2003; Masters 2002). Undesirable trees are cut down while selected trees are spared during weeding. Pollarding and pruning are popular in our study sites but they are considered to be 'subconscious' management practices (Lovett and Haq 2000), as they are usually undertaken with different intentions. Pollarding, for example, may be done to obtain branches for conversion to charcoal which is sold to cater

for immediate financial needs. Whereas fruit yield, tree age, vigour and health (absence of infestation with mistletoes) are important considerations for shea tree selection in West Africa (Lovett and Haq 2000; Maranz et al. 2003), only nut/oil yield, pulp taste, prolific fruiting and size of nuts influenced shea tree selection in Uganda. While the shea oil production is clearly the central focus of shea tree selection in Uganda, in West Africa, shea trees are viewed much more as important components of agroforestry production systems (Boffa et al. 2000). Subsequently, shea tree uses as well as the economic contribution to livelihoods of the local people are very important in the conservation of shea trees.

Since shea trees are economically and socially important to the local communities in the shea belt of Uganda, sustainable management and conservation of shea trees may be enhanced by promoting community involvement (Lufumpa 2005). Local communities are recognised as a central locus in conservation, in view of the decline in funding for conservation and increasing poverty in Africa (Agrawal and Gibson 1999). Given that traditional and economic motivations are important in balancing livelihoods and natural resource use by local communities, we propose a conservation framework based on shea tree use, traditional management and market access. We hope that this conservation framework will influence the direction and execution of shea tree conservation. The locus of the proposed framework is the local community (farmers) whose impact on tree resources is critical. The framework aims to devise management strategies geared towards fostering a linkage between contemporary conservation and the local communities whose livelihood is, in a large part, met by shea tree products and services.

2.4.3 Market-based shea tree conservation framework

Our framework fronts shea tree products and traditional uses/beliefs as the basis of conservation (Fig. 2.4). At the base of this framework (first tier) are traditional knowledge and shea tree uses and beliefs. Our view is that the conservation context of traditional customs and beliefs is borne out of the reciprocal service between shea tree use and associated beliefs or customs. Since the service functions provided by traditional beliefs and customs can be harnessed as an incentive for conservation of shea trees, our emphasis in this tier is in recognising the conservation value of traditional beliefs or customs and therefore protecting them from erosion by proclaiming their virtues.

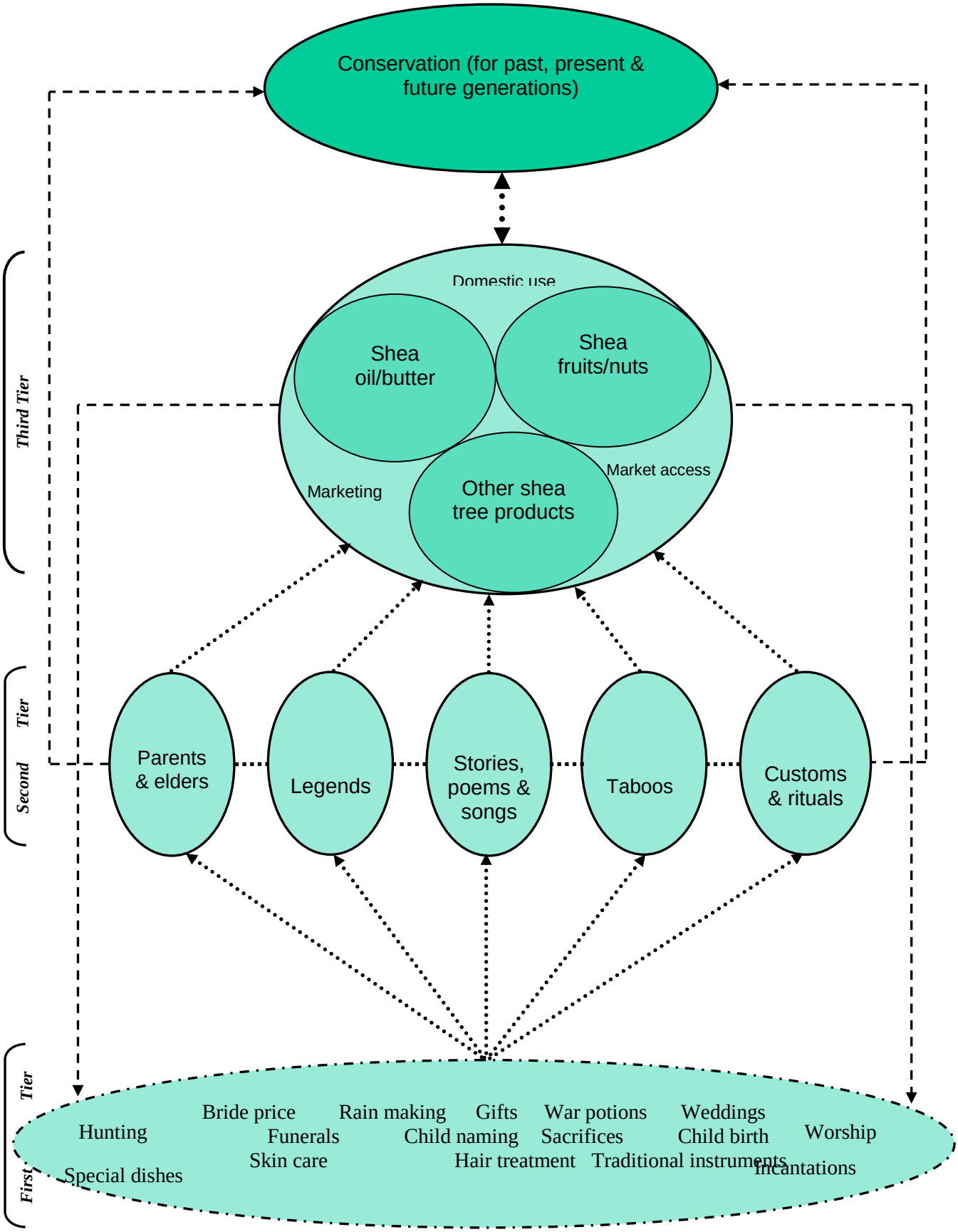


Figure 2.4. Conceptual framework of the proposed market- and community-based conservation of shea trees

The second tier of the framework utilises the five traditional knowledge transfer mechanisms (parents and elders; legends; stories, poems and songs; taboos; as well as customs and rituals). Through these, conservation efforts are targeted at the major products and services from shea trees. The traditional uses of shea trees draw their importance from folklore, chiefs, elders and depth of their integration into culture and tradition.

The third tier revolves around three main categories of shea tree products: (1) shea oil (and its cultural service functions), (2) shea fruits and nuts and (3) other products and services (such as poles and shade). However, for the framework to be effective, marketing and market access of shea tree products are vital. The sale of shea tree products does not feature prominently in the interview responses of our study, but there is a vibrant and profitable market, particularly for shea oil (Ferris et al. 2004). In addition, shea oil (from subspecies *nilotica*) contains a high oleic acid content (Maranz et al. 2003; Okullo et al. 2010a), making it highly suitable for use in cosmetics manufacture. Its pulp also contains as much as thrice the potassium content of bananas (Maranz et al. 2003; Okullo et al. 2010b). The market potential of shea products has, however, not been sufficiently exploited (Ferris et al. 2004; Lovett et al. 2000) and there is only an intermittent and low-scale trade in shea oil/butter. Lybbert et al. (2002) argue that it is important to include marketing and market access as a kind of market-based conservation strategy to complement traditional conservation practices in order to improve benefits accruing from the conservation. Hence, we include market access and marketing of shea tree resources in the framework. These are currently not given proper attention in local shea tree agroforestry and conservation in Uganda. We believe that this component will play an important role in the success of the proposed framework. Poor marketing of shea tree products is currently one of the causes of destructive exploitation of shea trees in Uganda (Ferris et al. 2004).

This framework is based on the belief that people need to be active participants in conservation decisions, and traditional knowledge has to be accepted and/or regularised as a component of conservation. This framework demonstrates that conservation does not need to undermine the traditional wisdom base; rather traditional knowledge can be used and complemented with contemporary methods to produce an acceptable and working conservation framework. Implementation of this framework will ensure that shea tree management maintains and enhances the sustainable flow of benefits to the communities.

2.5 Conclusion

Shea trees are important to the rural communities of the shea belt of Uganda as they have a range of domestic and socio-cultural uses. Thirty-six different uses of shea trees and their products have been identified in this study. Shea oil is the most important product, but products such as pulp and nuts were mentioned as important. The main traditional management practices include weeding, sparing on farm, natural coppicing, pruning and seasonal burning. Most of the practices are aimed at improving the production of shea oil, and traditional conservation through taboos, rituals and beliefs is still extant in the communities. The conservation of shea trees can therefore be achieved through an integrated approach that incorporates the traditional practices, marketing and market access into a holistic management mechanism. Traditional practices can ultimately play an important conservation role and should be given more attention by policy makers and conservation managers.

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CHAPTER THREE

FOLK CLASSIFICATION OF SHEA BUTTER TREE (*VITELLARIA PARADOXA* SUBSP. *NILOTICA*) ETHNO-VARIETIES IN UGANDA[4]

Abstract

Folk knowledge has been the basis for selection and improvement of many food crops such as potatoes, sorghum, yams, cassava and rice. In Uganda, there is strong potential to utilize folk knowledge to select and domesticate the shea butter tree (*Vitellaria paradoxa* C.F. Gaertn. subsp. *nilotica* (Kotschy) A.N. Henry & Chithra & N.C. Nair), an important economic tree species. Farmers report high variation in fruit yield, tree form and pulp taste. In this study, we documented shea tree folk classification by interviewing 300 respondents, 15 focus groups and 41 key informants across three farming systems of Uganda. Data were analyzed using Kruskall-Wallis and Spearman's tests, Chi-square, Multivariate, Factor and Discriminant Function Analyses. Folk classification and nomenclature of shea tree ethno-varieties is based on fruit/nut organoleptic (color and taste) and morphological attributes. Interestingly, despite the socio-cultural importance of shea oil, it does not feature as a factor in the folk classification and nomenclature of shea tree ethno-varieties. There was no significant difference in classification knowledge across the three farming systems (Kruskal – Wallis $\chi^2 = 28$, df = 28, $p > 0.05$; Spearman's $R > 0.8$, $p < 0.0001$) although there was significant influence from ethnicity of the respondents (Pillai's trace = 0.817, $p < 0.001$). While this study provides a record of shea tree ethno-varieties and associated classification criteria, there is need to validate these 'ethno-varieties' using detailed morphological, biochemical and molecular analyses.

Keywords: *Vitellaria paradoxa*, shea tree, ethno-variety, folk classification, organoleptic properties, local knowledge

3.1 Introduction

Vitellaria paradoxa C.F. Gaertn. subsp. *nilotica* (Kotschy) A.N. Henry & Chithra & N.C. Nair (shea butter tree) is a small to medium-sized deciduous tree that occurs in a wide swathe

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of territory above 1° N in tropical Africa (Hall *et al*. 1996). All over its distribution, from Uganda in the south to Senegal in the west, it is mostly known for its nuts that produce abundant oil (Hemsley 1968). The oil is used for cooking, cosmetic and medicinal ointments, as a hair cream, and for illumination and water proofing (Abbiw 1990, Lamien *et al*. 1996, Maranz & Wiesman 2003). The fruit pulp can be eaten fresh when ripe (Lovett & Haq 2000, Lovett *et al*. 2000). Due to its importance in rural farmers' economies, the shea tree is a species with great value for domestication (Bounkoungou *et al*. 1998, Franzel *et al*. 1996, Maghembe *et al*. 1998).

As domestication is a long-term process involving selection, improvement and integration into various agroforestry practices (Akinnifesi *et al*. 2006), it is normally imperative to understand the target plant's desirable traits. These traits, such as fruit size and taste, are often available in traditional (folk) knowledge (Ghimire *et al*. 2004, Kebebew *et al*. 2001, Thrupp 1989). Folk knowledge is important in identifying the value of farmers' varieties for breeding programs (Appa Rao *et al*. 2002). Indeed, folk knowledge has been documented and used as a basis for the domestication/ breeding of many plant species including potatoes in Chile (Quiros *et al*. 1990), *Sorghum bicolor* (L.) Moench in Ethiopia (Mekbib 2006), *Colocasia esculenta* (L.) Schott in China (Jianchu *et al*. 2008), *Manihot esculenta* Crantz in Brazil (Sambatti *et al.* 2001) and *Oryza sativa* L. in Laos (Appa Rao *et al.* 2002).

Many studies have shown that folk biological classification and local knowledge systems are highly organized and culturally structured (Berlin *et al*. 1973, Berlin 1992, Brown 1993, Holman 2002, Raven *et al.* 1971). These studies outlined the principles of folk classification and showed that folk taxonomies are arranged in hierarchical levels, the most common of which are the life form (e.g., trees, vines, grasses), generic (e.g., corn, bamboo) and specific (e.g., common bean) levels. Depending on the cultural significance of an organism, the folk-specific level may be further differentiated into folk-varietal levels (Berlin 1992, Hunn 1982). All these hierarchical levels are related to each other by class inclusion while they also share taxonomic, linguistic, biological and psychological properties. Besides these hierarchical levels, sometimes farmers have developed names that refer to particular parts or stages of a crop (partonomy) (Brown *et al*. 1976). Berlin *et al*. (1973) showed that there is consistency in folk classifications across many traditional communities – 'his theory of universality'. This universal cognition implies that folk classification knowledge is not arbitrary but explains biological realities that are held by traditional societies (Holman 2002).

Folk knowledge is however very dynamic and is strongly influenced by indigenous creativity, innovation and contact with other knowledge systems (Kakudidi 2004). Moreover, folk knowledge is strongly rooted in geographical and cultural cognition and representation (Komáromi 2009, Mazzocchi 2006). Because of its oral nature, folk knowledge is very vulnerable to degradation (Kakudidi 2004, Madhav *et al.* 2000) and even complete loss (Ramirez 2007, Reyes-Garcia *et al.* 2005). Even then, traditional communities all over the world have utilized local knowledge systems to sustain their survival for millennia (Krishna 2006, Mazzocchi 2006). The present work examined the folk classification and local knowledge of shea tree ethno-varieties in Uganda.

The prefix 'ethno' refers to traditional knowledge and cognition available in a given culture (Sturtevant 1964). The concept of 'ethno-variety' can therefore be defined as the infra-specific diversity, especially in crop plants, as understood and managed by farmers (Rivera *et al.* 2006). In this paper therefore, a grouping of shea trees identified by farmers under a single name within a particular ethnic group is referred to as an 'ethno-variety'. Farmers point to the existence of high variation in fruit yield, shape, canopies, flowering and fruiting of shea trees in Uganda. This folk knowledge is however not yet adequately documented (Okullo 2004, Okullo *et al.* 2004). The objectives of this study, therefore, were to: (1) document folk knowledge of shea tree ethno-varieties including classification, naming and associated criteria; (2) examine the consistence of folk classification across the three farming systems of the shea tree range, and (3) unravel the underlying factors that influence folk classification of shea trees in Uganda. A sound knowledge of shea tree classification and nomenclature based on folk knowledge will provide a useful baseline for the development of efficient selection strategies for the breeding and domestication of shea trees in Uganda.

3.2 Methods

3.2.1 Study Area

This study was carried out in three farming systems (Teso, Northern and West Nile) of Uganda where the shea butter tree is predominantly found (Figure 3.1). Within these farming systems, the ecological conditions (soil types, topography and rainfall) and farming practices are fairly homogeneous. Rainfall in the Teso farming system is bimodal and ranges from 800 to 1300 mm annually with one long dry season that lasts from December to March (Ebanyat *et al.* 2010, Gavigan *et al.* 2009). The Northern and West Nile farming systems have a less

pronounced bimodal rainfall pattern with a mean precipitation of between 800 - 2000 mm annually (Mwebaze 1999, NEMA 1997, UBOS 2006).

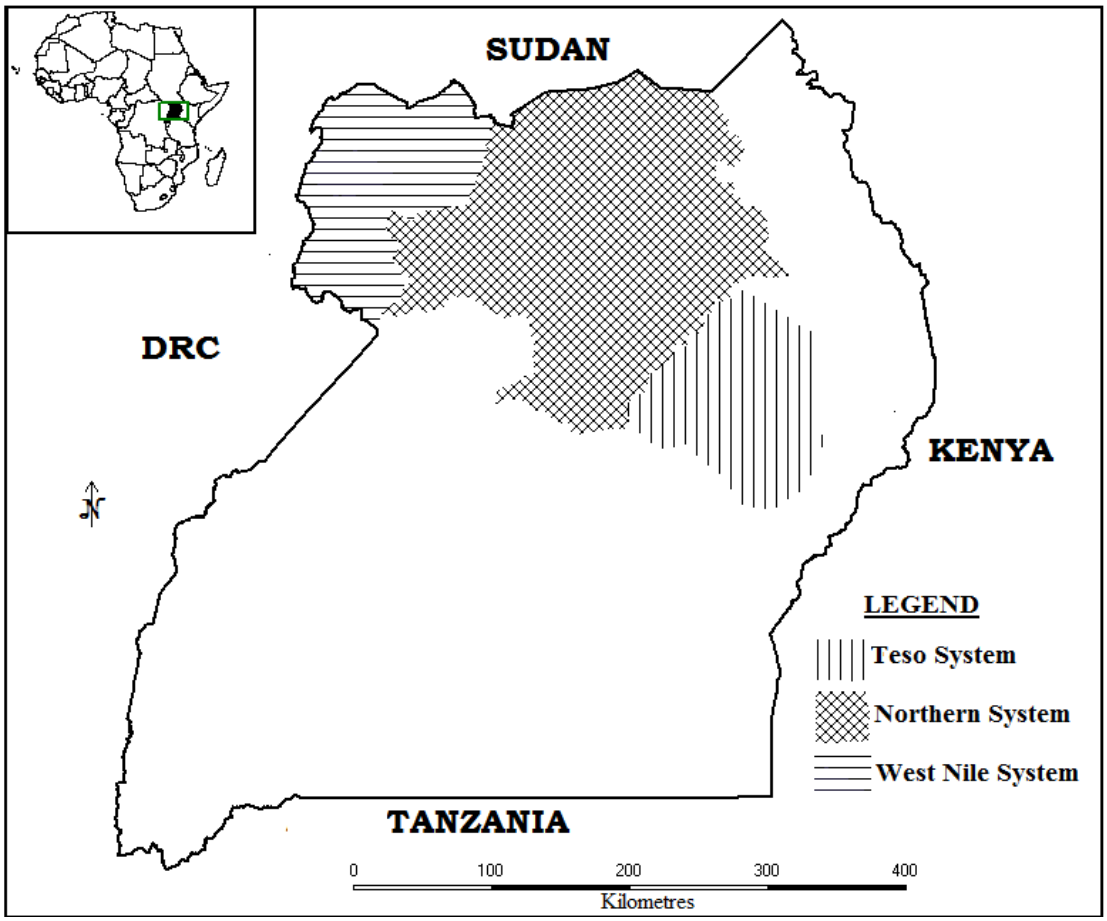


Figure 3.1. Farming systems of the shea belt of Uganda. Inset: Map of Africa showing location of Uganda.

All three farming systems are agro-pastoral with heavy dependence on subsistence mixed annual cropping and livestock production (Oluka *et al.* 2005, Okorio *et al.* 2004). The main crops grown are cotton (*Gossypium hirsutum* L.), sunflower (*Helianthus annuus* L.), cassava (*Manihot esculenta* Crantz), finger millet (*Eleusine coracana* (L.) Gaertn), sorghum (*S. bicolor* (L.) Moench), groundnuts (*Arachis hypogaea* L.), simsim (*Sesamum indicum* L.) and sweet potatoes (*Ipomoea batatus* (L.) Lam) (MAAIF & MFPED 2000).

Customary land tenure is the most predominant type of land ownership in these farming systems (Kamanyire 2000). This tenure system recognizes the right of individuals to possess and till the land but under superintendence of the family, clan or community. Some natural

resources such as shea trees, therefore, are common property and belong to the whole community rather than the individuals on whose land such trees may be found.

3.2.2 Selection of field study sites

Data collection was conducted between August and December 2008. First, a reconnaissance visit was made to the three farming systems during which at least four key informants from each of the three farming systems were identified with the help of local leaders, technical staff and elders. Through secondary sources, key informant responses and personal observations, purposive sampling was used to identify 12 sub-counties with high populations of shea trees in the three farming systems. A sub-county is the lowest administrative unit in Uganda at which local development planning and budgeting are carried out. Within the sub-county, random sampling was done at household level. Due to the ethnic homogeneity of the Teso farming system, only one sub-county was selected for data collection. However, the Northern and West Nile farming systems encompass many ethnic groups. Therefore, data collection was conducted in seven sub-counties of the Northern system and four sub-counties of the West Nile system. This made it possible to capture folk knowledge from eight large ethnic groups within the shea belt of Uganda.

3.2.3 Data Collection

Following the reconnaissance visit, a detailed survey using participatory rural appraisal (PRA) tools (Martin 1995) was conducted. A total of 15 focus group discussions (FGDs) and 41 key informant interviews (KIIs) were conducted across the three farming systems during which a semi-structured questionnaire was refined. All communication with the key informants, questionnaire respondents and during focus group discussions were done in the local languages of the ethnic groups studied. The focus groups and key informants were instrumental in elucidating the criteria for local nomenclature and shea tree folk classification in the different ethnic groups sampled. Questionnaire pre-test was then done in a sub-sample of the target communities using cognitive interviewing (Fisher & Geiselman 1992, Tourangeau 1984). A total of 300 respondents from the selected sub-counties were then interviewed using the pre-tested questionnaire. The questionnaire sought responses on shea tree varieties according to respondents' criteria, the different classification criteria and the underlying factors that influence the folk classification schemes. The questionnaire was administered to only one respondent from each randomly selected household in the three farming systems. Respondents from each household were selected on the basis of gender,

such that if a respondent chosen in the first household was a male, the next respondent chosen in the next household would be a female. This was done so as to capture as much information as possible from the two gender categories.

3.2.4 Data Analysis

All data analyses were performed using the Statistical Package for Social Scientists (SPSS) version 16.0. Shea tree folk classification across the three farming systems was assessed using the Kruskal-Wallis test (Kruskal & Wallis 1952). To understand the criteria upon which folk classifications are based, a principal axis factor analysis was performed. Factor analysis is based on the assumption that all variables are correlated to some degree (Ho 2006). Factor analysis is an appropriate tool as it can group similar variables into meaningful groups based on the patterns of responses across all respondents (Hair *et al.* 1998). According to Kaiser (1960), all factors with eigen- values greater or equal to 1.0 and a minimum correlation coefficient greater than 0.33 contain a substantial amount of variation and should therefore be retained for further analysis. Factors were therefore retained only if they had eigen-values greater than or equal to 1.0 and a minimum correlation coefficient greater than 0.33. In order to minimize cross loading of variables, all variables with communalities less than or equal to 2.0 were excluded from the analysis. To achieve the best grouping patterns, the Oblimin oblique method with Kaiser Normalization was used to rotate the factors. Rotation methods ensure that more discrete components with minimum overlapping are extracted from the variables (Hair *et al.* 1998).

A chi-square analysis was performed to establish whether there was a significant relationship between respondent's age, education, ethnicity and gender on the one hand and local knowledge on the other. Local knowledge was determined by the numbers of ethno-varieties, rituo-cultural uses of shea products, and different fruit/nut organoleptic (taste and color) properties a respondent could mention. Relationships that were significant at the 95% confidence level ($p < 0.05$) were then subjected to a multivariate analysis of variance (MANOVA) for further analysis of the effect of age, education, ethnicity and gender on local knowledge. MANOVA was selected as an appropriate method because of its comparative efficiency in testing for overall (multivariate) responses (Warton & Hudson 2004). Significant effects from the multivariate analysis of variance were subjected to a discriminant function analysis to assess relationships in shea tree traditional knowledge of the different ethnic groups that were sampled.

3.3 Results

3.3.1 Socioeconomic characteristics of the respondents

Eight ethnic groups were sampled of whom the majority (61.2%) had attained primary school educational level. Most of the respondents (57%) were young (less than 40 years old) while 81% were married peasant farmers practicing subsistence agriculture for their livelihoods (Table 3.1).

3.3.2 Folk classification criteria and ethno-variety nomenclature

The classification of shea tree ethno-varieties was found to be based on nine criteria (Table 3.2). Across the three farming systems, the shea tree folk classification criteria were similar (Kruskal – Wallis χ^2 = 28, df = 28, $p > 0.05$) suggesting a common naming/classification system in the shea tree belt of Uganda. Pair-wise comparisons indicated a strong correlation (Spearman's $r > 0.8$, $p < 0.0001$) of classification knowledge across the three farming systems. Among all the ethnic groups sampled, the use of fruit and nut size, pulp taste and nut color were very common (Table 3.2). Interestingly however, despite the socio-cultural importance of shea oil/butter in all the ethnic groups of the shea tree belt, all focus groups mentioned that oil/butter properties are not utilized in shea tree characterization. Rather, a linkage was made between nut color and oil/butter yield. Farmers indicated that it is possible to tell high oil yielding nuts on the basis of their color. Thus, dark brown nuts were said to yield more oil than light brown nuts. In terms of fruit yield by individual trees, the focus groups mentioned that the highest fruit yield was usually obtained from trees that produced small fruits. Indeed, across the three farming systems, all respondents expressed knowledge of specific shea trees that produced fruits with particular properties such as small sized fruits and nuts, little pulp and astringent pulp.

To understand the basis of ethno-variety classification by farmers, a principal axis factor analysis (n = 300) utilizing all classification variables yielded a 3-factor solution explaining 51.5% of the total variance. A subsequent analysis excluding all variables with communalities equal to or less than 2.0 yielded a 3-factor solution. However, the scree plot (Figure 3.2) showed that only 2 factors could be clearly extracted and explained. A 2-factor solution explaining 54% of the total variation was therefore extracted. The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy was 0.747 and the Bartlett's test of sphericity yielded a chi-square (χ^2) of 33.55 (with df = 21 and $p < 0.001$).

