
Project no. **INCO-CT-032037**

Project acronym: **INNOVKAR**

# Project title: **Innovative Tools and Techniques for Sustainable Use of the Shea Tree in Sudano-Sahelian zone**

**SIXTH FRAMEWORK PROGRAMME**
**PRIORITY [#]**

**Contract for:**
**SPECIFIC TARGETED RESEARCH**

# Publishable Final Activity Report

Period covered: **01/12/2006 TO 30/11/2011**

Date of preparation: **January 2013**

Start date of project: **December 2006**

Duration: **60 months**

Project coordinator name: **Jean-Marc BOUVET**