Table 3.1. Socio-economic and demographic characteristics of the respondents (n=300) to a detailed survey about folk classification of shea butter tree (*Vitellaria paradoxa* C.F. Gaertn.subsp. *nilotica* (Kotschy) A.N. Henry & Chithra & N.C. Nair) ethno-varieties in Uganda.

Category	**Farming System (percent responses)**			
	Teso	**Northern**	**West Nile**	**Total**
Age group				
<20	10.2	2.0	4.0	4.0
20 – 29	24.5	21.9	25.0	23.3
30 – 39	26.5	30.5	31.0	30.0
40 – 49	16.3	21.2	16.0	18.7
50 – 59	8.2	7.3	12.0	9.0
60 – 69	10.2	11.3	9.0	10.3
70 – 79	4.1	5.3	3.0	4.3
80+	0.0	0.7	0.0	0.3
Ethnic group				
Lango	0.0	33.8	1.0	17.4
Acholi	0.0	33.1	0.0	16.7
Thur	0.0	33.1	0.0	16.7
Iteso	100.0	0.0	0.0	16.4
Madi_Okollo	0.0	0.0	48.5	16.1
Madi	0.0	0.0	47.5	15.7
Lugbara	0.0	0.0	2.0	0.7
Kuku	0.0	0.0	1.0	0.3
Gender				
Female	53.1	37.1	42.0	41.3
Male	46.9	62.9	58.0	58.7
Marital status				
Married	79.6	86.3	74.2	81.2
Single	12.2	5.0	20.2	11.2
Widowed	8.2	6.5	4.5	6.1
Divorced	0.0	2.2	1.1	1.4
Education level				
None	24.49	14.86	4.12	12.93
Primary	51.02	62.84	63.92	61.22
Secondary	18.37	18.24	20.62	19.05
Vocational	6.12	4.05	9.28	6.12
Occupation				
Peasant	93.33	92.00	70.41	84.98
Informally employed	6.67	2.67	22.45	9.90
Formally employed	0.00	3.33	6.12	3.75
Student	0.00	2.00	1.02	1.37

Table 3.2. Shea tree (*Vitellaria paradoxa* C.F. Gaertn.subsp. *nilotica* (Kotschy) A.N. Henry & Chithra & N.C. Nair) ethnovarieties among different ethnic groups in the shea tree belt of Uganda.

Property	**Ethno-variety name from the different linguistic groups sampled**						**Literal**
Trait	**Acholi**	**Ateso**	**Lango**	**Madi**	**Madi-Okollo**	**Thur**	**meaning**
Fruits/nuts							
Size	Abor, Aboro, Abunkuru, Ajigi, Aliboro, Alwoyo, Atitina	Eitiyo	Ajigi, Aliboro, Alindiri	Ciria, Totokwiloro	Nyiri	Abor	Small or tiny
		Naburon		Futua, Futugo, Ritugo	Luara, Mbele, Wangaraka		Big
Shape	Acula, Aloto	Nasomel	Acula	Coloa	Julu		Oval or elliptic
	Alulung	Nalungur	Alulung	Gburu, Gburua	Gburua, Ngulu		Round
Pilosity	Ajayer	Nacekan, Nacekum	Ajayer				Hairy
Nuts							
Color	Elila	Nairionon	Col	Enyi, Onia			Dark brown
		Narengan	Rema	Ikaa			Light brown
Pulp							
Quantity		Ikinei		Ngorokwa			Little
Taste	Limi	Ewiny	Abono, Lim	Limi	Mbilimbili	Lim	Sweet
		Egeget	Yao-amakudong	Etria	Etria		Astringent
		Epiana		Asa	Asa		Tasteless
Hardness	Acogo, Atega, Atekateka	Nakweet, Okorocho	Acogo, Yao-atek	Okpoa			Hard
	Apocopoco, Yao-ayom	Naboroch, Oborocho, Opirisia	Apocopoco	Kpetea			Soft
Colour			Aremo				Pink
Habitat							
Edaphic conditions			Wi-gweng				Stony soils

The first factor clearly revealed that organoleptic and morphological properties of shea tree drupes are very important in folk classification (Table 3.3). Five variables (nut size, nut color, fruit shape, fruit size and pulp taste) loaded onto this factor at a value of 0.5 and greater (Table 3.3). There was strong support (Cronbach's alpha = 0.74) for organoleptic and

morphological properties as a factor in shea tree classification. This factor had an eigen value of 2.55 and explained 36.4% of the total variance.

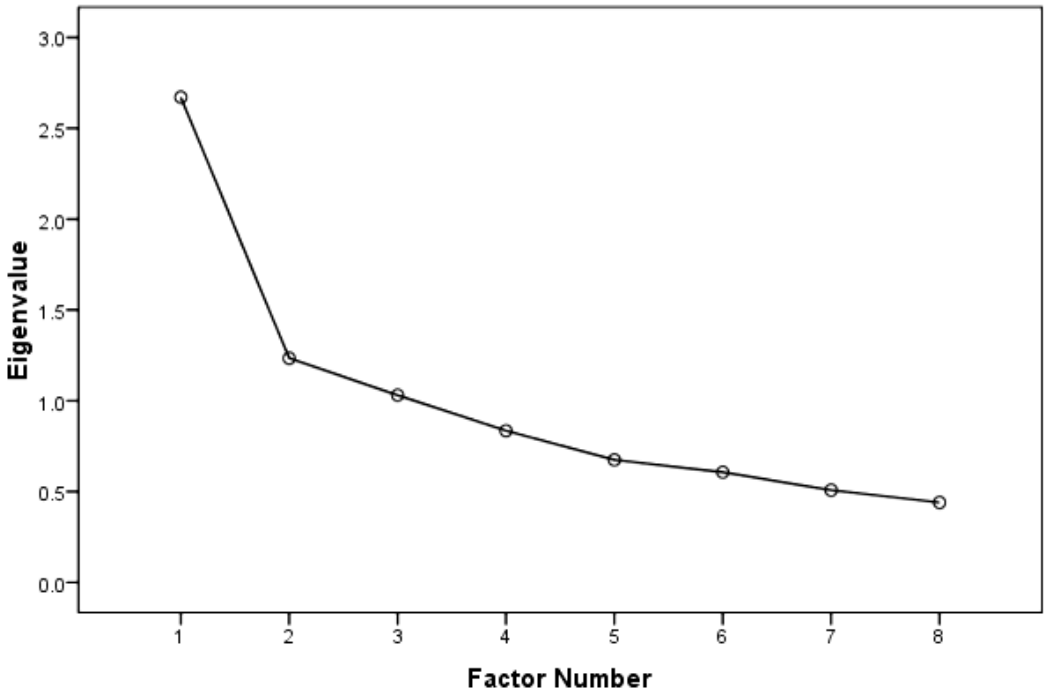


Figure 3.2. Scree plot showing eigen values of eight factors that influence shea tree (*Vitellaria paradoxa* C.F. Gaertn.subsp. *nilotica* (Kotschy) A.N. Henry & Chithra & N.C. Nair) classification criteria in Uganda.

The second factor showed that utilitarian values are important in shea tree classification. Two variables (domestic and rituo-cultural uses) loaded onto this factor with values of 0.4 and greater. The eigen value for this factor was 1.23 and explained a further 17.6% of the total variance. The variables that loaded onto this factor related to various socio-cultural values of the shea tree and its products (Table 3.3). These values relate to the use of the shea trees and their products for domestic and rituo-cultural practices such as in food festivals, rituals (such as those of rainmaking, marriage and funerals) and taboos (such as those against felling of shea trees, climbing and picking of shea nuts at night). The Cronbach's alpha reliability for this factor was however very low at 0.25.

Table 3.3. Factors that influence shea tree (*Vitellaria paradoxa* C.F. Gaertn.subsp. *nilotica* (Kotschy) A.N. Henry & Chithra & N.C. Nair) folk classification in the shea tree belt of Uganda.

Classification variable	Factor	
	1 **Drupe morphology and organoleptic properties**	**2** **Socio-cultural values**
Nut size	.687	
Nut color	.623	
Fruit shape	.609	
Fruit size	.608	
Pulp taste	.510	
Domestic uses		.450
Rituo-cultural uses		.431

3.3.3 Consistence of folk classification knowledge in the shea tree belt of Uganda

A Multivariate Analysis of Variance (MANOVA) revealed that there was significant difference between independent variables (respondents' education, ethnicity and sex) in relation to the derived dependent factors (shea tree classification, fruit/nut nomenclature and rituo-cultural uses). Only ethnicity significantly influenced all three dependent factors that measured folk classification knowledge (Pillai's trace = 0.817, $p < 0.001$) (Table 3.4). A test of between-subjects effects showed that there was a significant difference in effect of respondents' education and shea tree classification on the one hand ($p = 0.019$) and respondent's education and fruit/nut nomenclature and rituo-cultural uses on the other ($p > 0.05$).

A discriminant function analysis of ethnicity on folk classification knowledge showed that all three canonical discriminant functions that were used in the analysis were significant ($p < 0.001$) (Table 3.5). The first discriminant function measured shea tree ethno-variety nomenclature across the different ethnic groups and accounted for 62.3% of the total variation while the second function measured rituocultural uses of shea tree products across the different ethnic groups and accounted for 32.8% of the total variation. The third discriminant function measured drupe (fruit/ nut) classification across the different ethnic groups and accounted for 4.9% of the total variation.

Table 3.4. Multivariate tests of the effect of respondents' education, ethnicity and sex on traditional shea tree (*Vitellaria paradoxa* C.F. Gaertn.subsp. *nilotica* (Kotschy) A.N. Henry & Chithra & N.C. Nair) classification knowledge in Uganda.

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	.031	2.710	3.000	258.000	.046
	Wilks' Lambda	.969	2.710	3.000	258.000	.046
	Hotelling's Trace	.032	2.710	3.000	258.000	.046
	Roy's Largest Root	.032	2.710	3.000	258.000	.046
EDUC	Pillai's Trace	.037	1.642	6.000	518.000	.133
	Wilks' Lambda	.963	1.642	6.000	516.000	.133
	Hotelling's Trace	.038	1.642	6.000	514.000	.133
	Roy's Largest Root	.031	2.712	3.000	259.000	.045
ETHN	Pillai's Trace	.817	13.911	21.000	780.000	.000
	Wilks' Lambda	.360	15.106	21.000	741.387	.000
	Hotelling's Trace	1.313	16.049	21.000	770.000	.000
	Roy's Largest Root	.792	29.419	7.000	260.000	.000
SEX	Pillai's Trace	.017	1.525	3.000	258.000	.208
	Wilks' Lambda	.983	1.525	3.000	258.000	.208
	Hotelling's Trace	.018	1.525	3.000	258.000	.208
	Roy's Largest Root	.018	1.525	3.000	258.000	.208
EDUC * ETHN	Pillai's Trace	.132	1.199	30.000	780.000	.215
	Wilks' Lambda	.873	1.198	30.000	757.957	.216
	Hotelling's Trace	.140	1.196	30.000	770.000	.218
	Roy's Largest Root	.077	2.005	10.000	260.000	.033
EDUC * SEX	Pillai's Trace	.042	1.846	6.000	518.000	.088
	Wilks' Lambda	.958	1.859	6.000	516.000	.086
	Hotelling's Trace	.044	1.871	6.000	514.000	.084
	Roy's Largest Root	.044	3.762	3.000	259.000	.011
ETHN * SEX	Pillai's Trace	.039	.682	15.000	780.000	.804
	Wilks' Lambda	.962	.680	15.000	712.626	.806
	Hotelling's Trace	.040	.678	15.000	770.000	.807
	Roy's Largest Root	.026	1.337	5.000	260.000	.249
EDUC * ETHN * SEX	Pillai's Trace	.065	.959	18.000	780.000	.506
	Wilks' Lambda	.936	.959	18.000	730.219	.507
	Hotelling's Trace	.067	.958	18.000	770.000	.507
	Roy's Largest Root	.044	1.921	6.000	260.000	.078

Table 3.5. Canonical discriminant functions of shea tree (*Vitellaria paradoxa* C.F. Gaertn.subsp. *nilotica* (Kotschy) A.N. Henry & Chithra & N.C. Nair) folk classification among the different ethnic groups in the shea tree belt of Uganda.

Ethnicity	Discriminant Function		
	(1) Shea tree ethno-variety nomenclature	(2) Traditional / cultural uses	(3) Shea fruit/nut organoleptic / morphological attributes
Acholi	-.831	-.529	.000
Iteso	2.274	-.391	.153
Kuku	.251	-.133	-1.727
Lango	-.953	-.248	-.060
Lugbara	.340	1.283	-.382
Madi	.323	.278	-.607
Thur	-.583	-.760	.264
Madi-Okollo	-.213	1.629	.266

There was aggregation among ethnic groups based on the three discriminant functions. The Iteso were discriminated and grouped separately on the basis of shea tree ethno-variety nomenclature (canonical function variate 1 as shown in Table 3.5 and Figure 3.3). The Lugbara and Madi – Okollo were grouped together and separated from the rest on the basis of rituo-cultural use of shea tree products (canonical function variate 2). The rest of the ethnic groups, i.e. the Acholi, Lango, Kuku, Madi and Thur constituted a separate group on the basis of drupe (fruit/ nut) classification. Within this group, the Kuku were more distantly discriminated with a much higher negative value (Table 3.5).

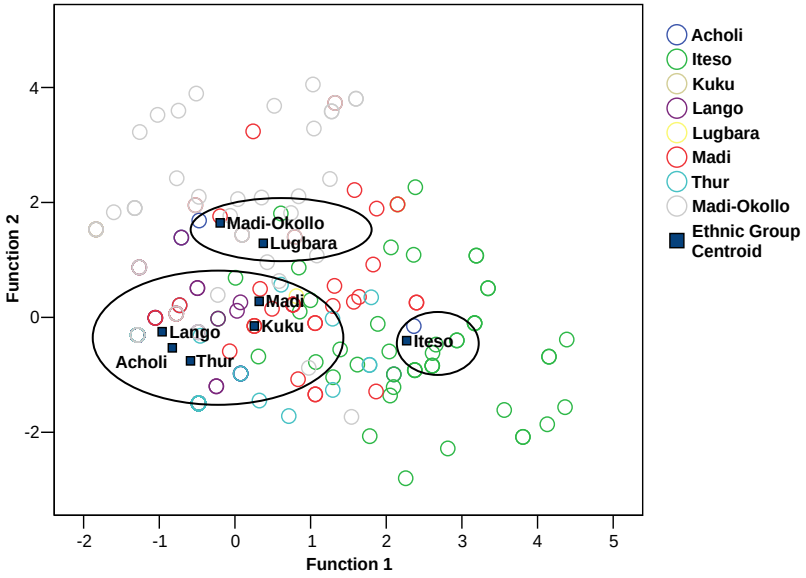


Figure 3.3. Ethnic grouping of shea tree (*Vitellaria paradoxa* C.F. Gaertn.subsp. *nilotica* (Kotschy) A.N. Henry & Chithra & N.C. Nair folk classification in Uganda from canonical discriminant function analysis.

3.4 Discussion

Traditional classification systems are usually developed for species that have attained high utilitarian and cultural significance (Kakudidi 2004, Montoya et al. 2003, Volpato *et al*. 2004). However, the existence of traditional classification systems is being threatened by a range of exogenous factors including wars, internal displacement, modern education and commerce, and international movements of people. Therefore, the need to capture this knowledge has never been more urgent. In an expansive area such as the shea tree belt of Uganda, there is likely to be a slow erosion of folk knowledge. However, once a pattern of agreement or congruence *sensu* Romney and Weller (1984) can be established, it is then possible to document the available knowledge. The results from this study show that the criteria used for shea tree classification are basically similar in the three farming systems of the shea tree belt of Uganda. Both during the focus group discussions, key informant interviews and questionnaire surveys, the respondents were able to identify individual shea trees by their fruits and nuts and not by merely looking at the trees.

Across the three farming systems, the consistent use of fruit, nut and pulp characteristics indicates an infusion of similar classification knowledge across the shea tree belt of Uganda. This was found in all the age groups in contrast to the general trend in variation in local biological knowledge, for example, in medicinal plants where particular knowledge may be held by particular age groups, men and a few families (Subramanyam *et al*. 2008, Teklehaymanot 2009). The MANOVA analysis showed that folk classification knowledge was not influenced by education or gender. This can be explained by the socio-economic discourse and rich culture of knowledge transmission across age, gender and social categories in the communities of the shea tree belt of Uganda (Wood 2008).

Considering that peasant farmers in this area of Uganda base their way of life, social structure and many rituals on subsistence agriculture, folk classification knowledge is highly developed and shared by many ethnic groupings. The Acholi people of the Northern farming system, for example, have been recorded to transmit traditional knowledge across all social and gender categories through dances, rituals, stories and legends (Cordileone 2008). It is therefore possible to transmit knowledge of different shea tree ethno-varieties from parents, elders and peers. Children are wont to gain most of the knowledge of shea tree ethno-varieties, their attributes and management through personal experiences and oral transmission

during shea nut collection. Children therefore grow up with this knowledge which is also maintained in social networks like peers, neighbors, relatives and even sometimes strangers. In some instances, for example, during the civil war between 1986 and 2004 many communities in the shea tree belt of Uganda were resettled in Internally Displaced Persons (IDP) camps, therefore there was increased interaction between elders and young people from diverse villages thereby enhancing propagation of folk knowledge. Although the civil war seriously disrupted the social order in these areas, it is possible that there was enhanced knowledge sharing among people across age and gender categories. Indeed, some focus group discussions and interviews for this study were conducted in IDP camps.

With regards to shea tree folk classification across Uganda's shea tree belt, all the ethno-variety nomenclature recorded across the three farming systems is based on morphological and organoleptic traits. For example, **Limi** is distinguished from **Asa** on the basis of pulp taste, **Alulung** is distinguished from **Acula** on the basis of fruit or nut shape. Classification is based on a single feature without reference to super-ordinate categories. Therefore, **Limi** (=sweet) is simply named so and is universally understood to refer to sweet pulp varieties. Reference to the fruit (a super-ordinate category to the pulp) is not included in the nomenclature. Similar optional binomialisation has been reported by Hays (1976) among the Ndumba people of Papua, New Guinea. Only one ethno-variety, **Wi-gweng**, is distinguished on the basis of habitat. This latter case presents a problem as environmental conditions, such as soil types, can be variable across the shea tree belt. There was, however, a tendency to use different names to refer to the same ethno-variety. In most cases, these semantics are used in different localities and are a result of the high state of flux of some of the dialects in the shea tree region of Uganda. The Acholi, Lango and Thur ethnic groups, for example, belong to one large cultural grouping referred to as 'Luo'. From Table 3.2, it is apparent that their characterization criteria are very similar and differ only by spelling or pronunciation. For example, **Ajigi** (trees with small shea fruits/nuts) among the Acholi and Lango people are named as **Ajiki** among the Thur (Table 3.2).

Therefore, consistency in folk classification is highly influenced by ethnic groupings. The results from the canonical discriminant function analysis point to an inter-gradation of folk knowledge across the shea tree belt of Uganda. It is worth noting that this inter-gradation follows the common ethnic inter-gradation across the shea tree belt. From the Teso farming system in the east (Figure 3.1) occupied mainly by the Iteso ethnic group, taxonomic

knowledge is connected by the Thur people who speak a dialect that is closer to the Lango but with some lexical relationships to the Iteso and Karimojong people. The Lango and Acholi people share more than 95% of there lexicon and therefore have very similar ethno-varietal nomenclature. On the other hand, the Madi (Moyo) are not related to the Acholi at all, and are physically separated by the river Nile, but by virtue of their proximal location to each other, some of the ethno-variety nomenclature is shared such as **Limi** (Madi) and **Lim** (Acholi and Lango) to refer to shea trees that produce nuts with sweet pulp (Table 3.2). The intersection of shea tree folk knowledge of the Northern with the West Nile farming system through the Madi and Kuku people (Figure 3.3) shows that the Madi folk knowledge forms a continuum with that of the Acholi. In the west Nile farming system, the folk classification schemes of the Madi – Okollo is closer in similarity to the Lugbara with whom they inhabit Okollo county and are separated from the Madi of Moyo.

There is, however, a significant influence of ethnicity on ethno-variety nomenclature (Figure 3.3). The variation in folk knowledge among the various ethnic groups may be due to the intensity of utilization of the shea tree and its products. In the Teso region, for example, there is only moderate utilization of shea tree products while in the Northern and West Nile farming systems, the shea tree forms a central role in the culture of the people (personal observation). In the latter two farming systems, the shea tree is used for different purposes from food and drink, to household uses and beautification, weddings and funerals, and ritual sacrifices. Shea tree populations are therefore higher in the Northern and West Nile farming systems due to more awareness for their conservation.

Given that the shea tree products are equally important to all social groups, folk classification and knowledge of shea products domestic uses has not been structured according to social groups. Indeed, during the data collection exercise, detailed knowledge of different shea tree types and exact locations of particular trees was exhibited by individuals of all age and gender categories. The respondents also exhibited an acute awareness of ecological surroundings of the shea tree population. This is concordant with the findings of Choudhary *et al.* (2008) who reported that local people are usually very much aware of the ecological and phenological attributes of particular plants that are important for everyday life. The factor analysis showed good reliability, with a KMO measure of 0.747 which is a good indicator of distinct and reliable factors (Field 2005). From the factor analysis therefore, shea tree ethno-

variety classification can be said to be based mainly on morphological and organoleptic variation in shea tree drupes (fruits/nuts).

Although factor analysis revealed the use of rituo-cultural knowledge in folk classification, this factor is not reliable as a basis for classification on account of the very low and unstable Cronbach's alpha value ($\alpha = 0.25$). On the other hand, drupe properties (a factor that had a more reliable Cronbach's alpha value of 0.74) can be considered as the main basis of folk classification of shea tree ethno-varieties. Within the drupe or fruit characteristics, fruit/nut morphology and organoleptic attributes are the two most important factors for folk classification. This is concordant with many folk classifications all over the world. For example, Assogbadjo *et al.* (2008) reported the use of morphological classification system for baobab (*Adansonia digitata* L.) in West Africa. Results from similar studies have revealed the critical importance of organoleptic properties of plants in folk classifications (Heinrich 1998, Newmaster *et al.* 2006). In Mexico, the Popoluca people of southern Veracruz utilize organoleptic properties of plants to distinguish between medicinal and non-medicinal plants (Leonti *et al.* 2002). In the Himalayas of Nepal, Ghimire *et al.* (2004) suggested underlying chemical differences in medicinal plants on account of the folk classification based on organoleptic differences.

3.5 Conclusion

Although traditional classification methods are mainly qualitative, they are nevertheless valuable because they are based on long term observations often incorporating experiential learning and utilization of different tree products. Folk classification knowledge in Uganda indicates the presence of shea tree ethno-varieties based on fruit/ nut morphological and organoleptic properties. It is possible to use these ethno-varieties as a basis for further studies on the inherent variation among and within shea tree variants in Uganda. Ultimately this can lead to a domestication/ breeding program once the variation has been quantified and the variants or 'ethno-varieties' validated by detailed morphological, biochemical and molecular studies.

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CHAPTER FOUR

MORPHOLOGICAL VARIATION AMONG SHEA TREE ETHNO-VARIETIES IN UGANDA[5]

Abstract

Vitellaria paradoxa C. Gaertn. (shea butter tree) is an indigenous African tree species that is widely distributed in the dry areas of northern and eastern Uganda. The species is widely known for its oil which is used in cooking, cosmetics and traditional medicine. Local folk classification recognises the presence of different ethno-varieties on the basis of fruit and nut characters. In the present study, 176 trees representing 44 ethno-varieties from three farming systems of Uganda were assessed to determine the patterns of morphological variation and establish the congruence between morphological variation and folk classification. The results show high variation in pulp weight (CV = 35.9%), stem diameter (CV = 28.48%), fruit weight (CV = 27.81%) and canopy diameter (CV = 26.69%). There was a strong positive correlation between pulp and fruit weight ($r = 0.963$, $p < 0.001$), leaf length and leaf width ($r = 0.652$, $p < 0.001$) and between petiole length and leaf length ($r = 0.788$, $p < 0.001$). There was no underlying quantitative morphological structuring among the 44 ethno-varieties. Hierarchical cluster analysis using quantitative morphometric data produced three groups without clear aggregation based on ethnographic or geographic separation. However, a combination with qualitative traits as perceived by farmers provided good congruence with folk classification. Quantitative morphological data alone does not resolve any discrete forms of *V. paradoxa* that are related to folk classification. There is need to utilise biochemical and molecular markers to unravel the underlying variation for use in selection and improvement of shea butter tree ethno-varieties.

Keywords: ethno-varieties, folk classification, morphological variation, qualitative traits, quantitative traits, *Vitellaria paradoxa*

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4.1 Introduction

The shea butter tree (*Vitellaria paradoxa* C. F. Gaertn. – Family Sapotaceae) is a multipurpose tree species of the Sudano-Sahelian belt of Africa (Hall et al. 1996; Hemsley 1968). It is a semi-domesticated species distributed in a wide belt over 1 million km^2 i.e. between western Senegal and eastern Uganda (Hall et al. 1996; Sallé et al. 1991). It is currently divided into two subspecies (*V. paradoxa* subspecies *paradoxa* – found in Western Africa and *V. paradoxa* subsp. *nilotica* (Kotschy) A.N. Henry, Chithra & N.C. Nair – found in eastern Africa). The shea butter tree takes on various forms, which may be influenced by environmental factors, but nonetheless are recognised by farmers in variety classification. The fruits contain a large solitary seed (sometimes the seeds can be 2 or 3) and the cotyledons contain abundant fat and oil. Within Uganda, the sub-species *nilotica* covers the whole range of the savanna drylands above 1° North. The southern-most distribution is in Pallisa district in eastern Uganda while to the west, it occurs in Masindi and the Democratic Republic of Congo (DRC). To the north, its occurrence is contiguous with its southern Sudan distribution. The species therefore occurs in eastern, mid-western and northern Uganda. The highest density of *V. paradoxa* subsp. *nilotica* is reported to be in Otuke county in Lira district (Masters and Puga 1994).

Interest in this species arises out of the multiple uses of the tree and its products. The most important product is fat/oil that is extracted from the kernels of the shea fruit. This oil is used for a variety of purposes including cooking (Abbiw 1990), medicinal, hair and skin ointments and as a base for industrial manufacture of confectioneries (Cidell and Alberts 2006). It is also used for various traditional and social rituals and ceremonies such as marriages, funerals, coronations and rainmaking (Ferris et al. 2004; Gwali et al. 2012; Hall et al. 1996; Moore 2008).

Morphological characters have been widely used in descriptive accounts of this species (Hall et al. 1996; Hall and Hindle 1995; Hemsley 1968; von Gaertner 1807; Kotschy 1865; Andrews 1952; Pennington 1991) and in identifying useful phenotypes of *V. paradoxa* for domestication (Chevalier 1943, 1948; Diarrassouba et al. 2007; Ruyssen 1957; Sanou et al. 2005; Sanou et al. 2006; Ugese et al. 2010). Based on fruit and leaf variation, Chevalier (1943) distinguished eight varieties (i.e. *cuneata, ferruginea, floccosa, mangifolia, nilotica, parvifolia, poissoni* and *serotina*). Ruysen (1957) utilised tree shapes and sizes, fruits, nuts

and leaves to report *V. mangifolium* as a subspecies consisting of two varieties (*viridis* and *rubifolia*). Correlation between different morphological characteristics has been demonstrated in many West African countries such as Burkina Faso (Lamien et al. 2007), Cameroon (Diarrassouba et al. 2007), Ghana (Lovett and Haq 2000b), Mali (Sanou et al. 2005; Sanou et al. 2006) and Nigeria (Ugese et al. 2010). High variation has been reported for shea butter tree trunk, leaves, fruits and nut characters (Kelly et al. 2004; Lovett and Haq 2000a). Five phenotypes based on fruit morphology (round, ovoid, reversed pear, fusiform, and oblong) have been recorded for shea butter trees in Cameroon (Diarrasouba et al. 2007). Nut colour, crown shape and habitat types have been used in addition to dendrometric and fruiting variables to assess shea butter tree variation (Diarrasouba et al. 2007; Lamien et al. 2007).

However, the recognition of many different forms (ethno-varieties) purely on the basis of local selection criteria cannot produce an adequate understanding of the range of variation within the species. Folk taxonomy and phenotypic observations can only describe a small portion of the underlying genetic diversity present in a species. Nevertheless, it is important to document the folk taxonomy or classification systems of useful and yet undomesticated species as a first step in the selection process. In West Africa, farmers are reported to utilise this folk classification to actively select and preserve superior shea (Lovett and Haq 2000b) and other priority trees on farm (Assogbadjo et al. 2008). However, there is only scanty documentation of shea butter tree folk classification in Uganda. There are no studies in Uganda to-date that have quantified phenotypic variation of shea butter trees across their range. Although genetic diversity studies have been undertaken for shea butter trees across the whole range and have included Ugandan populations, the criteria for sampling did not include folk classification and phenotypic variation. Although the reproductive biology and flowering and fruiting of subspecies *nilotica* in Uganda were studied by Okullo (2004), additional studies are needed to consider folk classification, phenotypic and genetic variability of subspecies *nilotica*. According to many authors (Kovach et al. 2007; Sweeney and McCouch 2007; Vodouhé et al. 2011), folk classifications can be the basis for domestication or breeding programmes. Therefore, understanding the morphological variation of the ethno-varieties is important for the management, conservation and domestication of shea trees in Uganda. A sound knowledge of the relationships between folk taxonomy, phenotypic variation and the underlying genetic variability is useful in developing efficient selection strategies for shea butter trees in Uganda.

In the present study, we investigated the morphological variation among shea butter tree (*Vitellaria paradoxa* subsp. *nilotica*) ethno-varieties in Uganda on the basis of the recently documented folk classification of shea butter trees in Uganda (Gwali et al. 2011) and established the extent of congruence between morphological and folk classification.

4.2 Methods

4.2.1 Study Area

The study was conducted in the shea belt of Uganda. Sampling was carried out in the Teso, Northern and West Nile farming systems (Fig. 4.1). The vegetation in the study area is classified as *Combetrum* / *Vitellaria* and grass savannas (Langdale-Brown et al. 1964). The rural people are agro-pastoralists and depend on subsistence mixed annual cropping as well as livestock production for their livelihoods (Okorio et al. 2004; Oluka et al. 2002). The rainfall pattern is bimodal in the Teso farming system with a mean annual precipitation of 1300 mm (Ebanyat et al. 2010; Gavigan et al. 2009); while the Northern and West Nile farming systems receive less markedly bimodal rainfall, consisting mainly of one major rainfall season per year, with mean annual precipitation of 800 mm (Mwebaze 2010).

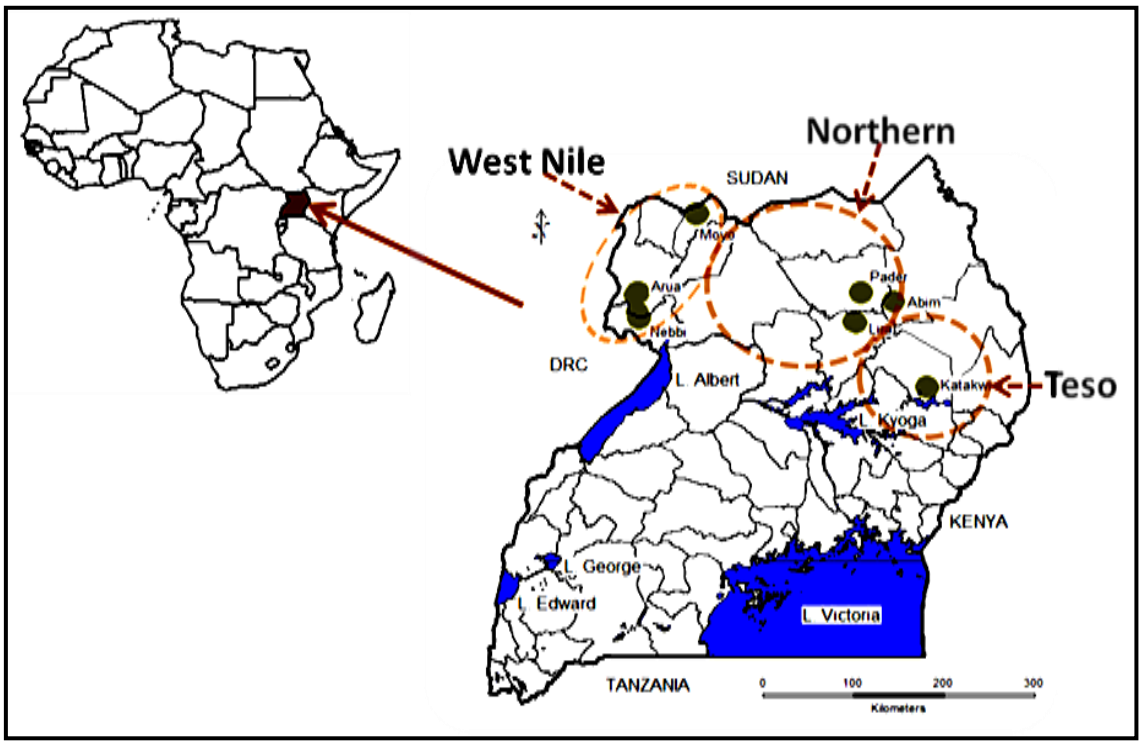


Fig. 4.1. Location of the seven study sites (shaded circles in map) in the three farming systems (bounded by dark dashed lines in map) of the shea belt of Uganda. Inset: Map of Africa showing location of Uganda.

4.2.2 Plant material used and their characteristics

Shea butter trees come in all sorts of shapes. Many trees have a porous crown with spreading branches, sometimes branching very low (Fig. 4.2). Sometimes, the crown takes on a rounded shape with drooping or sagging branches. Some trees have conical shapes, with the branches growing skyward thereby forming an "inverted cone-shaped" crown. At seedling level however, it is not possible to know which tree shape it will grow into. Nursery trials conducted in the present study show some ethno-varieties grow more vigorously than others. However, farmers use mainly fruit/nut morphological and organoleptic properties for classification of shea butter tree phenotypes (Gwali et al. 2011). In this study, we investigated 176 shea butter trees representing 44 ethno-varieties from the three farming systems. The different ethno-varieties and characteristics used by farmers to classify them as documented by Gwali et al. (2011) are presented in Table 4.1.

4.2.3 Data collection

Morphological variation was investigated based on a maximum of five trees sampled from each ethno-variety in each farming system. A total of 40 – 50 mature leaves and 20 – 30 fallen ripe fruits were collected from each tree between April and June 2009. Only 3 – 5 leaves were taken from the distal end of each leafing twig on a tree to ensure comparability between sampled trees. The location of each tree was geo-referenced with the aid of a global positioning system (GPS). Data were collected from six qualitative and 14 quantitative phenotypic and morphological variables (Table 4.2). Diameter at breast height (DBH) was measured at 1.3 m high using a diameter tape while a measuring tape was used to determine the canopy diameter (Table 4.2). Canopy diameter was measured in the N – S and E – W directions after which a mean value was derived for each tree. Leaf morphometric parameters were determined using a graduated rule or calliper. Fruit and nut weight was measured using a Mettler PJ3600 Delta Range 0.001 g sensitive balance.

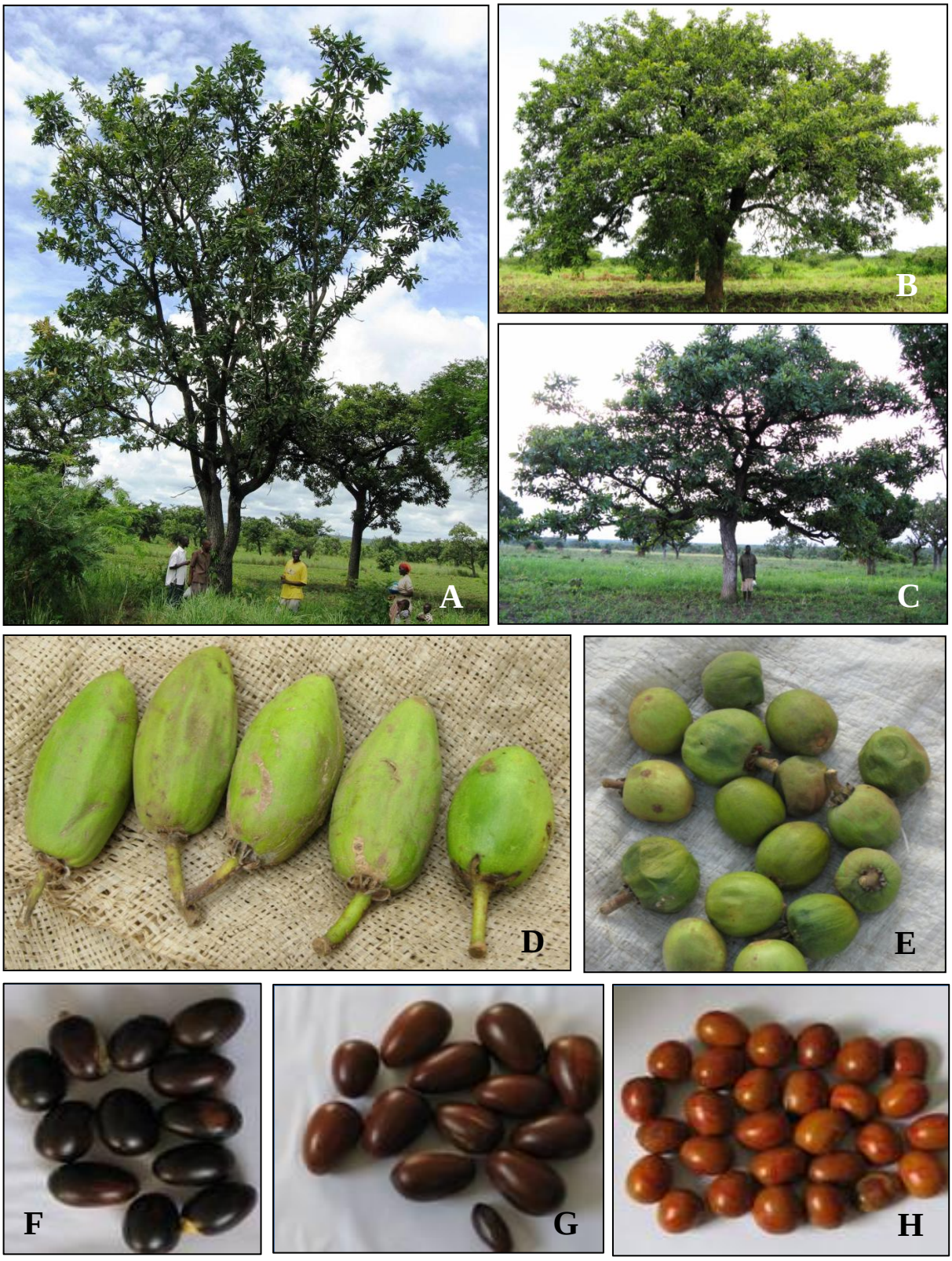


Fig. 4.2. Diversity of shea butter tree (A-C) and fruit (D-E) shapes and nut sizes / colours / shapes (F-H).

Table 4.1. List of ethno-varieties (including their corresponding descriptors) documented by Gwali et al. (2011) and utilised in assessment of morphological variation

Descriptor		Ethno-variety	Provenance	Farming_System
Pulp taste	Astringent pulp	Egeget	Katakwi	Teso
		Etria	Moyo	West_Nile
		Menacwot	Abim	Northern
		Nyangili_Macwot	Nebbi	West_Nile
		Udanyo_Macwot	Nebbi	West_Nile
	Sweet pulp	Abono	Lira	Northern
		Ewiny	Katakwi	Teso
		Limi	Moyo	West_Nile
		Mbilimbili	Arua	West_Nile
	Tasteless pulp	Asa	Arua/Moyo	West_Nile
		Epiana	Katakwi	Teso
Pulp quantity	Little pulp	Ajiki	Abim	Northern
		Upende_Aboro	Nebbi	West_Nile
	Much pulp	Amoo	Abim	Northern
		Mudaa	Moyo	West_Nile
Fruit/nut size	Big fruits/nuts	Enyii	Moyo	West_Nile
		Mbele	Arua	West_Nile
	Small fruits/nuts	Abor	Abim	Northern
		Alindiri	Lira	Northern
		Ciria	Moyo	West_Nile
		Nyiri	Arua	West_Nile
		Yao_Matino	Pader	Northern
Fruit/nut shape	Oval/elliptical fruits/nuts	Acula	Abim/Lira	Northern
		Aloto	Pader	Northern
		Coloa	Moyo	West_Nile
		Julu	Arua	West_Nile
		Nasomel	Katakwi	Teso
	Round fruits/nuts	Alulung	Lira	Northern
		Gburua	Moyo	West_Nile
		Mangulungulu	Nebbi	West_Nile
		Mulunge	Pader	Northern
		Nalungur	Katakwi	Teso
		Ngulu	Arua	West_Nile
Pulp hardness	Hard pulp	Acogo	Lira	Northern
		Ngorokwa	Moyo	West_Nile
		Nyangili_Acogo	Nebbi	West_Nile
		Yao_Atega	Pader	Northern
	Soft pulp	Apocopoco	Lira	Northern
		Mayom	Nebbi	West_Nile
		Upende_Appi	Nebbi	West_Nile
Fruit hairiness	Hairy fruit	Ajayer	Lira	Northern
		Layer	Pader	Northern
		Nacekum	Katakwi	Teso
		Nyangili_Ayir	Nebbi	West_Nile

Table 4.2. Quantitative and qualitative variables and measurement methods and equipment used for this study

Variables and units	Code	Equipment/method used
Pulp taste	PlpTst	Farmers' perception
Fruit size	FrtSze	Farmers' perception
Pulp quantity	PlpQty	Farmers' perception
Fruit pubescence	FrtPbs	Farmers' perception
Fruit shape	FrtShp	Farmers' perception
Pulp eubstance	PlpEbst	Farmers' perception
Canopy diameter (m)	CanDia	Measuring tape
Diameter at breast height (cm)	DBH	Measuring tape
Fruit length (cm)	FrtLgth	Vernier calipers
Fruit width (cm)	FrWdth	Vernier calipers
Fruit weight (g)	FrWght	0.001 g sensitive balance
Nut weight (g)	NtWght	0.001 g sensitive balance
Pulp weight (g)	PlpWght	0.001 g sensitive balance
Nut length (cm)	NtLgth	Centimetre rule
Nut width (cm)	NtWdth	Centimetre rule
Leaf length (cm)	LfLgth	Centimetre rule
Leaf width (cm)	LfWdth	Centimetre rule
Petiole length (cm)	PtlLgth	Centimetre rule
Lamina base angle (degrees)	LmBseAng	Protractor
Lamina apex angle (degrees)	LmApxAng	Protractor

4.2.4 Data analysis

4.2.4.1 Basic statistical parameters

Means and standard deviations were calculated for each quantitative variable of all the leaf and fruit samples of each ethno-variety. Quantitative variables were tested for normality at the 95% confidence level using the one-sample Anderson – Darling (A–D) test in Minitab version 14. Only eight out of the 14 quantitative variables were normally distributed at the 95% confidence level. To achieve normality, the data set was transformed using the Box-Cox transformations within the control charts command of Minitab at a Lambda value of -0.13 (Fig. 4.3).

Pair wise comparisons of all variables were calculated using Minitab 14.0. First order (Pearson's) and second order (partial) correlation coefficients were computed. Partial correlation analysis is aimed at finding correlation between two variables after removing the effects of other variables. Partial correlation helps to spot correlations explained by the effect of other variables as well as to reveal those correlations masked by the effect of other

variables (Pillai and Mohankumar 2010). Partial (second order) correlations were calculated from the bivariate correlations using the following formula:

$$r_{ijk} = \frac{r_{ij} - (r_{ik} \times r_{jk})}{\sqrt{(1 - r_{ik}^2) \times (1 - r_{jk}^2)}}$$

Where r_{ijk} refers to the partial correlation coefficient between variable *i* and *j* controlling for *k* (ethno-variety). r_{ij}, r_{ik} and r_{jk} are Pearson's correlation coefficients between variables i, j and K (ethno-variety).

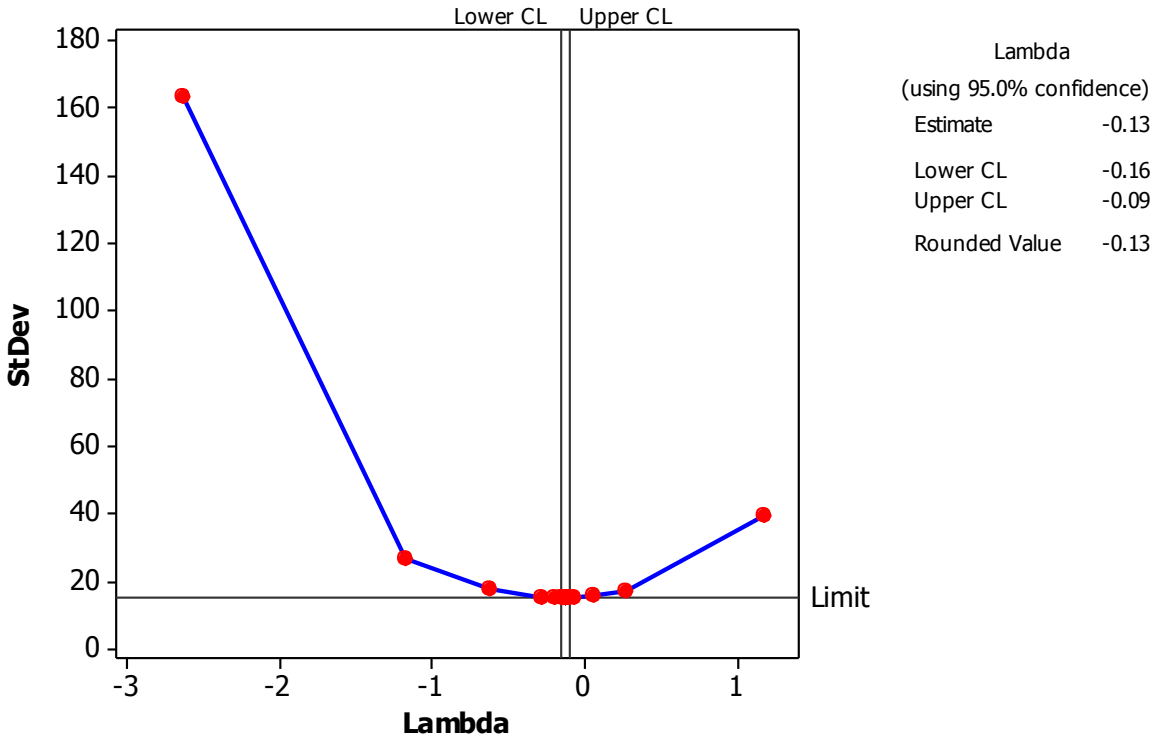


Fig. 4.3. Box-Cox plot of estimated lambda value based on standard deviations at 95% confidence for 14 morphometric variables

Principal Component Analysis (PCA) was performed on the quantitative variables to produce a few representative components of the variables and hence assess quantitative morphological similarity of different shea butter tree ethno-varieties. Only components with eigenvalues of more than 1.0 were extracted. Morphological variables that loaded PCA coefficient values greater than 0.3 were considered relevant for a particular principal component (Jeffers 1967). An agglomerative hierarchical cluster analysis (HCA) was then performed based on a pair-wise similarity matrix of 14 standardised variables using Ward's method and squared

Euclidean distance. A second hierarchical cluster analysis was performed in combination with the qualitative variables as perceived by farmers.

4.3 Results

4.3.1 Morphological variation of shea tree ethno-varieties

The highest variation was found in pulp weight (Table 4.3) followed by DBH, fruit weight and canopy diameter. The lowest variation was recorded in nut width, nut length and lamina apex angle. With the exception of petiole length, nut length and width, variation in fruit morphology was generally higher than that in leaf variables.

Table 4.3. Variation of morphological traits of *Vitellaria paradoxa* in Uganda

Variable	Minimum	Maximum	Mean	SE Mean	CV
Canopy Diameter (m)	4.40	21.75	13.44	0.27	26.69
Diameter at Breast Height (cm)	35.00	111.05	62.77	1.34	28.48
Fruit Length (cm)	2.43	5.18	3.52	0.04	13.66
Fruit Width (cm)	1.92	4.14	2.86	0.02	11.59
Fruit Weight (g)	11.70	53.75	24.22	0.51	27.81
Nut Weight (g)	5.06	19.48	9.50	0.16	22.73
Pulp Weight (g)	5.79	37.65	14.72	0.40	35.90
Nut Length (cm)	1.98	3.29	2.65	0.02	9.48
Nut Width (cm)	1.38	2.26	1.88	0.01	7.88
Leaf Length (cm)	15.08	33.31	24.05	0.27	15.16
Leaf Width (cm)	4.61	9.80	7.32	0.08	15.41
Petiole Length (cm)	2.82	10.78	6.10	0.10	21.03
Lamina Base Angle (degrees)	56.40	128.28	84.01	0.99	15.65
Lamina Apex Angle (degrees)	69.56	118.75	89.65	0.66	9.72

‘Apocopoco’ had the heaviest fruits, nuts and pulp at mean values of 43.94 g, 14.08 g and 29.86 g respectively). ‘Acula’ had the longest fruits with a mean value of 4.28 cm while the highest variation in fruit length was recorded in ‘Apocopoco’ (CV = 26.94%). The longest nuts were recorded in ‘Ewiny’ with a mean length of 2.95 cm. In terms of nut width, ‘Ewiny’ and ‘Nalungur’ had the widest nuts at mean values of 2.06 cm and 2.06 cm respectively. ‘Nyiri’, ‘Yao-matino’ and ‘Alindiri’ had the smallest fruits and nuts. Leaf lengths were higher in ethno-varieties from the West Nile farming system with ‘Gburua’ having the longest leaves at mean values of 28.69 cm. Other folk varieties with long leaves were ‘Coloa’ (mean = 7.74 cm) and ‘Ngorokwa’ (mean = 27.17 cm). The shortest leaves were recorded among ‘Nasomel’ and ‘Epiana’ of the Teso farming system. Ethno-varieties from the

northern and West Nile farming system also had wider leaves. 'Apocopoco' leaves had a mean width of 9.22 cm while 'Nyangili-Acogo' and 'Gburua' had leaves measuring 8.59 cm and 8.59 cm wide respectively.

Table 4.4. Pearson correlation test of morphological variables of shea tree ethno-varieties in Uganda. Codes in the table refer to variables named in table 4.2). Numbers below the diagonal blank line indicate Pearson's rank correlation coefficient. The numbers above the diagonal are related p-values.

	CanDia	*DBH*	*FrtLgth*	*FrtWdth*	*FrtWght*	*NutWght*	*PlpWght*	*NutLgth*	*NutWdth*	*LfLgth*	*LfWdth*	*PtlLgth*	*LmBseAng*	*LmApxAng*
CanDia		0.000	0.317	0.575	0.944	0.462	0.886	0.947	0.549	0.132	0.385	0.845	0.781	0.225
DBH	0.575		0.026	0.020	0.005	0.018	0.007	0.001	0.069	0.236	0.024	0.033	0.007	0.079
FrtLgth	0.076	-0.168		0.000	0.000	0.000	0.000	0.000	0.000	0.409	0.934	0.747	0.001	0.271
FrtWdth	0.043	-0.176	0.776		0.000	0.000	0.000	0.000	0.000	0.383	0.545	0.999	0.016	0.187
FrtWght	-0.005	-0.213	0.812	0.864		0.000	0.000	0.000	0.000	0.721	0.654	0.689	0.020	0.377
NutWght	-0.056	-0.178	0.586	0.695	0.800		0.000	0.000	0.000	0.723	0.774	0.960	0.122	0.328
PlpWght	0.011	-0.201	0.812	0.832	0.963	0.615		0.000	0.000	0.654	0.554	0.634	0.016	0.483
NutLgth	-0.005	-0.245	0.803	0.682	0.843	0.814	0.753		0.000	0.878	0.758	0.742	0.002	0.973
NutWdth	0.045	-0.138	0.447	0.624	0.640	0.755	0.514	0.713		0.834	0.756	0.794	0.949	0.916
LfLgth	-0.114	0.090	0.063	0.066	0.027	0.027	0.034	-0.012	0.016		0.000	0.000	0.811	0.041
LfWdth	-0.066	0.170	-0.006	0.046	0.034	0.022	0.045	-0.023	0.024	0.652		0.000	0.000	0.008
PtlLgth	-0.015	0.160	0.025	0.000	-0.030	0.004	-0.036	-0.025	0.020	0.788	0.425		0.171	0.329
LmBseAng	0.021	0.204	-0.252	-0.181	-0.175	-0.117	-0.182	-0.236	-0.005	0.018	0.270	0.104		0.000
LmApxAng	0.092	0.133	-0.083	-0.100	-0.067	-0.074	-0.053	0.003	-0.008	-0.154	0.200	-0.074	0.265	

There was strong correlation between most of the fruit and nut (length, width, weight) variables (Table 4.4 and Fig. 4.4). The strongest correlation was between pulp weight and fruit weight ($r = 0.963$, $p < 0.001$). While there was strong significant correlation between leaf length and leaf width ($r = 0.652$, $p < 0.001$) and between petiole length and leaf length ($r = 0.788$, $p < 0.001$), the rest of the relationships among the leaf variables were weak ($r < 0.5$). A partial (second order) correlation analysis controlling for ethno-varieties revealed only very little difference when compared to the first order correlation analysis. For example, compared to first order correlations, the second order (partial) correlation between pulp weight and fruit weight was 0.960 while that between petiole length and leaf length was 0.787, a difference of only 0.3% and 0.1% respectively. This means that quantitative morphological variation in shea butter tree ethno-varieties is not structured according to folk classification.

There were weak but significant correlations between most morphological and geographical variables (latitude, longitude and altitude) (Table 4.5). Only canopy diameter and lamina apex angle were not significantly related to any of the geographical variables. All fruit variables were negatively correlated with latitude but positively correlated with longitude and altitude. On the other hand, leaf variables were positively correlated with latitude but negatively correlated with longitude and altitude.

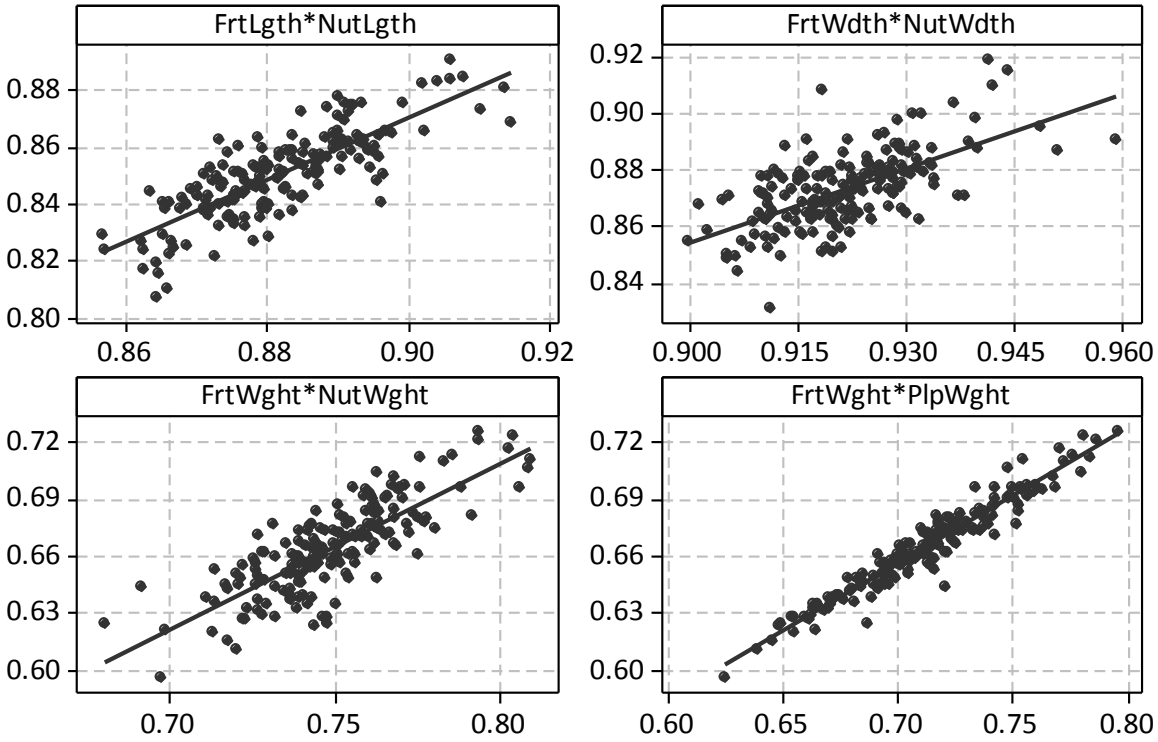


Fig. 4.4. Morphometric relationships between fruit variables

Table 4.5. Pearson's rank correlation coefficients between morphological variables and geographical variables (Latitude, Longitude and Altitude). Asterisks represent significant p values: *** p<0.001, **p<0.01, *p<0.05

	Latitude	*Longitude*	*Altitude*
Canopy Diameter (m)	0.035	-0.003	-0.066
Diameter at Breast Height (cm)	-0.160*	0.436***	0.325***
Fruit Length (cm)	0.075	-0.313***	-0.282***
Fruit Width (cm)	0.108	-0.258**	-0.210**
Fruit Weight (g)	0.214**	-0.355***	-0.365***
Nut Weight (g)	0.207**	-0.174*	-0.257**
Pulp Weight (g)	0.179*	-0.386***	-0.359***
Nut Length (cm)	0.235**	-0.351***	-0.371***
Nut Width (cm)	0.198**	-0.234**	-0.268***
Leaf Length (cm)	-0.325***	0.299***	0.331***
Leaf Width (cm)	-0.248**	0.293***	0.311***
Petiole Length (cm)	-0.244**	0.284***	0.302***
Lamina Base Angle (degrees)	-0.178*	0.256**	0.244**
Lamina Apex Angle (degrees)	-0.074	0.037	0.063

4.3.2 Congruence between morphological variation and folk classification

Principal component analysis of 14 quantitative variables extracted four components (with eigenvalues ≥ 1) explained 77% of the total variation in shea butter tree ethno-varieties. The first component explained 39.4% of the total variation and received relatively high negative loadings from fruit variables (Table 4.6). The second component explained a further 16.8% of the total variation and received high positive loading from leaf variables. The third component received relatively high loadings from tree (canopy diameter and DBH) variables and explained another 11.8% of the total variation while the fourth component received loadings from lamina characters (base and apex angles) and explained 9.1% of the total variation available.

Table 4.6. Principal component analysis (PCA) of 14 quantitative variables of shea tree ethno-varieties in Uganda. Relative loading on the first four PCA axes are indicated in bold face.

PCA	**Axis**			
	PC1	**PC2**	**PC3**	**PC4**
Eigenvalue	5.5229	2.3472	1.6462	1.2712
Explained proportion of variance (%)	39.4	16.8	11.8	9.1
Cumulative proportion of variance (%)	39.4	56.2	68	77.1
Variable	**PCA Coefficients**			
Canopy Diameter (m)	0.008	0.008	**-0.620**	-0.410
Diameter at Breast Height (cm)	0.116	0.193	**-0.570**	-0.289
Fruit Length (cm)	**-0.365**	0.016	-0.037	-0.135
Fruit Width (cm)	**-0.379**	0.034	-0.046	-0.046
Fruit Weight (g)	**-0.411**	0.011	-0.039	0.020
Nut Weight (g)	**-0.361**	0.020	-0.026	0.103
Pulp Weight (g)	**-0.383**	0.011	-0.037	-0.017
Nut Length (cm)	**-0.389**	-0.022	-0.032	0.050
Nut Width (cm)	**-0.315**	0.034	-0.111	0.159
Leaf Length (cm)	-0.016	**0.590**	0.218	-0.149
Leaf Width (cm)	-0.001	**0.526**	-0.025	0.237
Petiole Length (cm)	0.007	**0.547**	0.121	-0.168
Lamina Base Angle (degrees)	0.105	0.182	-0.270	**0.521**
Lamina Apex Angle (degrees)	0.042	0.030	-0.367	**0.558**

For further analysis of quantitative variation among the ethno-varieties, we considered the first two principal components which carry most of the variation and are utilized in folk classification. Hierarchical cluster analysis (HCA) using all variables that contributed to PC1 and PC2 showed no clear aggregation on the basis of ethnographic (folk classification) or

geographic separation across the three farming systems (Fig. 4.5). Ethno-variety membership widely overlapped in separate clusters. Examination of the dendrogram (Fig. 4.5) revealed three groups (at a similarity level of 51.5%):

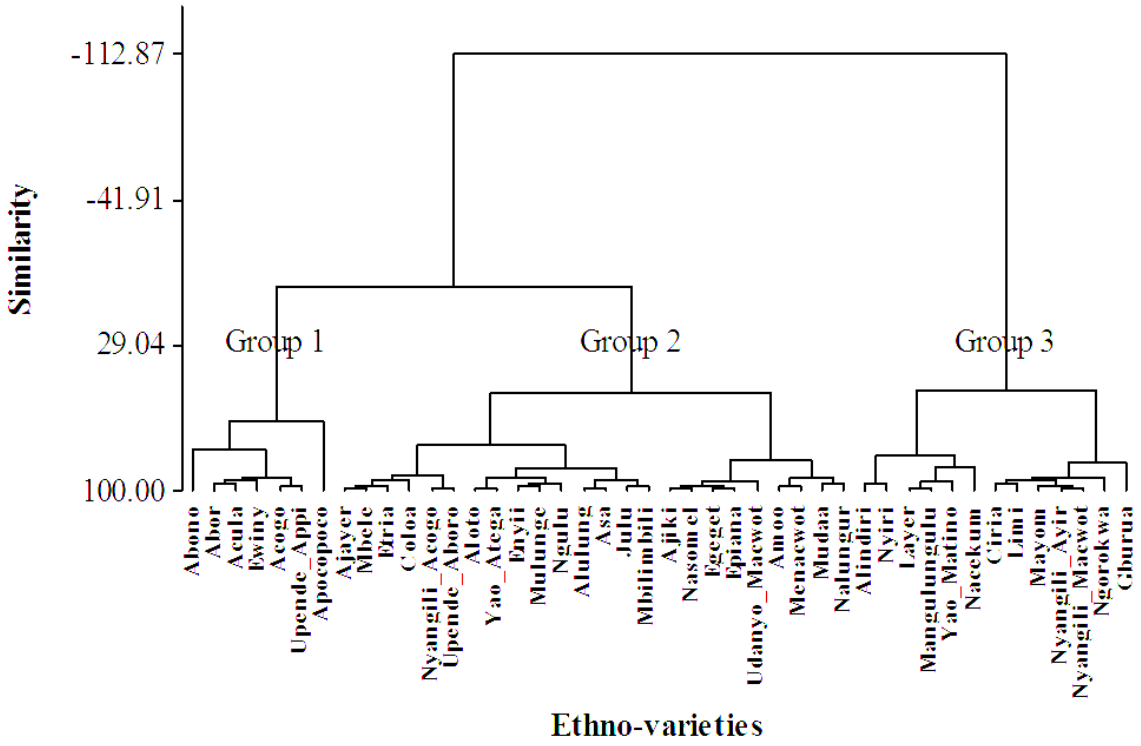


Figure 4.5. Dendrogram from cluster analysis of seven fruit (PC1) and three leaf (PC2) quantitative variables based on Ward's linkage and squared Euclidean distances.

1) Group one consisted of seven ethno-varieties that are characterized by possession of heavy fruits. The mean fruit, pulp and nut weights in this group were over 29 g, 16.6 g and 10.8 g respectively. With the exception of 'Ewiny' and 'Upende-Appi' from the Teso and West Nile farming systems, the rest of the ethno-varieties in this group are from the northern farming system.

2) Group two comprised of ethno-varieties that are characterized by possession of short leaf petioles. Membership of this group was dominated by ethno-varieties from the Teso and northern farming systems. Mean petiole length as well as leaf length and width ranged from 4.5 – 6.9 cm. The shortest petioles were recorded in ethno-varieties from the eastern fringes of the northern farming system and the Teso farming system

namely, ‘Ajiki’, ‘Nasomel’, ‘Egeget’, ‘Epiana’, ‘Menacwot’ and ‘Nalungur’ (Fig. 4.5). Petiole lengths among these ethno-varieties ranged from 4.5 to 5.5 cm.

3) Group three consisted of 13 ethno-varieties that are characterized by possession of small fruits/nuts and long leaf petioles. This group was separated into two sub-groups. One sub-group consists of small-sized and light-weight fruits and nuts, namely, ‘Alindiri’, ‘Nyiri’, ‘Layer’, ‘Mangulungulu’, ‘Yao-matino’, ‘Nyiri’ and ‘Nacekum’. The second sub-group comprised long-leaved ethno-varieties with petioles that measured over 7 cm long. With the exception of ‘Nacekum’, the rest of ethno-varieties in this group are from the northern and west Nile farming systems.

Inclusion of qualitative traits as perceived by farmers produced a dendrogram with eight clear groups with considerable congruence to folk classification (Fig. 4.6):

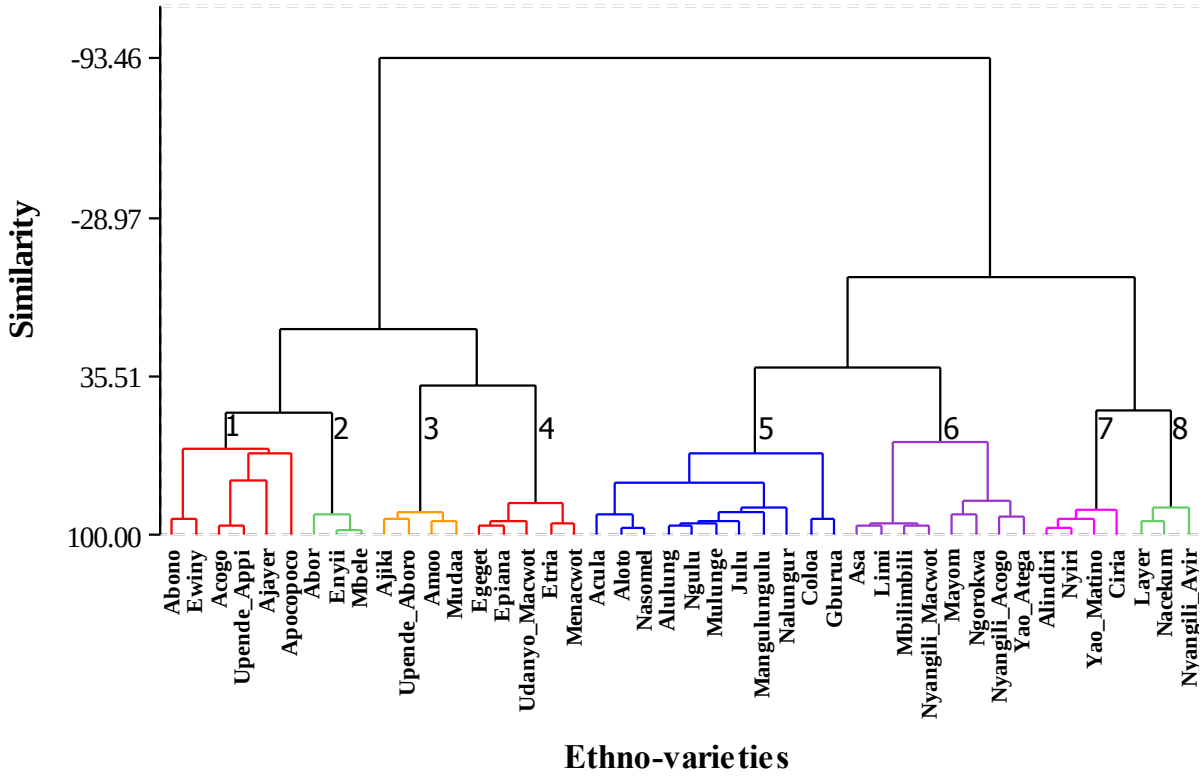


Figure 4.6. Dendrogram from cluster analysis of seven fruit (PC1) and three leaf (PC2) quantitative variables in combination with six qualitative variables based on Ward’s linkage and squared Euclidean distances.

a) Cluster one comprised six ethno-varieties grouped together on the basis of fruit weight. Fruit, nut and pulp weights of these ethno-varieties were the heaviest, having mean values ranging between 28 – 44 g, 10.6 – 14 g and 16.7 – 29.9 g respectively.

b) Cluster two comprised three ethno-varieties grouped together on the basis of fruit size. However, while 'Enyii' and 'Mbele' were described by farmers as ethno-varieties that produced big fruits, they classified 'Abor' among those that produce small fruits. However, in the morphometric analysis, 'Abor' fruits were considerably longer and broader (4 × 3 cm).

c) Cluster three comprised of four ethno-varieties grouped together on the basis of pulp quantity. Farmers described 'Amoo' and 'Mudaa' fruits as containing a lot of pulp while those of 'Ajiki' and 'Upende-aboro' were described as containing little pulp.

d) Cluster four comprised of five ethno-varieties grouped together on the basis of pulp taste. The fruit pulp of 'Egeget', 'Etria', 'Menacwot' and 'Udanyo-macwot' were described by farmers as astringent while that of 'Epiana' was described as tasteless. Due to morphometric variation, some ethno-varieties that were classified by farmers according to pulp taste were grouped in different clusters.

e) Cluster five comprised of 11 ethno-varieties grouped together on the basis of fruit and nut shape. Due to ethnographic limitations, farmers broadly grouped fruit and nuts shapes into two, that is, round and oval or elliptic.

f) Cluster six comprised of eight ethno-varieties grouped together on the basis of pulp taste and eubstance, that is, the hardness or softness of ripe fruit pulp. This cluster was made up of two sub-clusters. One sub-cluster comprising 'Asa', 'Limi', 'Mbilimbili' and 'Nyangili-macwot' can be defined by pulp taste while another sub-cluster comprising 'Nyangili-acogo', 'Ngorokwa' and 'Yao-atega' can be defined by pulp hardness.

g) Cluster seven comprises four ethno-varieties grouped together on the basis of fruit size. From morphometric assessment, 'Alindiri', 'Nyiri', 'Yao-matino' and 'Ciria' ethno-varieties had the lowest mean length and breadth values for fruits and nuts, ranging from 2.6-2.0 × 2.2-2.5 cm and 2.3-2.4 × 1.6-1.8 cm respectively.

h) Cluster eight comprised of three ethno-varieties grouped together on the basis of fruit pubescence. Fruits of 'Layer', 'Nacekum' and 'Nyangili-ayir' were described by farmers as pubescent.

4.4 Discussion

4.4.1 General trends in morphological variation

The primary aim of this study was to establish the patterns of morphological variation in shea butter tree ethno-varieties in Uganda. Although, at first, this seemed at odds with folk descriptors of ethno-varieties which span beyond morphology, a pattern of morphological variation was eventually discerned. Up to 23% of the total variation was not explained by tree, fruit and nut characteristics. Variation was higher in fruit and nut variables compared to leaf variables. Principal Component and Hierarchical Cluster analyses in the present study showed that fruit and nut traits were the most important in quantification of morphological variation. Fruit and nut traits have been found to be important in structuring morphological variation in many tropical trees, such as *Tamarindus indica* L. (Fandohan et al. 2011) and *Balanites aegyptiaca* (L.) Delile (Abasse et al. 2011). It is clear therefore that fruit and nut traits provide a very considerable opportunity for identification and selection of plus trees for domestication.

The presence of bigger and heavier fruits in the Northern farming system, and larger-leaved trees in the West Nile farming system compared to the smaller-leaved trees of the Teso farming system as revealed by correlation and cluster analyses presents an interesting pattern. This pattern may be explained by environmental and abiotic factors such as rainfall, temperature, soil and altitude. Generally, rainfall in the shea butter tree belt of Uganda decreases from south to north (Kamanyire 2000). The Teso system, the most southerly of the three farming systems, is characterized by two distinct rain seasons separated by a four month dry spell. Meanwhile the West Nile and northern systems are characterized by uni-modal rains and a more intense dry season than the Teso system. In the West Nile system, high altitude areas receive much more rainfall (Ebanyat et al. 2010; Mwebaze 2010; Kamanyire 2000; Kabeere and Wulff 2008). In West Africa, shea butter tree agromorphological variables have also been found to be related to climatic gradients, with larger trees being more common in the drier areas (Sanou et al. 2006). The variation in fruit and nut variables recorded here are very close to those estimated by Sanou et al. (2006) in Mali indicating that the magnitude

of morphological variability within East and West African shea butter tree populations may be similar. Other tropical fruit trees that exhibit a similar variability pattern include *Balanites aegyptiaca* and *Tamarindus indica* (Soloviev et al. 2004).

There was a small but significant relationship between geographical coordinates (latitude, longitude and altitude) and morphological variables. In Uganda, climatic regimes are strongly associated with geographical location, with areas at low latitudes and high longitudes receiving higher rains and experiencing lower temperatures. Conversely, areas with higher latitudes and lower longitudes receive relatively lower rainfall and experience higher temperatures (Kamanyire 2000). Therefore, in spite of the significant albeit low correlation between morphological and geographical factors, it can be postulated that environmental influences play a role in determining the morphological properties of shea butter tree organs. Other factors that may explain morphological variation include natural selection (Irwin 2000; Tremblay et al. 2010; Darwin 1869), selection by humans (Vaughan et al. 2007) and gene flow mediated random genetic drift (Abasse et al. 2011).

4.4.2 Congruence between morphological variation and shea tree folk classification

There was lack of clear separation between ethno-varieties from different farming systems in this study suggesting that shea butter tree ethno-varieties are not aggregated on the basis of ethnographic separation. While ethnographic separation gives rises to variations in folk classification systems, these systems usually arise out of the recognition of and need to maintain diversity. Sometimes folk classification is done for no apparent value but for culinary purposes (Boster 1985). This may partly explain the lack of clear aggregation in morphometric variation on the basis of ethnographic separation. In addition, the use of morphological markers to study folk classification is bedevilled with numerous problems (Quiros et al. 1990). In most cases, farmers may use a single name to describe several phenotypes or several names for a single phenotype (Quiros et al. 1990). In the present study, farmers were observed to utilize a single rather than a combination of morphological traits to describe an ethno-variety. This is usually due to the need to utilize the most important traits that can conveniently communicate and describe particular crop varieties (Bellon 2002). It is, however, possible that these single traits used in folk classifications are genetically inherited hence their stability for use in folk classifications.

While the use of single trait descriptors may be important in folk classifications, anthropogenic and environmental influences may be under-played in such ethno-variety descriptions. There is high flux and movement of shea fruits/nuts by humans, which facilitates high levels of gene flow and low differentiation in traits of closely related offspring. Hence, shea butter trees have a high level of gene flow due to their out-crossing nature (Hall et al. 1996). This then presents the scenario of unresolved alliances in morphological variation among farmers' ethno-varieties as found in the present study. Similarly low levels of phenotypic differentiation have also been recorded in other African out-crossing and long-living tropical trees (Abasse et al. 2011; Bani-Aameur and Ferradous 2001; Kadzere et al. 2006; Leakey et al. 2005a; Leakey et al. 2005b).

Hierarchical cluster analysis on the basis of fruit and leaf quantitative variables revealed three groups. As pointed out from the discussion on general trends in morphological variation above, these groups are neither congruent to folk (ethnographic) classification nor to geographical separation. Therefore, the cause of the inherent differences enunciated by folk classifications may be due to the allogamous nature of shea butter trees. It is known that cross pollination between shea butter trees can give rise to highly phenotypic variants that are largely influenced by environmental factors (McGowen et al. 2010; Williams and Conner 2001). However, phenotypic variation could as well be genetic (Masood et al. 2005). This therefore makes it difficult to identify shea butter tree varieties on morphological evidence alone. However, a combination of quantitative and qualitative variables in the present study resulted into a more powerful resolution of clusters that are highly congruent to folk classification (Fig. 4.6). Given the lack of clear correspondence in quantitative morphology with an apparently distinct folk classification, there is need to utilize a combination of morphometric and farmers' perspectives of variation. However, given that even a combination of quantitative and qualitative variables may sometimes group similar ethno-varieties into different clusters as seen in Fig. 4.6, it is important to utilize more powerful biochemical and molecular markers in strategies for wider scale domestication programs.

4.5 Conclusion

While folk knowledge in Uganda classifies shea butter trees into 44 ethno-varieties based mainly on fruit and nut morphological traits, evidence presented here shows that there are no discrete groupings from quantitative morphological variation that are congruent with local folk classification. This is possibly due to the plasticity of morphological characters due to

environmental variability, the use of a single rather than multiple traits in folk classification and the allogamous nature of shea butter trees. A combination of quantitative and qualitative traits greatly enhances the resolution of inherent variation that is congruent to folk classification. Therefore, quantitative traits alone cannot be used to explain folk classification of shea butter tree ethno-varieties. There is need to utilise even more powerful biochemical and molecular markers to establish the fixed variation and hence validate the present folk classification of shea treeshea butter trees in Uganda.

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CHAPTER FIVE

FAT CONTENT AND FATTY ACID PROFILES OF SHEA TREE ETHNO-VARIETIES IN UGANDA

Abstract

Fat content and fatty acid composition are important nutritional properties of shea fruits. Farmers in Uganda report the presence of local shea tree ethno-varieties, but it is necessary to evaluate the economic importance of these ethno-varieties by investigating their relative fat content and fatty acid composition. The objectives of this study were to describe the fat content and fatty acid composition of shea tree ethno-varieties in Uganda and investigate the variation in their fat content and fatty acid composition. Near Infrared Spectrophotometry (NIRS) was used to determine the fat content as well as the fatty acid composition of 44 ethno-varieties. Wet chemistry (soxtec petroleum – ether fat extraction and gas chromatography) methods were used to validate the results from NIRS. Gravimetric determination of moisture content was also performed. Fat and moisture content ranged from 43.9 to 58.4% and 4.4 to 8.7% respectively while fatty acid composition was dominated by oleic (47 - 62%) and stearic acid (25 - 38%). Other fatty acids present were palmitic, vaccenic, linoleic, linolenic and arachidic acids. There was no significant difference in stearic, palmitic and oleic acid composition between ethno-varieties although significant variation in fat content, vaccenic and linoleic acid was observed between some ethno-varieties. This lack of significant difference in fat content and fatty acid composition was attributed to local site, climatic and tree-to-tree differences. These findings can be utilized for the selection of ethno-varieties that are suitable for commercial production of shea oil in Uganda.

Keywords: Shea butter tree; *Vitellaria paradoxa*; *nilotica*; fatty acids; near infrared spectrometry, provenance, Teso, West Nile

5.1 Introduction

The shea butter tree (*Vitellaria paradoxa* C. F. Gaertn. – Family Sapotaceae) (Hemsley, 1968) occurs over a wide geographical range of the savanna parklands in Sudano-Sahelian Africa. Its distribution extends from Senegal in West Africa to Uganda and Ethiopia in the eastern part of Africa. Taxonomically, *V. paradoxa* is a monospecific genus with two

subspecies: *paradoxa* and *nilotica* (Hall, *et al*., 1996; Hall & Hindle, 1995; Hemsley, 1968). The ranges of the two subspecies do not overlap, with subspecies *paradoxa* occurrying exclusively in west and central Africa (from Senegal to Cameroon) while subspecies *nilotica* occurs only in east Africa (specifically in the Central African Republic, Sudan, Uganda and south-eastern Ethiopia). The tree is important for shea oil, which is produced from dried kernels of the nuts (Hall, *et al*., 1996).

Shea oil is an important source of β-carotene (a source of vitamin A), vitamin F (Kar & Mital, 1981; Wiesman, *et al*., 2003) and tocopherols (or vitamin E) (Alander, 2004; Maranz, et al., 2003; Moharram, *et al*., 2006). In addition, shea oil is one of a few sources of fat in the local diets of the local farming communities of the savanna parklands of Africa (Maranz, *et al*., 2004; Schreckenberg, 2004). Apart from its use in cooking (Abbiw, 1990; Lamien, *et al*., 1996), shea oil is utilized in various social and cultural rituals such as traditional initiation, marriages, births, rainmaking, traditional worship, funerals and coronation rituals (Goreja, 2004; Moore, 2008). Shea oil is also used in traditional medicine, for example, in the treatment of wounds (Egunyomi, *et al*., 2009; Jiofack, *et al*., 2010; Lawal, *et al*., 2010) and its importance to local communities is further manifested by its use in local poetry, song and dance (Fardon, 1990; Kuwabong, 2004).

Shea oil attracts attention for industrial and commercial uses on account of the dominant fatty acids it contains. Shea oil is principally composed of palmitic, stearic, oleic, linoleic, and arachidic acids (Akihisa, *et al*., 2010; Davrieux, *et al*., 2010; Hall, *et al*., 1996; Maranz, *et al*., 2004). Stearic and oleic acids are the two dominant fatty acids, accounting for 85-90% of the total fatty acid composition. The relative proportion of these two fatty acids defines the two subspecies of shea trees (Davrieux, *et al*., 2010). Stearic acid dominates in subspecies *paradoxa*, and is specifically ideal for use as an industrial base for confectionary and food products (Kraft & Lynde, 2005). On the other hand, oleic acid dominates in subspecies *nilotica* and is specifically ideal as a raw material for cosmetic and personal care applications, especially due to its high content of un-saponifiable components (Alander, 2004). Variation in fat content and fatty acid composition, therefore, significantly influences the marketability, including the nutritional value and shelf life of shea tree nuts and oil (Beuchat & Worthington, 1978). Human consumption of certain fatty acids, such as oleic acid, is associated with health benefits such as the lowering of blood cholesterol levels (Williams, 2000). Many food industries, therefore, undertake expensive hydrogenation processes to alter

fatty acid composition of various foods to suit consumer requirements (Ursin, 2003). Identification of ethno-varieties and provenances with high fat content and desired fatty acid profiles would therefore transform the shea oil industry in Uganda to a lucrative venture or enterprise. Fat content and fatty acid composition therefore provide a good opportunity to characterize particular shea trees with desirable traits for possible production linked to particular markets.

However, while recent studies suggest variable fat content and fatty acid composition in shea oil (Akihisa, *et al*., 2010; Badifu, 1989; Davrieux, *et al*., 2010; Glew, *et al*., 1997; Maranz, *et al*., 2004; Sawadogo & Bezard, 1982), little is known about such variation in shea tree phenotypes which farmers identify as distinct ethno-varieties. Most studies have often treated shea trees in Uganda as an entity without segregation into ethno-varieties. The objectives of this study were to describe the fat content and fatty acid composition and investigate the variation in fat content and fatty acid composition content of shea tree ethno-varieties in Uganda. It is important to understand the variation in fat content and fatty acid composition of shea tree ethno-varieties as this knowledge can be useful in shea tree improvement programmes.

5.2 Methods

Shea trees in Uganda have been reported by Gwali, *et al*. (2011) to comprise of 44 ethno-varieties. According to the authors (Gwali, *et al*. ibid), shea tree folk classification is based on fruit organoleptic (taste and colour) and morphological (size and shape) properties and can be categorised under 13 broad descriptors (Table 5.1). In this study, 44 ethno-varieties documented by Gwali, *et al*. (op. cit.) were assessed for fat content as well as fatty acid composition.

5.2.1 Sample collection and preparation

A total of 40-50 ripe fruits were collected from 180 trees belonging to 44 ethno-varieties recognized by local farmers in the northern, Teso and West Nile farming systems of Uganda. These farming systems form the distributional range of shea trees in Uganda. The vegetation in these farming systems is classified as *Combetrum* / *Vitellaria* and grass savannas (Langdale-Brown, *et al*., 1964). Each fruit was de-pulped and the nuts oven dried at 55° C for 2 to 3 days. The oven dried nuts were ground for 8 seconds to a coarse powder in a Verwoerk grinder. The powder was then finely ground in an SEB Moulin Prepline 850 coffee grinder to

produce a consistent fine powder. Samples that were not satisfactorily ground by the coffee grinder were subjected to finer grinding under liquid nitrogen. Samples were then stored at -20° C until required for analysis.

Table 5.1. List of ethno-varieties and their corresponding descriptors

Trait	Descriptor	Ethno-variety
Pulp taste	Astringent pulp	Egeget, Etria, Menacwot, Nyangili_Macwot, Udanyo_Macwot
	Sweet pulp	Abono, Ewiny, Limi, Mbilimbili
	Tasteless pulp	Asa, Epiana
Pulp quantity	Little pulp	Ajiki, Upende_Aboro
	Much pulp	Amoo, Mudaa
Fruit/nut size	Big fruits/nuts	Enyii, Mbele
	Small fruits/nuts	Abor, Alindiri, Ciria, Nyiri, Yao-Matino
Fruit/nut shape	Oval/elliptical fruits/nuts	Acula, Aloto, Coloa, Julu, Nasomel
	Round fruits/nuts	Alulung, Gburua, Mangulungulu, Mulunge, Nalungur, Ngulu
Pulp hardness	Hard pulp	Acogo, Ngorokwa, Nyangili_Acogo, Yao_Atega
	Soft pulp	Apocopoco, Mayom, Upende_Appi
Fruit hairiness	Hairy fruit	Ajayer, Layer, Nacekum, Nyangili_Ayir

5.2.2 Analytical procedures

Fat content and fatty acid composition were determined using non-solvent (Near Infrared Spectrophotmetry or NIRS), solvent (Soxtec petroleum-ether and gas chromatography) and gravimetric methods. Near Infrared Spectrophotometry, the main analytical procedure in this study, is a non-solvent, rapid, non-destructive and quantitative method that is increasingly being employed in the analysis of fat content and fatty acid composition in food and forage samples (Dempsey, *et al.*, 1996). The solvent and gravimetric methods were utilized to validate the values obtained from near infrared spectrophotometry.

Approximately 3 – 8 g of fine shea nut powder were loaded into 50 mm diameter cells and analyzed for reflectance using a FOSS NIRSystems 6500 spectrophotometer (Foss NlRSystems, Silver Spring, MD) running ISI version 3.1 software (InfraSoft International, Port Matilda, PA, USA). Fat content and fatty acid composition values were determined by scanning each sample at a wavelength range of 400 nm to 2500 nm. Data were saved as

spreadsheets and analyzed using the Statistical Package for Social Scientists (SPSS) version 16.0.

To validate the data obtained through NIRS, gravimetric and wet chemistry methods described below were also employed:

Fat extraction by petroleum – ether was conducted in a semi-automatic Soxtec 2050 extractor (FOSS Analytical, Denmark). Approximately 4 g of sample were weighed and placed in 33 × 80 mm Whatman extraction thimbles (Carlo Erba Reactifs, SA). Fat extraction was conducted in 3 cycles at 100° C: extraction (60 min), rinsing (30 min), evaporation (25 min) and drying (5 min). Extracted fat was collected in pre-weighed aluminium cups and the fat content determined the formula below:

$$MG = \frac{w_i - w_{ii}}{m} \times 100$$

Where w_i = initial weight (in grams) of cup and sample, w_{ii} = final weight (in grams) of cup and sample after Soxtec extraction, m = weight of sample (in grams). The fat was then placed in vials and stored at -20° C until its use in gas chromatography.

Fatty acids were extracted by esterification of the triacylglycerols in shea oil using sodium methylate (CH_3NaO) under hexane (C_6H_{14}) to produce fatty acid methyl esters (FAME). Up to 3 ml of sodium methylate were added to a flask containing 2 to 3 drops of phenolphthalein and the mixture refluxed for about 10 min. Approximately 3 ml of hydrochloric methanol (Hcl-MeOH) were then added to the mixture until saponification of phenolphthalein. The mixture was again refluxed for 10 mins and allowed to cool. The FAME was then concentrated by adding 8 ml of hexane and 10 ml of distilled water. One microlitre (1µl) of the hexane – FAME mixture was then analyzed with a Thermo Focus Gas Chromatograph (Thermo Fisher Scientific, Inc. MA) fitted with a Flame Ionization Detector (FID) on a non-bonded CP SIL 88 column (Varian, Inc., CA). The injector and detector temperatures were set at 250° C and 270° C respectively. The oven temperature was allowed to build from 150° C to 225° C at a rate of 5° C per min. The temperature was then held at 225° C for 2 mins before cooling for the next run. The chromatographic peaks were identified by comparison with a coco-test standard and captured using Chrom-Card data system.

5.2.3 Data analysis

Pearson's bi-variate correlations were computed to determine the relationship between fatty acid composition and fat content. One-Way Analysis of Variance (ANOVA) was used to test for differences between mean values of fatty acids and fat content within ethno-varieties. Equality of variances was tested by the Levene's (Levene, 1960) and Welch test (Welch, 1951). Due to differences in sample sizes and variances, differences in mean values ($p = 0.05$) between ethno-varieties were analyzed using the Games – Howell multiple comparison post hoc test (Games & Howell, 1976). This test is useful in instances of unequal variances and also takes into account unequal group sizes (Tamhane, 1979). All data were analyzed using the Statistical Package for Social Scientists (SPSS) version 16.0.

5.3 Results

Fat content values ranged between 43.9% and 58.4% with an average value of 53.5% (Table 5.2). The highest fat content was observed in *Ajayer* (58.4% ± 2.2) and *Aloto* (58.4% ± 2.9). *Yao atega* (43.9% ± 5.2) and *Egeget* (43.9% ± 2.2) had the lowest fat content. Fat content did not differ significantly among the ethno-varieties assessed ($F_{40, 136} = 1.306$; $p = 0.132$). However, post-hoc analysis indicated that fat content varied significantly between *Ajiki* and nine other ethno-varieties, namely, *Limi, Mayom, Mbele, Ngulu, Nyangili-Acogo, Nyangili-Ayir, Nyangili-Macwot, Nyiri* and *Udanyo-Macwot.* Fat content was significantly higher in *Ajiki* compared to any of the listed ethno-varieties. Similarly, fat content was significantly ($p < 0.05$) higher in *Acula* and *Menacwot* compared to *Nyangili-Macwot.*

Table 5.2. Mean, minimum and maximum fat content and fatty acid composition of shea tree ethno-varieties (n=180) in Uganda.

Variable	Minimum	Maximum	Mean	StDev	CoefVar
Fat content (MG)	43.88	58.40	53.46	2.22	4.14
Palmitic acid (C16:0)	3.55	5.31	4.44	0.29	6.46
Stearic acid (C18:0)	25.31	38.48	32.49	1.99	6.13
Oleic acid (C18:1 n-9)	47.35	62.04	54.06	1.99	3.68
Vaccenic acid (C18:1 n-7)	0.29	0.62	0.42	0.07	15.52
Linoleic acid (C18:2cc)	4.72	8.97	6.21	0.54	8.65
Linolenic acid (C18:3 n-3)	0.13	0.37	0.25	0.05	19.08
Arachidic acid (C20:0)	0.76	1.12	0.98	0.07	7.26
Sat/Unsat acid ratio	0.43	0.81	0.62	0.06	8.91

Fatty acid profiles were dominated by two unsaturated fatty acids (oleic and linoleic acids), and two saturated (palmitic and stearic acids) (Figure 5.1, Tables 5.2 and 5.3). Over 87% of the fatty acid composition consisted of stearic acid (25 - 38%) and oleic acid (47 - 62%). Linoleic and palmitic acids were present in an average proportion of 6% and 4% respectively. The lowest values of linoleic (4.72%) and palmitic (3.55%) acids were observed in *Ajiki* and *Enyii*, while the highest values of linoleic (8.97%) and palmitic (5.31%) acids were observed in *Yao atega*. Vaccenic, linolenic and arachidic acids were present in percentages lower than 1%. Stearic and oleic acid composition were very strongly negatively correlated ($r = -0.944$, $p < 0.01$) while fat content was strongly correlated to linoleic acid content ($r = -0.690$, $p < 0.01$) (Table 5.4).

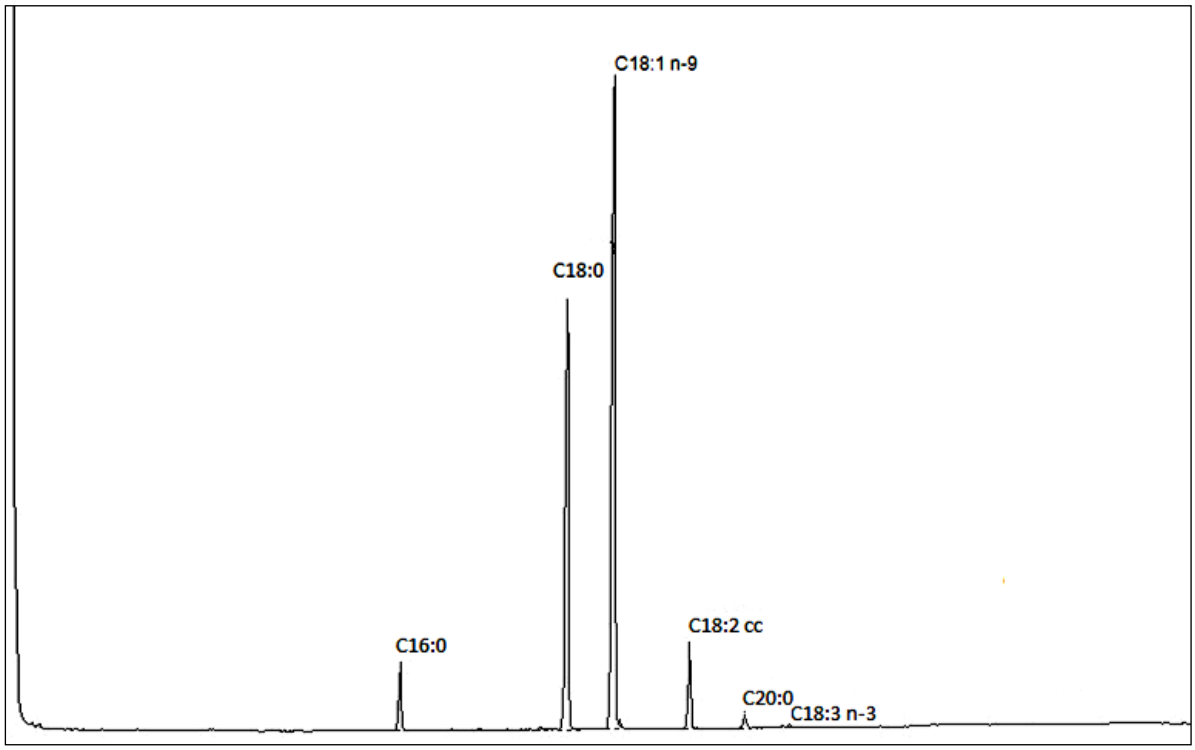


Figure 5.1. Typical gas chromatography fatty acid profile of a shea tree ethno-variety sample from Uganda.

There was no significant difference ($p > 0.05$) in stearic, oleic and palmitic acid composition between ethno-varieties. Similarly, the saturated to unsaturated acid ratio was not significantly different among the different ethno-varieties. Vaccenic acid composition was significantly more in *Yao matino* compared to either *Nacekum, Ngorokwa, Nyangili Macwot, Mangulungulu* or *Nyiri.*

Table 5.3. Percent composition of fatty acid, fat and moisture content of shea tree ethno-varieties in Uganda (n = 176)

Ethno variety	MG	C16:0	C18:0	C18:1n-9	C18:1n-7	C18:2cc	C18:3n-3	C20:0	Sat/Unsat
Abor	54.22	4.58	33.23	53.34	0.46	6.29	0.29	0.97	0.64
Acogo	53.62	4.62	32.07	54.49	0.51	5.95	0.30	0.94	0.62
Acula	55.27	4.38	32.66	54.48	0.45	5.83	0.23	0.95	0.62
Ajayer	55.22	4.33	34.09	52.99	0.45	5.85	0.26	0.97	0.66
Ajiki	56.37	4.31	33.85	53.87	0.50	5.42	0.30	0.95	0.65
Alindiri	52.58	4.67	32.80	53.62	0.46	6.30	0.29	0.96	0.63
Aloto	54.36	4.48	31.65	54.94	0.46	6.23	0.26	0.93	0.60
Alulung	52.79	4.56	32.10	54.18	0.45	6.28	0.29	0.99	0.62
Amoo	53.64	4.31	30.23	56.80	0.45	6.30	0.30	0.93	0.56
Apocopoco	54.29	4.40	34.10	53.04	0.46	5.77	0.24	0.96	0.67
Asa	54.15	4.33	32.77	53.73	0.39	6.24	0.23	0.94	0.63
Ciria	53.64	4.60	30.85	55.72	0.48	6.35	0.24	0.94	0.58
Coloa	53.20	4.53	32.32	54.34	0.43	6.24	0.24	0.99	0.62
Egeget	51.43	4.30	30.65	55.36	0.41	6.82	0.29	0.97	0.57
Enyii	54.86	4.25	32.66	54.30	0.39	6.01	0.24	1.00	0.63
Epiana	52.34	4.16	32.19	53.98	0.35	6.88	0.24	1.05	0.61
Ewiny	52.96	4.16	31.30	55.36	0.40	6.42	0.25	1.02	0.59
Gburua	54.82	4.48	31.82	55.18	0.45	6.02	0.24	0.97	0.60
Julu	53.76	4.50	33.40	53.01	0.43	6.16	0.21	0.94	0.65
Layer	53.21	4.43	33.04	53.23	0.43	6.37	0.29	0.97	0.64
Limi	52.86	4.49	31.65	55.17	0.42	6.05	0.23	0.97	0.60
Mangulungulu	52.43	4.47	32.56	53.75	0.35	6.29	0.19	0.97	0.63
Mayom	53.67	4.54	33.27	53.55	0.42	6.03	0.24	1.00	0.64
Mbele	53.36	4.29	34.87	51.40	0.38	6.30	0.22	1.04	0.69
Mbilimbili	54.12	4.34	33.09	53.74	0.47	5.98	0.22	0.98	0.64
Menacwot	55.89	4.48	32.51	54.87	0.41	5.50	0.22	0.93	0.62
Mudaa	53.50	4.36	31.56	55.12	0.41	6.31	0.23	0.98	0.59
Mulunge	54.95	4.52	32.46	54.12	0.47	5.92	0.25	0.96	0.63
Nacekum	51.29	4.46	32.08	53.61	0.39	6.92	0.27	1.02	0.61
Nalungur	52.60	4.46	31.34	54.80	0.39	6.56	0.26	0.98	0.59
Nasomel	52.09	4.38	30.61	55.41	0.40	6.82	0.25	0.95	0.57
Ngorokwa	53.03	4.41	31.69	55.04	0.36	6.22	0.21	1.00	0.60
Ngulu	53.41	4.49	34.17	52.45	0.44	5.96	0.22	0.98	0.67
Nyangili_Acogo	52.03	4.52	33.50	52.70	0.42	6.25	0.26	1.06	0.66
Nyangili_Ayir	53.11	4.28	32.51	53.97	0.40	6.47	0.24	1.02	0.62
Nyangili_Macwot	51.96	4.57	32.93	53.28	0.39	6.35	0.23	1.04	0.64
Nyiri	52.89	4.51	32.31	53.93	0.42	6.12	0.25	0.96	0.62
Udanyo_Macwot	52.87	4.64	31.38	55.51	0.39	5.70	0.19	0.92	0.60
Upende_Aboro	53.35	4.28	35.10	51.38	0.36	6.08	0.20	1.06	0.70
Yao_Atega	51.25	4.49	31.39	54.10	0.45	7.02	0.32	0.94	0.60
Yao_Matino	53.69	4.62	31.54	54.57	0.48	6.20	0.30	0.92	0.60

The ethno-variety *Ajiki* contained significantly more vaccenic acid compared to *Mangulungulu.* Linoleic acid content was significantly less in *Menacwot* compared to either *Asa, Alindiri, Nacekum, Nyangili Ayir* or *Nyangili Macwot.* Similarly, the linoleic acid content in *Acula* was significantly less than that in *Nacekum* or *Nyangili Macwot.* The ratios

of saturated to unsaturated fatty acids for all the ethno-varieties were all below 1.0 due to the dominance of stearic and oleic acids. The lowest saturated to unsaturated acid ratios were observed in *Amoo* (0.56 ± 0.09), *Egeget* (0.57 ± 0.08) and *Yao_Atega* (0.60 ± 0.08). The highest saturated to unsaturated acid ratio was recorded in *Upende aboro* (0.70 ± 0.07).

Table 5.4. Correlation of the most important fatty acids in shea tree ethno-varieties in Uganda (n=180).

Variable	MG	C16:0	C18:0	C18:1n-9	C18:1n-7	C18:2cc	C18:3n-3	C20:0
C16:0	-0.008							
C18:0	.280**	0.127						
C18:1n-9	-0.061	-.199**	-.944**					
C18:1n-7	.369**	.455**	0.01	0.09				
C18:2cc	-.690**	-.166*	-.461**	.205**	-.382**			
C18:3n-3	-.150*	.192**	-0.094	0.001	.316**	.274**		
C20:0	-.406**	-.379**	.308**	-.356**	-.494**	.168*	-.176*	
Sat/Unsat	.234**	.227**	.991**	-.962**	0.018	-.441**	-0.076	.291**

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

5.4 Discussion

Fat content values were not significantly different among the various ethno-varieties. For example, the highest fat content was recorded in the *Ajiki, Menacwot and Acula* whose distribution is separated only by about 100 km from ethno-varieties such as *Nacekum* and *Egeget* which had the lowest fat content. These observations imply that fat content may be responsive to local site differences.

With regard to fatty acid composition, all ethno-varieties exhibited a pattern similar to what has been reported in earlier investigations (Akihisa, *et al*., 2010; Badifu, 1989; Davrieux, *et al*., 2010; Maranz, *et al*., 2004; Mbaiguinam, *et al.*, 2007; Okullo, *et al*., 2010). The dominant fatty acids, whose content was not significantly different among ethno-varieties, were oleic and stearic acids. Since the stearin and olein fraction determines the usefulness of an oil for either cosmetic or pharmaceutical use, all these ethno-varieties can be considered to have similar potential for these uses. All ethno-varieties had proportionally higher oleic acid, in addition to other unsaturated fatty acids.

The mean saturated to unsaturated fatty acids ratio in this study ranged from 0.43 – 0.81, implying that unsaturated fatty acids are predominant in all these ethno-varieties. It is known that oils with a high proportion of unsaturated fatty acids can be heated to high temperatures without smoking, which leads to faster cooking time and utilization of less oil (Miller, *et al.*, 1987). In addition, since unsaturated fatty acids are particularly required for normal body functioning (Harper & Jacobson, 2001; Williams, 2000) due to their high stability (Cunnane, *et al*., 1993), ethno-varieties with considerable quantities of these possess high commercial potential. This implies that ethno-varieties with high saturated to unsaturated ratios, such as *Upende aboro* would be very ideal for industrial production that aims for more stable as well as long storing oil.

5.5 Conclusion

This study shows that the mean fat content of shea tree ethno-varieties in Uganda ranged from 43.9 to 58.4%. Meanwhile, fatty acid composition was dominated by oleic (47 - 62%) and stearic acid (25 - 38%). Other fatty acids found included palmitic, stearic, oleic, vaccenic, linoleic, linolenic and arachidic acids. Pair-wise comparisons showed no significant variation in stearic, palmitic and oleic acid composition. However, significant variation in fat, vaccenic and linoleic acid was observed between some (and not all) ethno-varieties. The possible cause of this variation could be local site, climatic and tree-to-tree differences. Given the importance fatty acid composition and saturated-to-unsaturated acid ratio, these data could be useful in the selection of ethno-varieties that are suitable for commercial production of shea nut oil in Uganda.

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CHAPTER SIX

GENETIC DIVERSITY IN SHEA TREE ETHNO-VARIETIES ASSESSED WITH MICROSATELLITE MARKERS

Abstract

Shea trees (*Vitellaria paradoxa* C. F. Gaertn.) are classified locally into several folk or ethno-varieties by farmers in Uganda. Local perceptions of plant variability are the precursors of many valuable plant varieties. In this study, the genetic basis of folk classification was assessed using microsatellite markers. A total of 118 individual shea trees constituting 28 ethno-varieties sampled from three farming systems of Uganda were analysed at 10 microsatellite loci. The number of alleles amplified per microsatellite locus ranged from 6 to 13 with an average of 9.5, with a total of 106 alleles identified. Observed (H_o) and expected heterozygosity (H_e) per locus ranged between 0.366 to 0.934 and 0.580 to 0.840 respectively. Mean H_o and H_e values for all loci across all ethno-varieties were 0.634 and 0.690 respectively. Analysis of molecular variance indicated that most of the variation (86.90%) occurred within individual trees; 8.43% was found among individual trees within ethno-varieties while 4.67% was found among ethno-varieties. The in-breeding index ($f = 0.088$), fixation index ($\theta_P = 0.047$), gene flow value ($N_m = 2.59$) and cluster analysis show that all shea tree ethno-varieties were a single out-crossing population with very low genetic differentiation and high gene flow. The low differentiation in shea tree ethno-varieties was most likely due to the utilisation of non-genic traits in folk classification.

Keywords: *Vitellaria paradoxa*, microsatellites, gene flow, genetic diversity, heterozygosity, ethno-varieties

6.1 Introduction

Vitellaria paradoxa (‘shea butter tree’ in English or simply ‘shea tree’ and ‘karite’ in French) is an indigenous African tree of the Sudano-Sahelian belt (Hall, *et al*., 1996; Hemsley, 1968). Its distribution spans 20 African countries, from Senegal in West Africa to Uganda in eastern Africa (Hall, *et al*., 1996). The species is taxonomically divided into two subspecies, *paradoxa* (found in West and central Africa) and *nilotica* (found in east Africa). Shea trees bear fruits whose nuts are cherished locally and internationally for their high oil content. It is the most important source of oil for the people in the shea belt (Gwali, *et al*., 2012; Moore,

2008). The shea fruit pulp is eaten as a food supplement especially during the dry seasons when shea tree fruits tend to mature and ripen (Maranz, *et al*., 2004). In some African countries such as Burkina Faso, there is some local processing of fruit jam from shea fruit pulp (Sanou & Lamien, 2011). Shea trees are also utilised for medicinal and cultural purposes, such as in the treatment of wounds, making of funeral beds, pregnancies, births and weddings (Goreja, 2004; Gwali, *et al*., 2012; Hall, *et al*., 1996; Ferris, *et al*., 2004). There are reports of the use of shea oil in war rituals by some traditional communities in Uganda (Sturges, 2008). Shea trees are therefore economically important to the local economies of many African traditional communities.

Due to the high degree of socioeconomic and cultural significance of shea trees, a system of folk classification below the subspecies level has evolved among local communities in Uganda. A recent survey of folk classification of subspecies *nilotica* in Uganda indicated that local people classify shea trees into 'ethno-varieties' based on criteria such as fruit pilosity; fruit/nut size and shape; nut color; pulp color, quantity, taste and eubstance (hardness or softness) as well as habitat (Gwali, *et al*., 2011). Shea trees that produce fruits with sweet pulp are, for example, classified as a distinct variety from trees that produce fruits with astringent pulp. Similarly, trees that bear large fruits are recognized as different from trees that bear small fruits. Since these phenotypic traits are usually products of long term adaptation, it is important to understand how genetically fixed they are.

Genetic markers have shown important and critical application in designing effective conservation and breeding programmes (Veasey, *et al*., 2008). Unfortunately, shea tree genetics, especially of farmers' varieties, is still poorly understood. So far, several authors have studied the existing diversity and pattern of genetic variation within shea trees at different geographic levels using isozymes (Lovett & Haq, 2000), neutral DNA markers (Bouvet, *et al*., 2004; Fontaine, *et al*., 2004; Allal, *et al*., 2008; Bouvet, *et al*., 2008; Cardi, *et al*., 2005; Kelly, *et al*., 2004; Logossa, *et al*., 2011) or a few genes involved in oil production pathways (Allal, *et al*., 2011)..

Microsatellites have distinguished themselves as excellent molecular markers in characterising genetic diversity, population history and signature of selection in a range of plant species (Dhillon, *et al*., 2009; Varshney, *et al*., 2005; Gupta & Varshney 2000; Majourhat, *et al*., 2008). Most studies that have characterised the genetic variation in shea

trees have not focussed on unravelling the genetic basis of farmers' (folk) classifications. Since farmers criteria and perceptions in varietal selection are pivotal to crop improvement programmes (Chi, *et al*. 2007), it is necessary to identify and understand the genetic basis of shea tree ethno-variety classifications. This work represents the first molecular characterization of shea tree ethno-varieties. The main objective of this study, therefore, was to characterize shea tree ethno-varieties in Uganda using microsatellite markers. The specific objectives were to describe the distribution of genetic variation within and between shea tree ethno-varieties investigate whether the pattern of genetic variation is concordant with shea tree folk classification in Uganda.

6.2 Methods

6.2.1 Plant materials

Individual trees of putative shea tree ethno-varieties (Gwali, *et al*., 2011) were identified by key informants/respondents through interviews. A total of 118 trees representing 28 shea tree ethno-varieties were assessed (Table 6.1). Due to the difficulty in identifying an equal number of individual trees of each ethno-variety, sample sizes were uneven for the different ethno-varieties. Only ethno-varieties with at least three individuals were considered for inclusion in this analysis. Sampling from a similar or even fewer number of individuals is not uncommon in genetic characterization of farmer's varieties (cf. Pervaiz, *et al*., 2010; Turet-Sayar, *et al*., 2012; Meerow, *et al*., 2003). Two or three young leaves of healthy appearance were collected from each tree, cleaned with ethanol and placed in zip-lock bags containing silica gel (Weising, *et al*., 1995) in order to desiccate them. The specimen samples were stored at ambient temperature until further use.

6.2.2 Microsatellite marker analysis

Approximately 50 mg of silica dried leaves were placed in a 20 ml tube and flash frozen in liquid nitrogen. The super frozen leaf samples were ground to fine powder using an automatic grinder (Retsch® MM301, Haan, Germany) at a frequency of 30 Hz for 1 to 2 minutes. Total genomic DNA was extracted using the Dolezel – Matab method (Ky et al. 2000). Up to 1.8 ml of Dolezel buffer (pH = 9) containing 15 mM of Tris, 2 mM of Na_2EDTA, 80 mM of KCl, 20 mM of NaCl and deionised H_2O was autoclaved, mixed with 0.2% β-mercaptoethanol and 0.5% ×100-Triton™ and then added to the powder samples before centrifugation for 20 minutes at 4 000 g. Following centrifugation, the supernatant was discarded and the pellets were re-suspended in 1.3 ml of autoclaved lysis buffer (pH = 8) containing 0.1 M Tris-HCl

(pH = 8), 0.02 M Na_2EDTA (pH = 8), 1.25 M NaCl and de-ionised H_2O mixed with 4% Matab and 0.2% β-mercaptoethanol. DNA quantitation was performed on a Shimdazu BioSpec-Mini spectrophotometer (Shimadzu Corporation, Tokyo, Japan) at a wavelength of 260 and 280 nm. All DNA samples were diluted with de-ionised water to a working concentration of 50 ng/µl. The samples were then stored at -20° C for further use.

Table 6.1. Folk nomenclature and literal meanings of shea tree ethno-varieties from three farming systems assessed in this study

Farming system	Ethno-Variety	Literal meaning
Northern	Layer	Hairy fruits
	Acogo	Hard pulp
	Yao_Atega	Hard pulp
	Ajiki	Little pulp
	Amoo	Much pulp
	Acula	Oval fruits or nuts
	Aloto	Oval fruits or nuts
	Abor	Small fruits or nuts
	Alindiri	Small fruits or nuts
	Apocopoco	Soft pulp
Teso	Egeget	Astringent pulp
	Nasomel	Oval fruits or nuts
	Nalungur	Round fruits or nuts
	Ewiny	Sweet pulp
	Epiana	Tasteless pulp
West Nile	Nyangili_Macwot	Astringent pulp
	Mbele	Big fruits or nuts
	Ngorokwa	Hard pulp
	Upende_Aboro	Little pulp
	Mudaa	Much pulp
	Julu	Oval fruits or nuts
	Coloa	Oval fruits or nuts
	Ngulu	Round fruits or nuts
	Nyiri	Small fruits or nuts
	Ciria	Small fruits or nuts
	Mayom	Soft pulp
	Limi	Sweet pulp
	Asa	Tasteless pulp

Ten SSR markers *mCIRVp167, mCIRVp168, mCIRVp134, mCIRVp113, mCIRVp175, mCIRVp191, mCIRVp14, mCIRVp28, mCIRVp159, mCIRVp181* (Allal et al. 2008) were used for this study. PCR amplification reactions were duplexed in a total volume of 10 µl

containing: 3.64 µl de-ionised H_2O, 5 µl 1× Qiagen Kit Multiplex buffer, 0.08 µM forward and 0.1 µM reverse primers, 0.1 µM Oligo M13 (BIOTROP) dye and 0.5 ng/µl of DNA. PCR amplifications were performed using an Eppendorf Mastercycler under the following reaction conditions: Step 1 (Denaturation at 95° C for 15 min, Hybridization at 67° C for 1 min 30 sec and Elongation at 94° C for 1 min 30 sec); Step 2 (Denaturation at 94° C for 30 sec, Hybridization at 65° C for 1 min 30 sec running 8 cycles with a drop of 2° C consecutively and Elongation at 72° for 1 min), and Step 3 (Denaturation at 94° C for 30 sec, Hybridization at 51° C for 1 min 30 sec running 24 cycles and Elongation at 72° C for 1 min). An additional elongation step was performed at 60° C for 30 min.

6.2.3 Electrophoresis of amplification products

After amplification, a 2 µl aliquot of the amplified SSR samples was combined with 6 µl of loading mix (loading blue + desmiling mix containing markers of the following sizes: 363 – 305 – 247 – 198 – 174 – 140 – 111 – 71 bp). The SSR sample mixes were then denatured on a thermocycler for 3 minutes at 74° C before electrophoresis. Electrophoresis was performed at 700 nm and 800 nm on a LiCor 4300 sequencer. Genotyping was performed using SAGA^{GT} version 3.1 software (LI-COR®).

6.2.4 Data Analysis

The mean number of alleles (A), proportion of polymorphic loci (P), mean number of alleles per polymorphic loci (A_p), expected heterozygosity (H_e), observed heterozygosity (H_o) and fixation indices were computed using the Genetic Data Analysis (GDA) software (Lewis & Zaykin, 2001). In GDA, Wright's conventional F-statistics i.e. F_{is} (the inbreeding coefficient of an individual relative to the subpopulation), F_{it} (the inbreeding coefficient of an individual relative to the total population) and F_{st} (the inbreeding among subpopulations relative to the total population) are computed as f, F and θ_P respectively. The GDA notation is henceforth used to describe these statistics. Polymorphic information content (PIC), which indicates the percentage contribution of each locus to the polymorphism in the analysis (Botstein et al., 1980), was calculated using the Microsatellite toolkit (Botsten, White, Skolnick, & Davis, 1980) in Excel 2007 (Microsoft® Corporation). Deviations from Hardy-Weinberg equilibrium were tested using the Markov chain method in Genepop version 4.1.0 (Rousset, 2008). Markov chain parameters were set at 10 000 dememorization steps for 20 batches at 5 000 iterations per batch.

To test for genetic variability, an Analysis of Molecular Variance (AMOVA) was performed using Arlequin version 3.5.1.3 (Excoffier & Lischer, 2010). Population genetic structure was assessed using hierarchical F-coefficients as implemented in GDA. Confidence intervals for the F-statistics at the 95% level were obtained by bootstrapping 20 000 times over the 10 loci that were used in this study. Gene flow was estimated using the following formula (Slatkin & Barton, 1989):

$$N_m = \frac{1-F_{st}}{4F_{st}}.$$

Relationships between individual trees were assessed based on a dissimilarity matrix constructed using the neighbour – joining method as implemented in DARwin 5.0 (Perrier & Jacquemoud-Collet, 2006). Missing data were handled by deletion of all units with missing data. Dissimilarity was calculated based on simple matching coefficients. Cluster analysis was performed using the un-weighted neighbour joining method (Nei, 1978) in DARWin 5.0.158. To test the reperesentativeness of the resulting dendrogram with the genetic structure in the data matrix, ana analysis of fit using the fit criterion option in DARWin 5.0.158 was performed. The resulting dendrograms were visualised in TreeView version 1.6.6 (Page, 1996).

6.3 Results

6.3.1 Allele frequency distribution

The mean percentage of polymorphic loci was 100% (0.99 criterions). All loci exhibited high polymorphism information content (PIC values ranged from 0.510 to 0.817). A total of 106 alleles were detected over the 10 SSR loci across the 28 shea tree ethno-varieties. The number of alleles (A) per locus ranged from 6 (*mCIRVp28*) to 13 (*mCIRVp14*) with an average of 9.5 alleles. The mean number of alleles per locus among the 28 ethno-varieties ranged from 2.7 within *Alindiri* and *Mbele* to 4.6 within *Acula.* A total of 19 private alleles were found among 14 ethno-varieties across seven of the 10 loci assessed in this study. Locus *mCIRVp191* and *mCIRVp14* (4 private alleles each) had the highest number of private alleles, followed by *mCIRVp175* with 3 private alleles. The ethno-variety *Ajiki* (3 private alleles) had the highest number of private alleles, followed by *Ciria, Limi* and *Mayom* with 2 private alleles each (Table 6.2).

Table 6.2. Genetic variability of 10 SSR loci assessed in this study. (n = sample size per locus, P = proportion of polymorphic loci, A = number of alleles per locus, A_p = number of alleles per polymorphic locus, H_e = Nei's (1978) un-biased estimate of the expected proportion of heterozygous loci per individual, H_o = observed heterozygosity, f = inbreeding coefficient of an individual relative to the sub-population, F = inbreeding coefficient of an individual relative to the total population, θ_P = inbreeding among sub-populations relative to the total population, PIC = polymorphism information content.

Locus	**n**	**P**	**A**	**A_P**	**H_e**	**H_o**	**f**	**F**	**θ_P**	**PIC**
mCIRVp167	111	1	8	8	0.676	0.459	0.292	0.322	0.043	0.628
mCIRVp168	106	1	10	10	0.777	0.934	-0.230	-0.202	0.023	0.742
mCIRVp134	93	1	7	7	0.580	0.366	0.272	0.374	0.140	0.510
mCIRVp113	105	1	8	8	0.720	0.619	0.119	0.142	0.025	0.670
mCIRVp175	98	1	10	10	0.656	0.561	0.123	0.146	0.026	0.597
mCIRVp191	96	1	12	12	0.826	0.510	0.378	0.383	0.009	0.798
mCIRVp14	113	1	13	13	0.832	0.779	0.019	0.065	0.047	0.808
mCIRVp28	113	1	6	6	0.700	0.611	0.058	0.130	0.077	0.646
mCIRVp159	112	1	9	9	0.664	0.670	0.016	-0.010	-0.026	0.629
mCIRVp181	113	1	12	12	0.840	0.823	-0.098	0.023	0.110	0.817
All	**106**	**1**	**9.5**	**9.5**	**0.727**	**0.633**	**0.088**	**0.131**	**0.047**	

6.3.2 Genetic diversity

Observed (H_o) and expected heterozygosity (H_e) were consistently high ($\geq$ 0.400) in all ethno-varieties and most loci, except for locus *mCIRVp134* (H_o = 0.366). H_o values ranged from 0.366 at locus *mCIRVp134* to 0.934 at locus *mCIRVp168* while H_e values ranged from 0.580 at locus *mCIRVp134* to 0.840 at locus *mCIRVp181* (Table 6.2). The highest H_o value across all populations (ethno-varieties) was 0.817 in *Nyangili Macwot* while the lowest value was 0.480 in *Egeget.* H_e values across all populations ranged from 0.579 in *Nasomel* to 0.833 in *Upende-Aboro* (Table 6.3). The mean H_o and H_e values for all loci across the 28 ethno-varieties were 0.634 and 0.690 respectively. Six loci departed significantly ($p < 0.05$) from Hardy-Weinberg equilibrium when tested for heterozygous deficiency: *mCIRVp113* (*Egeget*), *mCIRVp134* (*Ciria, Egeget*), *mCIRVp167* (*Apocopoco*), *mCIRVp175* (*Nyiri*), *mCIRVp191* (*Acula, Epiana, Layer*) and *mCIRVp28* (*Apocopoco*). Heterozygote excess was not observed at any locus.

Table 6.3. Genetic variability of 28 shea tree ethno-varieties assessed in this study.

Ethno-variety	H_e	H_o	f	Private alleles		
				Locus	Allele	frequency
Nyangili Macwot	0.827	0.817	0.030	-	-	-
Nyiri	0.712	0.805	-0.150	mCIRVp191	294	0.100
Ngorokwa	0.780	0.767	0.022	-	-	-
Acula	0.713	0.736	-0.036	mCIRVp175	268	0.167
Upende Aboro	0.833	0.733	0.174	mCIRVp175	222	0.250
Asa	0.674	0.723	-0.080	-	-	-
Ciria	0.713	0.700	0.031	mCIRVp168	164	0.125
				mCIRVp113	201	0.125
Ajiki	0.680	0.678	-0.001	mCIRVp159	287	0.200
				mCIRVp191	270	0.100
				mCIRVp191	282	0.100
Acogo	0.690	0.667	0.046	-	-	-
Mayom	0.667	0.667	0.000	mCIRVp175	210	0.167
				mCIRVp191	286	0.167
Ngulu	0.813	0.667	0.216	mCIRVp113	213	0.167
Aloto	0.651	0.653	-0.003	-	-	-
Layer	0.693	0.645	0.074	-	-	-
Apocopoco	0.716	0.640	0.162	mCIRVp159	275	0.100
Coloa	0.720	0.633	0.146	mCIRVp14	260	0.333
Mudaa	0.679	0.625	0.097	-	-	-
Abor	0.657	0.617	0.086	-	-	-
Mbele	0.637	0.617	0.027	-	-	-
Ewiny	0.635	0.600	0.062	mVIRVp14	258	0.100
Nasomel	0.579	0.577	0.002	-	-	-
Amoo	0.615	0.570	0.080	-	-	-
Epiana	0.712	0.565	0.226	-	-	-
Nalungur	0.589	0.560	0.055	-	-	-
Yao Atega	0.707	0.550	0.274	mCIRVp14	236	0.167
Julu	0.693	0.500	0.326	mCIRVp14	234	0.167
Alindiri	0.650	0.483	0.323	-	-	-
Limi	0.617	0.483	0.267	mCIRVp167	249	0.167
				mCIRVp167	233	0.167
Egeget	0.678	0.480	0.317	mCIRVp168	150	0.100
Mean	**0.690**	**0.634**	**0.099**			

The values of f, F and θ_P ranged from -0.230 (*mCIRVp168*) to 0.406 (*mCIRVp08*), -0.202 (*mCIRVp168*) to 0.521 (*mCIRVp08*) and -0.026 (*mCIRVp159*) to 0.194 (*mCIRVp08*) respectively (Table 6.2). Heterozygote deficiency was reflected in the mean inbreeding

coefficient (f = 0.088). The highest inbreeding coefficient (f) among the ethno-varieties was found in *Alindiri* (0.323) while the lowest was in *Nyiri* (-0.150). Global f, F and θ_P values were 0.088, 0.131 and 0.047 respectively. Bootstrap analysis at 95% confidence interval with 20 000 replicates showed that F and θ_P were significantly ($p < 0.05$) greater than zero while f was not, suggesting a panmictic mating system among the sampled trees. The proportion of genetic variation due to differences in ethno-varieties ($\theta_P = 0.047$) indicates that most genetic variation (95.3%) occurs within rather than between the ethno-varieties. Results from AMOVA analysis (Table 6.4) showed that within this (95.3%) variation, 8.4% is found among individual trees within ethno-varieties while 86.9% is found within individual trees. In addition, evidence for low differentiation is further indicated by the high level of gene flow ($N_m = 2.59$).

Table 6.4. Analysis of molecular variance (AMOVA) results for 118 individuals from 28 *V. paradoxa* subspecies *nilotica* ethno-varieties based on 10 SSR markers

Source of variation	**Sum of squares**	**Variance components**	**Percentage variation**
Among ethno-varieties	136.724	0.170	4.673
Among individual trees within ethno-varieties	293.53	0.307	8.428
Within individual trees	339	3.166	86.899
Total	**769.253**	**3.64317**	

Cluster analysis generated a tree (Figure 6.1) with a tree edge length sum of 17.07, mean error of 0.0008, mean absolute error of 0.0586, maximum absolute error of 0.26, mean square error of 0.0055 and co-phenetic r of 0.8496. A co-phenetic value greater than 0.8 indicates the phenogram truly represents the genetic structure in the data matrix. The cluster analysis doid not reveal any clearly defined clusters implying that the ethno-varieties as perceived by farmers is not congruent with the genetic diversity of shea trees. The phenogram (Figure 6.1) shows that the tree genotypes were separable into three broad clusters with no clearly defined ethno-variety membership..

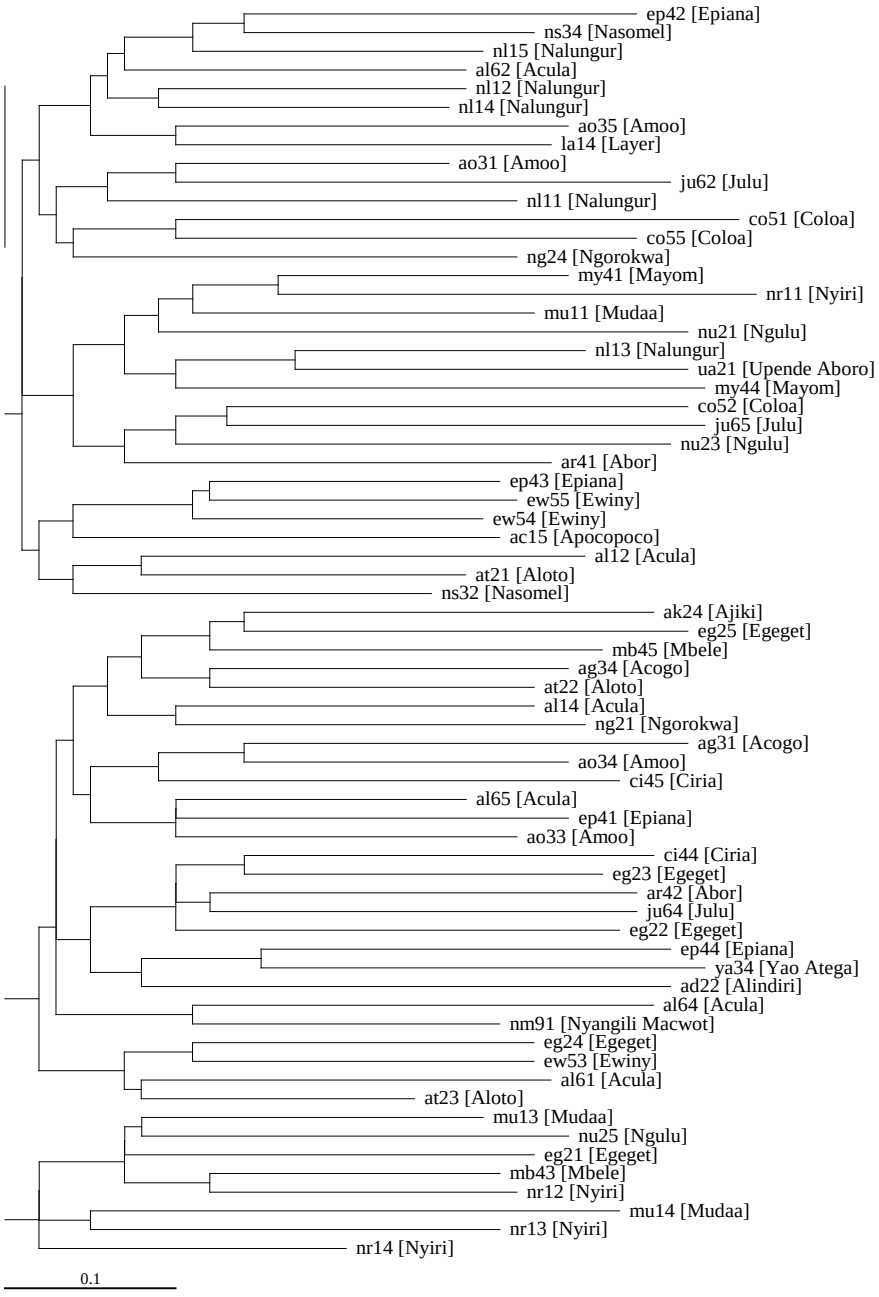


Figure 6.1. Neighbour-joining phenogram (based on a dissimilarity matrix and simple matching coefficients) showing relationships of 67 shea tree genotypes classified by local communities as distinct ethno-varieties. Names in parenthesis refer to local ethno-variety names (Table 6.1) while the codes before the ethno-variety names refer to individual tree identification codes.

6.4 Discussion

Assessment of genetic diversity is essential for the design of effective conservation and breeding programmes for locally identifiable plant varieties (Kresovich & McFerson, 1992). It is important to consider locally observable variation in genetic diversity assessments (Arias-Reyes, *et al*. 2000). However, while folk classifications are crucial in guiding selection and breeding studies, they are usually influenced by various socio-economic factors (Nuijten & Almekinders, 2008). In the case of shea tree ethno-varieties, the classification system in Uganda is constructed around the fruit, which is the most important commercial product, especially as a source of shea oil (Gwali, *et al*., 2011). Fruit traits, such as size and shape, are however very plastic due to influence from environmental factors (Aabd, *et al*., 2011; De Smedt, *et al*., 2011; Dhillon, *et al*., 2009).

Microsatellite markers have demonstrated their superiority in screening for genetic differentiation and diversity in several tree species (Dhillon, *et al*., 2009; Fares, *et al*., 2009; Majourhat, *et al*., 2008). In this study, shea tree ethno-varieties were found to be highly polymorphic (51 – 82%). High genetic diversity has been documented in economically important fruit tree varieties, such as *Olea europaea* L. (Claros, *et al*., 2000; Rekik, *et al*., 2008; Wiesman, *et al*., 1998), *Pistacia vera* L. (Fares, *et al*., 2009) and *Mangifera indica* L. (Kashkush, *et al*., 2001). In shea trees, high genetic diversity has been observed for populations in Mali (Kelly, *et al*., 2004; Sanou, *et al*., 2005) and Ghana (Lovett & Haq, 2000). High genetic diversity is associated with mainly out crossing species (Hamrick & Godt, 1996; Lacerda, *et al*., 2001; Nybom, 2004). The low inbreeding coefficient (f = 0.088) which was not significantly different from zero therefore suggests that shea tree ethno-varieties are largely panmictic (randomly mating) i.e. selfing is either very weak or absent. In addition, the estimated gene flow (N_m = 2.59) was high reflecting a substantial amount of gene flow among the ethno-varieties.

The levels of polymorphism, inbreeding and gene flow observed for shea ethno-varieties in the present study shows these shea tree ethno-varieties are dynamic in gene exchange and possible influence from human practices such as selection by farmers. Studies on the reproductive biology of *V. paradoxa* indicate that the species is an out-crossing tree (Hall, *et al*., 1996; Lovett & Haq, 2000; Okullo, *et al*., 2003; Sanou, *et al*., 2005), pollinated mainly by insects such as bees and butterflies and dispersed barochorously and secondarily

zoochorously by bats, monkeys but also by birds (Hall, *et al*., 1996; Okullo, *et al*., 2004, 2005). Out-crossing species generally have low levels of differentiation on account of high levels of pollen flow (Hamrick & Godt, 1990; Hamrick, *et al*., 1992; Muir, *et al*., 2004). The fruits of *V. paradoxa* consist of a round or oval berry consisting of a sweet pulp, which highly influences the barochorous seed dispersal mechanism (Jordano & Godoy, 2000). Therefore, in addition to bat pollination, farmers (especially women and children) collect shea fruits for nut extraction and subsequent production of shea butter/oil. In many instances, the fruit pulp is eaten fresh (especially during famine periods) and when the nuts are discarded, this contributes to seed dispersal. This movement of fruits and dispersal greatly facilitates high gene flow

Results from AMOVA within and between the 28 shea tree ethno-varieties suggest that there are high levels of genetic variation within individual trees compared to that among the ethno-varieties. The lack of genetic differentiation between ethno-varieties can be attributed to the use of a single rather than a combination of traits to describe an ethno-variety (see detailed description in Gwali, *et al*., 2011*a*). For example, the trees whose fruit pulp is sweet are described as an ethno-variety (e.g. *Ewiny* and *Limi*), yet this does not exclude the possibility that the same trees may also produce fruits which are oval or round in shape, and are then classified as different ethno-varieties (e.g. *Acula* or *Nalungur*). The use of a single rather than a combination of locally observed traits creates a folk classification system that is not congruent with the genetic composition of the individual shea trees. In addition, traits used in folk classification may be influenced by environmental factors thereby contributing to differences in local perceptions of variation across different localities.

In addition, low genetic differentiation shown by a low θ_P value and lack of clear grouping in cluster analysis indicates that shea tree ethno-varieties as perceived by farmers are basically similar genotypes described with different names in different locations, since farmers name their varieties according to their own experiences (Veasey, *et al*., 2008). This implies that the numerous ethno-variety names used in shea tree folk classification do not always reflect real genetic diversity. Even though some private alleles were found thus presenting an opportunity for distinguishing among ethno-varieties, further investigation involving much heavier sampling is required for a definitive account of these private alleles, especially in view of the small sample sizes in this study.

A similar lack of concordance between folk classification and genetic variation was observed in *Adansonia digitata* L. in West Africa (Assogbadjo, *et al*., 2009). In addition, several studies have shown that phenotypic traits may not necessarily be correlated to genetic variation (Perrie & Brownsey, 2005). Maranz and Wiesmann (2003) reported a relationship between environmental variables (rainfall and temperature) and variation in shea tree phenotypic traits, such as fruit size and shape, pulp sweetness and oil content.

6.5 Conclusions

Shea trees in Uganda possess high levels of genetic diversity which is useful in the long term survival of the species. Most genetic variation was found within individual tree genotypes rather than between ethno-varieties. It was, therefore, not possible to distinguish the present shea tree ethno-varieties as perceived by framers using SSR fingerprinting. This is most likely due to the use of single, non-genetic traits in ethno-variety classification. The classification of shea trees into ethno-varieties is therefore not congruent with the genetic variation present in shea trees in Uganda. We conclude that these ethno-varieties are arbitrarily defined subgroups of a single large randomly mating population. The observable variation is mainly phenotypic, continuous and plastic as a cause of micro-environmental factors.

6.6 References

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CHAPTER SEVEN

GENERAL DISCUSSION

7.1 Overview

The need to design strategies for pre-breeding of shea trees (*Vitellaria paradoxa*) is crucial for domesticating and conservating this species. This is vital considering the increasing economic opportunities that could be derived from shea tree products on the international and local markets. The major commercial product from shea trees is the butter or oil that is extracted from the kernel. Farmers recognize the presence of different shea tree ethno-varieties and use this apparent variation for pecuniary purposes such as trade and family exchange. Indeed, farmers in Uganda's shea tree parklands have started to appropriate ownership rights over shea trees on their land contrary to the past when shea trees were communally owned (Owona, 2008).

Inadequate characterization of shea tree germplasm for useful traits remains a serious constraint to the use and improvement of the species. In addition, traditional management practices and local knowledge of variation in the species are needed for ex-situ and in-situ conservation. This study examines whether different shea tree ethno-varieties are arbitrarily defined subgroups of a single large randomly mating population of shea trees in Uganda or not. The following questions were addressed: 1) What management and conservation practices do farmers carry out on shea trees? 2) What criteria do farmers use to identify differences in shea tree varieties? 3) Are there significant differences in chemical composition of shea nuts from different shea tree ethno-varieties? 4) Do the different shea tree ethno-varieties exhibit significant genetic variation?

The thesis documents the traditional management and folk classification system of shea trees in Uganda and characterises shea tree ethno-varieties using classical morphological, chemical and molecular tools. This is an important step in pre-breeding since the development of domestication or conservation programmes can be based on locally observed variation (Quiros, *et al*., 1990). The extent to whicthe key questions are addressed is discussed in sections 7.2 – 7.4 below.

7.2 What management and conservation practices do farmers carry out on shea trees?

Traditional management practices are a component of traditional knowledge and form a knowledge – practice - belief complex (Berkes, 1999). Hence, an assessment of the knowledge pool in the shea tree zones revealed several traditional management practices including pruning, weeding, selective sparing, burning, coppicing, pollarding, manuring, mulching and staking (Chapter 2). These traditional management practices are also reported for shea trees elsewhere in Africa (Boffa, 1999; Boffa, *et al*., 2000; Djossa, *et al*., 2008; Hall, *et al*., 1996; Lovett & Haq, 2000; Maranz & Weisman, 2003; Masters 2000). However, some practices such as pollarding and pruning, although popular for the provision of other products such as firewood and charcoal in the shea parklands of Uganda, can be regarded as subconscious management practices (sensu Lovett & Haq, 2000*b*) since they are intended to achieve alternate objectives. Despite the presence of various management practices (Chapter 3), shea trees are wild or at best, semi-domesticated if they grow in farmers' fields. Therefore, the diversity of management practices serves to show the strong connection to shea trees by farmers in their areas.

In addition to the economic factors, cultural practices are important motivations in the management of shea trees in Uganda. Use of shea trees by local communities is strongly linked with cultural practices, taboos and rituals which are rooted in traditional spiritual belief systems. The cultural practices are social mechanisms that govern the traditional management practices (Berkes, *et al*., 2000). They also offer an example of how traditional knowledge can inform, complement, and strengthen shea tree management. Taboos and cultural rituals are therefore an important influence on the diversity of uses of shea trees and their products. Ethnic groups in the northern and West Nile farming systems, for example, reported a higher diversity of shea trees uses compared to the Iteso of Teso farming system on account of the diversity of cultural practices. The usage of taboos and rituals points to a long experience and empirical knowledge of shea trees, since experience and history informs how local farmers manage their natural resources (Mueller & Mechler, 2005; Tanyanyiwa & Chikwanha, 2011). Therefore, cultural practices are an inherent feature of traditional shea tree management and use in Uganda.

Contrary to the widely held view that more traditional knowledge resides with elderly persons (Camejo-Rodrigues, *et al*., 2003; Johnson, 1992), middle aged respondents in Uganda (20 and 40 years) reported a higher diversity of shea tree uses compared to

respondents aged less than 20 years or over 50 years. Thus, middle aged people can be considered as a useful repository of traditional knowledge. This is important as it shows that traditional knowledge of shea trees is not at great risk of erosion and is still available for traditional conservation purposes. The transmission of traditional knowledge was mainly attributed to parents and elders, but it is probable that the large pool of knowledge of shea trees and their products by the middle aged people may be due to more frequent use of and daily experiences. Frequent exposure to the natural environment is a major avenue for acquiring knowledge of traditional uses (Pilgrim, 2006). It also possible that middle aged respondents readily provide responses during interviews. Elicitation of responses from elderly persons is also more difficult and in some instances may be affected by memory loss (Grady & Craik, 2000). Moreover, some of the knowledge, especially regarding uses, may be non-traditional, gleaned from external sources which may not be accessible to the more elderly and younger persons in the community.

However, there was no difference in traditional knowledge about shea trees between men and women. Similarly, there was no significant difference in local knowledge of shea tree uses between the different ethnic groups in the shea belt of Uganda contrary to the known influence of ethnicity on traditional knowledge of natural resources (Ayantunde, *et al.*, 2008; Guimbo, *et al.*, 2011). Similar observations on knowledge of shea tree uses have been made in Ghana (Lovett & Haq, 2000*b*) and Burkina Faso (Elias, 2010). The uniformity in traditional knowledge between men and women, and between ethnic groups is explained by the similarity of use values of shea trees across the farming systems assessed. The ethnic groups depend on similar means of subsistence, with the shea tree providing the single largest source of vegetable oil. The geographic proximity of different ethnic groups helps with knowledge diffusion. Ethnic groups in close geographic proximity share cultural traits since they interact regularly (Cavalli-Sforza & Feldman, 1981). The utilization of shea tree products, such as fruit collecting, oil processing and marketing is, however, highly gendered (Elias, 2010; Elias & Carney, 2007).

7.3 What criteria do farmers use to identify different shea tree ethno-varieties?

Traditional knowledge and management practices of crops derive from highly organized and culturally structured classification systems that are socially accepted by given communities (Berlin, 1992; Berlin, *et al*., 1973; Brown, 1993; Holman, 2002; Raven, *et al*., 1971). In the present study, the local perspective of shea tree diversity was documented by assessing the

species' folk classification into ethno-varieties. An ethno-variety was considered to be a group of shea trees identified by farmers under a single name within a particular ethnic group (sensu Berlin, *et al*., 1992) (Chapter 3). All ethnic groups consistently defined ethno-variety names on the basis of fruit, nut and pulp traits, suggesting that classification knowledge is similar across the shea belt of Uganda.

Phenotypic diversity was also reflected in the multiplicity of names which farmers utilized to refer to the same ethno-variety. Among the Acholi and Lango ethnic groups, for example, shea trees that bear small and tiny fruits/nuts are either referred to as Abor or Aliboro while among the Madi, they are either referred to as Ciria or Totokwiloro. These cases of semantics were encountered in all the ethnic groups and may be a representation of inter-generational, inter-ethnic-linguistic or locality differences. This semantics may also be attributed to the high state of flux of some of the dialects and close lexical relationships among the different ethnic groups.

Fourty four ethno-varieties were recorded indicating a high level of diversity in shea trees and showing how farmers value shea tree resources through specific nomenclature. Ethno-variety nomenclature was based on fruit/nut organoleptic and morphological traits. In contrast, utilitarian and socio-cultural values of shea trees were not as influential in ethno-variety nomenclature. Thus folk classification utilises physically apparent differences (morphology, taste and colour) rather than utilitarian values. Moreover, ethno-variety nomenclature criteria, as inferred from the literal meanings, were consistent among ethnic groups. This nomenclature system serves a regional and trans-generational communication and knowledge diffusion and has been recorded in other crop species, such as potatoes in the Andes (Brush, 2004).

Folk classification was influenced by ethnicity, but not age and gender. Farmers from farming systems where there was higher traditional use and folklore of shea tree products generally enumerated more ethno-varieties. The northern and West Nile farming systems reported more ethno-varieties compared to the Teso farming system most likely due to the hgher diversity of traditional uses amd folklore in those systems. This suggests that folk classification is an inextricable component of traditional knowledge. Apart from ethnicity, other factors such as education level and gender did not have any significant influence on folk classification. It is, however, worthwhile to note that even for scarce ethno-varieties,

such as Aremo (a rare ethno-variety with red or pink fruit pulp) which were named by only a few informants, there was consistency in classification in the two farming systems (northern and Teso) where these were reported.

7.4 Are there significant differences in chemical composition of shea nuts of different shea tree ethno-varieties

Assessment of fat content and fatty acid variation in shea nut kernels of different ethno-varieties found high values of fat (44 – 58%), stearic (25 - 38%) and oleic (47 - 62%) acids. Fat content and composition of stearic and oleic acid were not significantly different among ethno-varieties. Indeed, individual trees from different ethno-varieties separated by a distance of only 100 km would have both the highest and lowest fat content, indicating that local site, climatic or tree-to-tree differences may be crucial (see chapter 5). This is not surprising since available local knowledge does not utilize fat/oil content in folk classification. Maranz and Wiesman (2003) were only able to find differences in fat content and fatty acid composition over the whole range of the species, with little or no differences reported within the subspecies range at the country level. In addition, fat content and fatty acid composition are closely correlated with environmental and genetic factors (Fieldsend & Morison, 2000; Abdallah, *et al*., 1998).

7.5 Do the different shea tree ethno-varieties different genetic variation?

With regard to characterization based on morphology and molecular markers, the results show no concordance with folk classification. A hierarchical cluster analysis of morphological traits revealed the presence of three groups based on fruit, nut and leaf traits (fruit and nut weight, size and shape, pulp quantity, taste and eubstance, and fruit pubescence). Similarly, a cluster analysis of microsatellite variation revealed three clusters with mixed ethno-variety membership of the clusters. In both the morphological and molecular analyses, cluster membership was overlapping, indicating a lack of concordance with folk classification. A refined morphological analysis including qualitative traits used in folk classification presented eight groups which were more concordant to the ethno-variety groupings. However, the apparent concordance of the refined morphological analysis is mostly likely due to the masking effect of the qualitative criteria that are used in folk classification rather than a representatation of actual concordant clustering. Analysis of Molecular Variance (AMOVA) showed that the principal source of genetic variation is encountered within (87%) rather than between (5%) ethno-varieties, indicating low

differentiation among ethno-varieties. This result is not surprising since shea trees are known to be allogamous, exhibiting a high degree of cross pollination. Indeed, this study shows that shea trees are highly polymorphic (51 – 82%), largely panmictic (f = 0.088) and dynamic in exchange of genetic material (Nm = 2.59). A similar lack of concordance between folk classification and genetic or morphological variation was observed in *Adansonia digitata* L. in West Africa (Assogbadjo, *et al*. 2009), potatoes of the Andes (Kandemir, *et al*., 2010). Similar studies elsewhere also indicate that there is high gene flow in this species (Kelly, *et al*., 2004; Logossa, *et al*., 2011; Sanou, *et al*., 2005).

Low levels of genetic differentiation between ethno-varieties can be attributed to panmixis, although it may also be attributed to the use of a single phenotypic rather than a combination of traits to describe an ethno-variety. In addition, the use of a single phenotypic trait to describe an ethno-variety, such as big fruit, does not exclude the possibility that this same trait (big fruit size) may be present in ethno-varieties that are described differently, such as sweet pulp. The observed phenotypic differences in shea trees may thus be the result of environmental rather fixed genetic effects. Therefore, the use of phenotypic data in folk variety identification is of great value but phenotypic variation is sometimes too plastic to account for any meaningful differences (Quiros, *et al*., 1990; Leemans, 1957; Aabd, *et al*., 2011; De Smedt, *et al*., 2011; Dhillon, *et al*., 2009). Both anthropogenic and environmental factors shape the morphology of shea trees (Lovett & Haq 2000; Sanou, *et al*., 2006). Selection by farmers tends to favour shea trees with particular traits (Boffa, 1995; Boffa, *et al*., 1996) while environmental influences on morphology have been recorded in other tropical tree species such as *Balanites aegyptiaca* and *Tamarindus indica* (Soloviev, *et al*., 2004). Other factors that may explain morphological variation include natural selection (Darwin, 1869; Irwin, 2000; Tremblay, *et al*., 2010), selection by humans (Vaughan, *et al*., 2007) and gene flow mediated random genetic drift (Abasse, *et al*., 2011).

Although shea tree ethno-varieties provide a considerable opportunity for identification and selection of plus shea trees for domestication, the use of phenotypic traits in folk classification may only represent physically observable rather than genetically fixed differences among individual ethno-varieties. Molecular technologies may present attractive possibilities for the characterization and identification of shea tree ethno-varieties for future improvement programmes. Specific primers have been developed and published for an array of microsatellite loci for this species. Since 14 ethno-varieties in this study contained private

alleles, these markers could be utilized for the development of Quantitative Trait Loci (QTL) maps hence providing an opportunity for identification of particular ethno-varieties on the basis of distinctive traits. However, since sampling of respective ethno-varieties was rather limited in the present study, heavier sampling of ethno-varieties identified in Chapter 3 should be done to verify the presence of these private alleles and their long-term and evolutionary contribution.

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CHAPTER EIGHT

CONCLUSIONS AND RECOMMENDATIONS

The present study is the first to examine farmers' perceptions of shea tree variation in Uganda. The focus of was the characterization of shea tree ethno-varieties and their phenotypic and molecular variation. Based on the findings, the concept of shea tree folk classification and ethno-varieties in Uganda and a conservation framework incorporating traditional as well as contemporary conservation approaches has been developed. In addition, shea tree ethno-varieties in Uganda have been identified and documented, and their variation assessed. Scientific knowledge in folk classification and traditional management of shea trees is therefore increased. This is important as it lays ground for further research and effective and efficient conservation of shea trees in Uganda. These results may also be useful in guiding the development of national policies and enhancing scientific innovation related to sustainable management and utilisation of shea trees.

8.1 Specific conclusions

The following specific conclusions have been drawn from this study:

1) Shea trees and their products are utilized for up to 36 different domestic and socio-cultural uses such as cooking, traditional ceremonies and rituals. These uses are derived from eight main shea tree products: oil, wood, whole trees, fruit pulp, stem bark, latex, leaves and nuts.

2) Shea oil is the most important shea tree product on account of its diversity of uses which range from domestic (cooking) to cultural (traditional marriages, funerals and festivals) and rituals (rain making, celebratingthe birth of twins, anointments).

3) The major shea tree traditional management practices are aimed at improving oil productivity and include weeding, sparing on farm, natural coppicing, pruning and seasonal burning.

4) Folk classification, which is consistent across different ethnic groups, indicates the presence of 44 ethno-varieties based on fruit/ nut morphological and organoleptic traits.

5) Morphological, chemical and genetic analysis of the shea tree ethno-varieties indicates that there are no discrete groupings that are congruent with local folk classification most likely due to the use of a single rather than multiple traits in folk classification, the plasticity of morphological characters that are used in folk classification and the allogamous nature of shea trees.

6) Shea nuts from all ethno-varieties have high fat content (43.9 to 58.4%), an attribute that is especially important for improved trade and industrial production.

7) Oil from all ethno-varieties is rich in unsaturated fatty acids, mainly oleic acid (47.35 – 62.04%) and linoleic acid (4.72 – 8.97%). In additon, the oil has a high unsaturated to saturated acid ratio (0.43 – 0.81) implying that all ethno-varieties produce oil that is particularly important for its health benefits due to its stability.

8) Shea tree ethno-varieties possess high genetic diversity (Ho = 0.634 and He = 0.690) which is an important attribute for the long term survival of the populations as a whole.

9) Genetic differentiation was low among the ethno-varieties (θ_P = 0.047) with most variation residing within individual trees (87%) rather than within ethno-variety groupings (8%) and between ethno-varieties (5%). This suggests that shea tree ethno-varieties as perceived by farmers are arbitrarily defined subgroups of a single large randomly mating population.

8.2 Recommendations

8.2.1 Further research

Folk classification of shea trees utilises mainly morphological but also organoleptic traits, such as, fruit size and weight, nut size and weight, pulp taste and colour. While these are sufficient for a folk classification system, there is need to establish the contribution of shea oil among these traits. Unfortunately, shea oil has traditionally been prepared out of bulked collections of shea nuts from various trees. Hence, it is not possible to ascribe particular attributes to oil from individual trees. It is however possible to investigate any differences in shea oil between different trees and ethno-varieties. Since oil is a major trade-able product

from shea trees, this will help to tag specific oil properties to particular trees and therefore promote the cultivation of trees with desired oil characteristics.

In addition, while the scope of this study was to document the folk classification of shea trees in Uganda and establish if there is any phenotypic or molecular evidence to confirm the folk classification, it was not possible to establish conclusively the different factors that influence farmers to ascribe different names to different varieties. In this aspect, soil and other environmental factors are important and should be further investigated to establish if there is any contribution they make to the perceived differences in shea tree ethno-varieties.

Future research could build on this traditional knowledge system and validate the ethno-variety classification system through much more intensive sampling of individual trees in each ethno-variety. This will build up on the formal characterisation of shea tree ethno-varieties in Uganda, in addition to understanding the infra-specific diversity present in shea tree populations in Uganda.

8.2.2 Industrial production

It is possible to identify and work effectively with traits associated with the breeding of shea trees so as to enhance industrial production. Besides, a verification and validation of these ethno-varieties is capable of producing next generation trade-able products from shea trees in Uganda. Ethno-varieties with sweet pulp could, for example, be developed for the production of jams with natural sugars (with no artificial ingredients). In addition, the conservation and management framework that was developed in this research would go a long way in conservation of shea trees, as well as fostering production that benefits the farmers and industrialists. Results from this research should therefore be utilized as a precursor for the development of industrial products from shea tree products from ethno-varieties.

APPENDIX 1

QUESTIONNAIRE FOR HOUSEHOLD INTERVIEWS

IDENTIFICATION AND DESCRIPTION OF LOCAL VARIETIES OF SHEA TREE (*VITELLARIA PARADOXA*) IN UGANDA

The aim of this study is to identify and characterise the local varieties of shea tree in the savannah parklands of Uganda. The specific objectives are:

1. To identify the existing local shea tree varieties based on farmers' knowledge;
2. To determine the farmers' criteria for local shea tree characterisation; and
3. To assess management practices undertaken by farmers on the shea resources.

GENERAL INFORMATION & INTERVIEW IDENTIFICATION

Questionnaire No: _____

Date of interview: _____

District: _____ Sub county: _____

Parish: _____ Village: _____

Additional notes:

Enumerator's name: _____

Checked by: _____ Date: _____

RESPONDENT'S CHARACTERISTICS

1) Name of respondent: _____
2) Gender (tick the most appropriate): Male □ Female □
3) Age: _____
4) Ethnic group and language of respondent: _____
5) Marital status (tick the most appropriate): Single □ Married □ Divorced □Widowed □
6) Education level reached (tick the most appropriate): None □Primary □Secondary □ University □

7) Main occupation (tick the most appropriate): Student □ Farmer □Trader/Business □ Informal (wage) work □Formally employed □

8) What are your main sources of income?

_____

INDIGENOUS KNOWLEDGE OF SHEA TREE VARIETIES

Section A: Land ownership and tenure

1) What is the size of your land in this village (acres)? _____

2) How did you obtain this land? (tick the most appropriate): Inherited □ Purchased □ Hiring/Renting □ Allocated/Given □

3) What proportion of your land is currently under cultivation and/or fallow?

_____

Section B: Shea tree abundance and population dynamics

1) Are there shea butter trees on your land? (tick the most appropriate): Yes □ No □

2) Approximately how many shea trees do you have on your farm?

_____

3) What are the other tree species that occur together with the shea butter tree on your land?

Tree name	In which language is this name?

4) Who in your household is the owner of the shea trees on your family land?

_____

5) What are the local subsistence uses of the shea butter tree and/or any of its products?

_____

_____

6) In what form is the shea butter tree and/or any of its products used to obtain economic/financial benefits?

_____

_____

7) What financial obligations are usually solved using income derived from shea products?

_____

_____

8) What are the environmental uses of the shea butter tree or any of its products?

_____

_____

9) What are the socio-cultural uses of the shea butter tree and/or its products?

_____

_____

10) What are the taboos and rituals that are related to the shea tree and/or its products?

_____

_____

11) What are the proverbs, stories or songs associated with the shea tree in your culture?

_____

_____

12) Do you think the population of shea trees on your farm has increased in the last ten (10) years or not? (Tick the most appropriate): Increased □ Decreased □

13) Why do you think this is so?

_____

_____

14) What proportion of shea trees (number of trees or percentage) in your farm were planted or found growing from natural regeneration?
Planted _____ Natural regeneration _____

15) If the shea trees are planted, where did you get the germplasm?

_____

16) What was your motivation/inspiration for planting shea trees on your land?

_____

Section C. Shea tree regeneration & domestication potential

1) Are there many young/regenerating shea trees in your land

_____

2) What could be the reason(s) for the situations in your responses to 1 above?

_____

3) What is the proportion of young shea trees compared to mature/old shea trees

_____

4) What can make people in your village to plant /domesticate shea trees?

_____

5) Where in your farm (location) would you consider to plant shea trees (Scattered, Boundary, Plantation)?

_____

Section D. Shea tree varieties & their characteristics

1) Mention the different varieties of shea butter tree & possible meanings of the variety names.

Variety name	**Language in which the name is**	**Possible meaning of the variety name**

2) For each variety, mention the months of the year when flowering, fruiting and fruit fall/harvesting take place

Variety	**Flowering (Months & Regularity)**	**Fruiting (Months)**	**Harvesting (Months & quantity)**

3) For each variety, describe the morphology of the following attributes (leaves, flowers, crown shape, trunk)

Variety	Leaves	Flowers	Crown shape	Trunk

4) For each variety, describe the morphology of the following attributes (Pulp, Kernel)

Variety	Pulp		Kernel	
	Quantity	Taste	Size & shape	Colour

5) Which of the varieties mentioned above is most preferred by farmers (rank in order of preference – from most preferred [5] to least preferred [1], giving reasons)

Variety	Ranking (5 – 1)	Reasons for rank order

6) What is the preferred habitat for the different varieties

Variety name	**Preferred habitat (e.g. rocky areas, wetland/lowland areas, dry upland areas, etc**

7) Which shea varieties are usually most maintained/conserved on farm?

_____

_____

8) How is the knowledge of varietal identification acquired? (Tick the most appropriate):
Parents □ Elders □ Age mates/friends □ Stories/folklore/songs □ Other (describe)

_____

MANAGEMENT PRACTICES OF LOCAL SHEA VARIETIES

1) What management practices do you carry out on the different varieties of shea tree on your farm?

Variety	**Management practice** ***Pruning=1 Weeding=2 Pollarding=3 Lopping=4 Coppicing=5 Other(specify)=6***	**Carried out by whom?** ***Men=1 Women=2 Children=3 All above=4***	**Months of the year in which they are carried out. (J,F,M,A,M,J,J,A,S,O,N, D) [relate each management practice with the month in which it is carried out]**

2) Mention the ecology and major stresses that face the shea varieties

Variety	**Relative abundance of the variety. Is it very common? (Yes=1, No=2)**	**Prevailing stresses 1=diseases (describe) 2=pests (describe) 3=other (describe)**

Finally:

Is there any comment or additional information that you would like to give about shea tree resources and their management in your village or the region?

THANK YOU FOR YOUR COOPERATION

APPENDIX 2:

FIELD DATA AND SAMPLE COLLECTION

DATA CAPTURE FORM

Instructions:

1. This form should be completed for each tree sampled
2. The fruit and leaf sample collection checklist section of this form require you to tick the check box to indicate that the respective activity has been accomplished.

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COLLECTOR AND SITE IDENTIFICATION

COLLECTOR'S NAME: COLLECTING DATE:

COLLECTION LOCATION: Village: Sub-county: District:

STATUS OF AREA (tick): ☐People's land ☐Protected area e.g. Forest Reserve

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CURRENT UTILISATION STATUS (tick): ☐ Cultivated land ☐ Fallow

---

SITE ACCESSIBILITY (tick): ☐Very accessible ☐ Accessible with difficulty ☐ Seasonally accessible

---

DISTANCE FROM NEAREST TRADING CENTRE (Name of centre; estimate distance in km):

VEGETATION TYPE (describe, indicate most common tree species):

==========

SHEA TREE VARIETY AND SAMPLE IDENTIFICATION

VARIETY: UNIQUE TREE ID No.:

GPS COORDINATES (decimal degrees): ALT. (m a.s.l.): ACC. +/- (m)

TREE SHAPE (see chart 1): DBH (cm): TREE HEIGHT (metres):

CANOPY DIAMETER 1 (m): CANOPY DIAMETER 2 (m):

CROWN CONDITION (see chart 2):

TREE PHOTOGRAPH NO.:

==========

FRUIT AND LEAF SAMPLE COLLECTION CHECKLIST

FRUIT SAMPLE COLLECTION (tick tasks already accomplished)

[30 ripe and fallen fruits will be collected for morphologic and chemical analysis]

☐ 30 ripe fallen fruits collected and placed in cool storage [see Part B (1) in the technical guidelines for fruit sample collection protocol]

☐ Label the fruit sample appropriately (use unique identification number above already given to tree)

LEAF SAMPLE COLLECTION (tick tasks already accomplished)

[50 leaves will be randomly collected for morphological analysis]

☐ 50 mature leaves randomly collected and placed in cool storage (see Part B (2) in the technical guidelines for leaf sample collection protocol)

☐ Label the leaf sample appropriately (use unique identification number above already given to tree)

LEAF SAMPLE COLLECTION (tick tasks already accomplished)

[Up to 4 youngest leaves will be randomly collected for genetic analysis]

☐ 3 – 4 youngest leaves randomly collected, placed in zip lock bags containing silica gel and then kept under cool storage (see Part B (3) in the technical guidelines for leaf sample collection protocol)

☐ Label the leaf sample appropriately (use unique identification number above already given to tree)

==========

COLLECTOR'S NOTES:

==========

APPENDIX 3

GUIDELINES FOR LEAF AND FRUIT SAMPLE COLLECTION

(Adapted from the Technical Guidelines for collection of Prunus bark and leaf samples for genetic analysis [2009 study conducted by the National Forestry Resources Research Institute with funding from Bioversity International])

A General guidelines

The objective of this study is to characterise local varieties of shea tree (*Vitellaria paradoxa* ssp. *nilotica*) on the basis of morphological, molecular and chemical traits. This investigation will provide valuable information for developing and designing breeding guidelines for the conservation and domestication of shea tree varieties for improved production, incomes and nutrition.

1. Number of sites, location and criteria for their selection

One sub-county was selected in each of the following districts on the basis of shea tree presence (Abim, Amuria, Arua, Katakwi, Lira, Moyo, Nebbi and Pader). In each of the sub-counties, one village (where contact persons for the project exist) will act as satellite centres from which collection visits will radiate throughout the sub-counties to areas with adequate densities of shea tree individuals (5 trees per variety) per site. The districts and satellite villages (and contact persons) are given below:

- Abim district – Arem Bwola village, Kanu parish & Lotuke subcounty (**Mr. Franco Eket** 0774447459 & **Mr. Patrick Lumumba** 0773986590)
- Amuria district – Akamurei & Angerepo villages, Acowa subcounty (**Mr. Paul Okiror** 0782224828)
- Katakwi district – Aparisia village, Omodoi sub-county (**Mr. Richard Michael Amuge** & **Mr. Richard Ecarit** 0751644104)
- Lira district – Okwongo village, Olarokwon parish, Adwari sub-county (**Mr. John Kennedy Opio** 0775985489 & **Mr. Patrick Abwango** 0715238422)
- Arua district – Uleppi trading centre, Uleppi subcounty (**Mr. Felix Etoma** 0773124852 & **Mr. Henry Asindua** 0782995891)
- Pader district - Lacankweri village, Lukole sub-county (**Mr. Ocaya LC1)**

- Moyo district – Oyajo village, Eria parish, Moyo sub-county (**Mr. Ratib Adrawa** 0782927300)
- Nebbi district – Palewo village, Kalwang parish, Nebbi subcounty (**Mr. Uma Philip Kigezi** 0772883320 & **Mr. Openytho Abok** 0772901219)

2. Passport data for sites:

The following passport data will be collected for each site:

- Record names of collector team.
- Record date of collection.
- Name of sites (village, sub-county and district)
- Status of the area (local people's land or protected area e.g. forest reserve)
- Current utilization of the area (cultivation) and level of threat to shea trees (e.g. felling)
- Name associated tree species with indication of abundance
- Site accessibility and distance from nearest trading centre
- Type of vegetation: short description with specification of the most common species

3. Criteria for the selection of trees

Five (5) trees of each variety have to be sampled from each shea tree population in each of the sample sites. The sample trees should preferably consist of older trees from which fruits are falling and the varieties can be ascertained by a local guide with certainty. Select the best representative trees in case you come across more than one tree of the same variety.

Validation of shea tree variety nomenclature was carried out between 8th and 15th May 2009 across all the districts mentioned in section 1 above. Therefore, collection efforts should be directed at collecting samples from the following varieties from each district as indicated in the table below. Samples may be collected from any other variety not listed below if the local guides feel such variety is very distinct.

District	Validated variety nomenclature
Katakwi & Amuria	**Ewiny** (sweet taste), **Egeget** (astringent taste), **Nasomel** (oval/elliptical shaped fruits/nuts), **Nalungur** (round fruits/nuts), **Nacekum** (Hairy fruits), **Epiana** (tasteless pulp)
Lira	**Alimdiri** (small fruits), **Acogo Apocopoco** (soft pulp), **Alulung** (round fruits/nuts), **Ajayer** (Hairy fruits), **Abono** (sweet pulp), **Acula** (oval/elliptical fruits/nuts), **Aremo** (red/pink pulp)
Abim	**Acula** (oval/elliptical fruits/nuts), **Abor** (small fruits), **Ajiki** (small fruits), **Amor, Yao-acot**
Pader	**Aloto** (oval/elliptical fruits/nuts), **Mulunge** (round fruits/nuts, **Layer** (hairy fruits), **Yao-atega** (hard pulp), **Yao-matino** (small fruit), **Aremo** (red/pink pulp)
Moyo	**Coloba or coloa** (oval/elliptical fruits/nuts), **Gburu** (round fruits/nuts), **Ngorokwa** (little pulp), **Enyii** (hairy fruits), **Ciria** (small fruits), **Mudaa** (soft pulp), **Urujua** (hard pulp), **Asa** (tasteless pulp), **Limi** (sweet pulp), **Etri** (astringent taste)
Arua	**Etri** (astringent taste), **Mbele** (big fruits & nuts), **Anyaka** (hairy pulp), **Ombati** (hard pulp), **Omemea** (little pulp), **Julu** (oval/elliptical fruits/nuts), **Ngulu** (round fruits/nuts), **Mbilimbili** (sweet pulp), **Kpetea** (soft pulp)
Nebbi	**Malim-lim** (sweet pulp), **Macwot** (Astringent pulp), **Nyangili** (hard pulp), **Madong** (big fruits), **Yao-mayer** (hairy fruits), **Mangulungulu** (round fruits/nuts)

4. Sampling checklist for each tree

Record all sampled data into a hardbound **field book**. Use a sharpened HB (No. 2) pencil for writing. The field book data will have to be digitised as soon as possible after return from the field to prevent loss of data. The field data book has to be kept for record purposes and therefore, handwriting has to be clear and legible.

a. All sampled trees have to be **marked permanently** (colour and metal tags)

b. Record the name of shea tree variety and assign each tree a **unique identification number**, this will be the initials of the sub-county and serial number (includes metal tags), e.g. first tree sampled from Omodoi sub-county would be OM1, next sampled tree would be OM2 etc. The same number must not be allocated to different trees.

c. Record **GPS coordinates** (decimal degrees) and **altitude** of sample tree (also record the accurateness of the readings, i.e. +/- in m).

d. Assess **shape of stem**/tree, using the assessment chart provided (Section C)

e. Record **dbh** and **height** of sample tree.

f. Take two measurements [in metres] of the canopy diameter of the sample tree (N – S & W – E) using measuring tape

g. Assess **crown condition** (Section D); if damaged, record on **which side** (orientation/ N, E, S, W) of the tree the crown is damaged.

h. Collect **fruits for chemical and morphological study.***

i. Collect **leaves for morphological study.***

j. Collect **leaf samples for DNA study.***

k. If possible, take 4 digital **photographs of each tree** from all directions (aspects) (note filenames and order of photos in the field book).

***see specific guidelines in the next section**

Record any useful characters you see.

B How to collect samples

1. Fruits for morphological and chemical study

i. Randomly collect 30 fallen fruits from each sample tree**.**

ii. Place the fruits in a dry polythene bag.

iii. Carefully label the bag with its sample number. Put a label inside the bag and another outside. The label number should be the same as that for the tree from which these samples are being collected.

iv. Place the fruit samples in a cool box containing ice cubes. Make sure to freeze the ice cubes at an appropriate place (freezer) whenever the ice becomes liquid.

v. Make an appropriate entry against the sampled tree in the field notebook showing that leaf samples for morphological study have been collected from the tree.

2. Leaves for morphological study

i. Randomly collect 50 oldest and mature leaves from each sample tree**.**

ii. Place the leaves in a paper bag, ensuring that they do not fold.

iii. Carefully label the paper bag with its sample number. Put a label inside the paper bag. The label number should be the same as that for the tree from which these samples are being collected.

iv. If possible, place weights on each paper bag to ensure flattening out of the leaf samples.

v. Make an appropriate entry against the sampled tree in the field notebook showing that leaf samples for morphological study have been collected from the tree.

3. Leaves for DNA study (to avoid contamination, **DO NOT** touch the leaf samples. Wear gloves (provided) at all times while processing these samples).

(i). Collect **3-4 young leaves (preferably recently fully or near full-expanded)**. These should be of healthy appearance and preferably **without damage** (signs of discoloration, insect feeding, dead parts, etc.). Young, fully opened reddish or pink leaves will be preferable. If not available, collect the youngest leaves from the most recent growth, in this case one leaf from different twigs of the same tree to make a sample of four leaves from an individual tree.

(ii). **Clean leaves** by wiping with a piece of cotton wool moistened with ethanol

(iii). **Break** leaves **into pieces**

(iv). Put **leaves** into **plastic zip-lock bags with silica gel** (ca. 50g); make sure the leaf pieces do not stick together, but are each separately covered by silica gel

(v). Immediately **close zip bag tightly.** Make sure to expel all air from the ziplock bags before closing it.

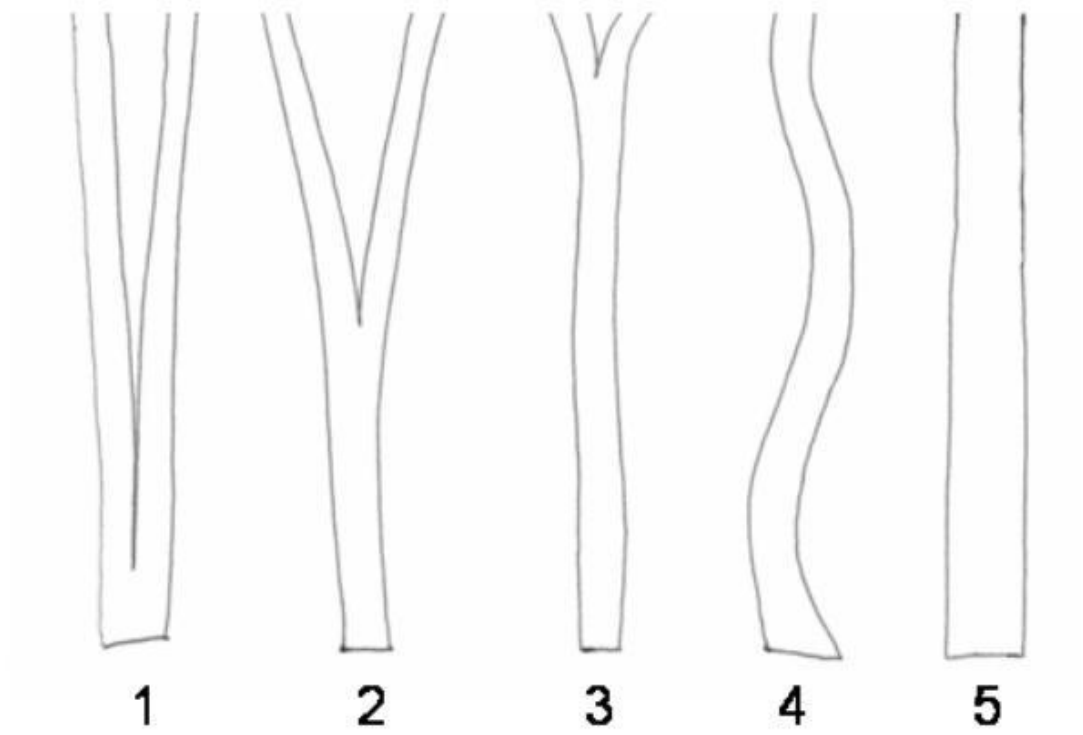
(vi). **Carefully label the zip bag** with its sample number (put label also inside the bag). The sample number should be the same number for the tree from which samples are being collected

(vii). **Samples should be bone dry within one day** at ambient temperature; **add extra silica gel if necessary** (if blue indicator turns pink)

(viii). Upon collection, **store** sample bags **in the provided plastic (cool) boxes**, make sure the lids of the boxes are firmly closed (airtight).

(ix). Indicate in the field note book a record of having collected leaves for DNA study against each sampled tree entry.

C: Assessment of stem form



1 – Forked from bottom

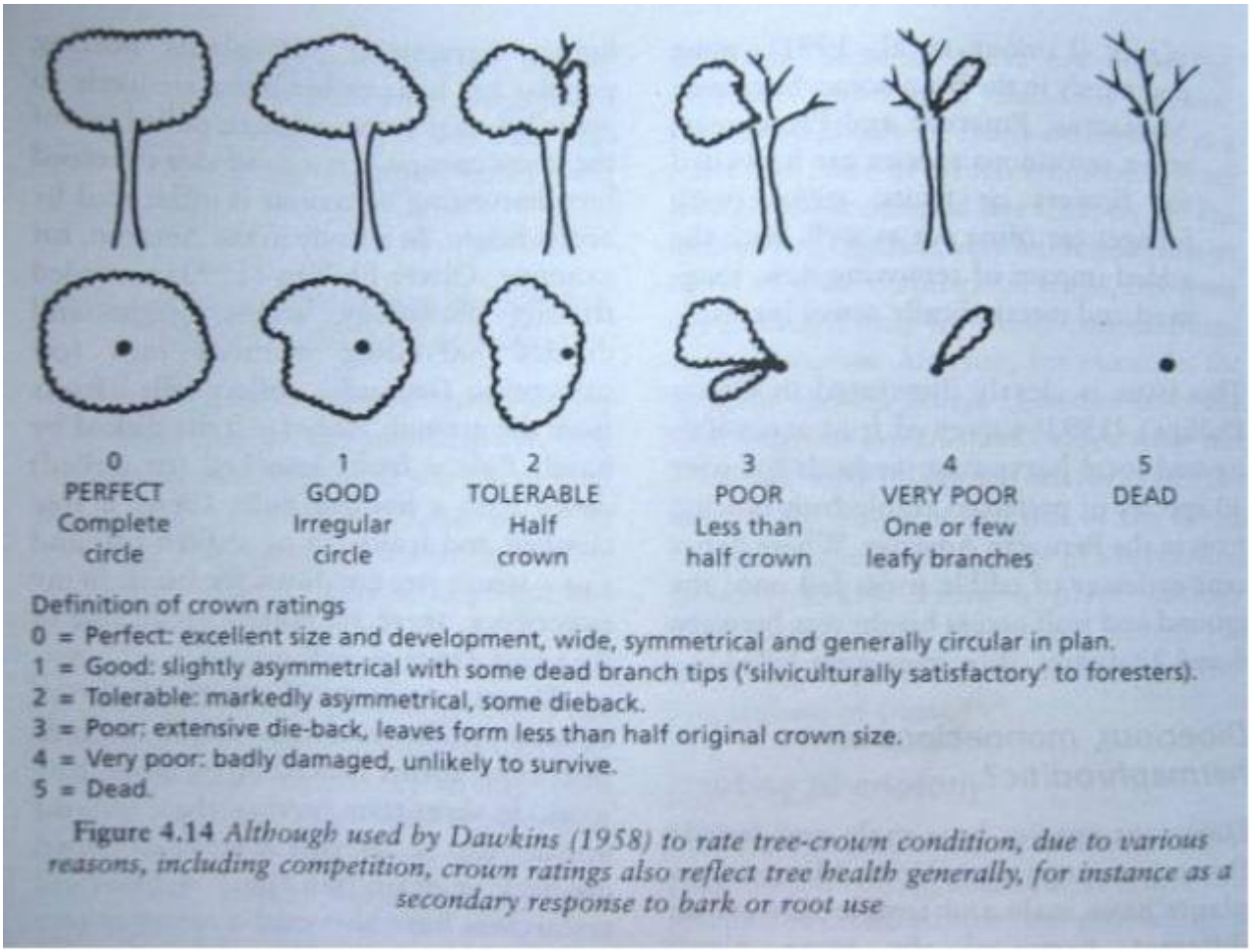
2 – Fork at less than 6 m height

3 – Forking starts above 6 m height

4 – Stem twisted

5 – Stem straight

D: Assessment of crown condition



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

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

APPENDIX 4

PUBLICATIONS AND DISSEMINATION

Papers published and/or accepted for publication

S Gwali, JBL Okullo, G Eilu, G Nakabonge, P Nyeko & P Vuzi (2011). Folk classification of shea butter tree (*Vitellaria paradoxa* subsp. *nilotica*) ethno-varieties in Uganda. *Ethnobotany Research & Applications* 9: 243-256

S Gwali, JBL Okullo, G Eilu, G Nakabonge, P Nyeko & P Vuzi (2012). Traditional management and conservation of shea trees (*Vitellaria paradoxa* subspecies *nilotica*) in Uganda. *Environment, Development and Sustainability* 14(3):347-363

S Gwali, G Nakabonge, JBL Okullo, G Eilu, P Nyeko & P Vuzi. Morphological variation among shea tree (*Vitellaria paradoxa* ssp. *nilotica*) ethnovarieties in Uganda. Accepted for publication by Genetic Resources and Crop Evolution (2012).

Magazine articles

S Gwali (2008). Famine foods for Uganda – domesticating the shea tree for food security. NaFORRI newsletter Issue 1, 2008. Page 3.

S Gwali (2011). Revealed: Folk classification and nomenclature of shea trees in Uganda. NaFORRI newsletter, Volume 2, Issue 1, June 2011. Pages 1 and 3.

Papers presented at scientific conferences/workshops

S Gwali, JBL Okullo and G Eilu (2012) Traditional practices in the conservation of shea trees (*Vitellaria paradoxa*) in Uganda: Lessons for conservation science. Paper presented at the 1st IUFRO – FORNESSA Regional Congress, World Agroforestry Center, Nairobi, Kenya, 25-29 June 2012.

S Gwali, G Nakabonge, JBL Okullo, G Eilu, P Nyeko and P Vuzi (2012). Does morphology provide any evidence about shea tree (Vitellaria paradoxa) folk classifications in Uganda? Paper presented at the 1st IUFRO – FORNESSA Regional Congress, World Agroforestry Center, Nairobi, Kenya, 25-29 June, 2012.

S Gwali, JBL Okullo and G Eilu (2011). Traditional shea tree conservation practices in Uganda: Lessons for conservation science. Paper presented at the INNOVKAR Scientific Conference, Splendid Hotel, Ouagadougou, Burkina Faso, 21-30 October, 2011.

S Gwali, G Nakabonge, JBL Okullo, G Eilu, P Nyeko, & P Vuzi (2011). Morphological variation among shea tree (*Vitellaria paradoxa* ssp. *nilotica*) 'ethno-varieties' in Uganda. Paper presented at the INNOVKAR Scientific Conference, Splendid Hotel, Ouagadougou, Burkina Faso, 21-30 October, 2011.

S Gwali, JBL Okullo, G Eilu, G Nakabonge, P Nyeko and P Vuzi (2011). Folk classification of shea butter tree (*Vitellaria paradoxa* subsp. *nilotica*) ethno-varieties in Uganda. Paper presented at the joint scientific conference of the Association for Tropical Biology and Conservation (ATBC) and African section of the Society for Conservation Biology (SCB), Arusha, Tanzania, 12-16 June, 2011.

S Gwali, G Nakabonge, JBL Okullo, G Eilu, SB Tumwebaze, P Nyeko and P Vuzi (2011). Morphological variation among shea tree (*Vitellaria paradoxa* ssp. *nilotica*) ethno-varieties in Uganda. Paper presented at the 3rd Makerere Annual Research and Dissemination Conference, Hotel Africana, Kampala, 11-12 April, 2011

S Gwali Characterisation of shea tree (*Vitellaria paradoxa* ssp. *nilotica*) ethno-varieties in Uganda. Paper presented at the 3rd EU-INNOVKAR Shea Tree Project Steering Committee Meeting, Kampala, Uganda, 5 – 10 July, 2009.

Poster presentations

S Gwali, JBL Okullo and G Eilu (2009). Towards the verification of folk classification of shea tree varieties in Uganda. 3rd EU-INNOVKAR Shea Tree Project Steering Committee Meeting, Kampala, Uganda, 5 - 10 July, 2009.

S Gwali, JBL Okullo and G Eilu (2009). Traditional practices in shea tree (*Vitellaria paradoxa*) conservation in Uganda. Second World Congress on Agroforestry, World Agroforestry Centre, Nairobi, Kenya, 23 – 28 August, 2009.

S Gwali, JBL Okullo and G Eilu (2009). Traditional shea tree conservation practices in Uganda: Lessons for Conservation Science. IUFRO Regional Conservation Science – Policy Conference, University of Ghana, Legon, Accra, Ghana, 28-30 January, 2009.

S Gwali, JBL Okullo and G Eilu (2009). Towards the verification of folk classification of shea tree varieties in Uganda. 3rd EU-INNOVKAR Shea Tree Project Steering Committee Meeting, Kampala, Uganda, 5-10 July, 2009.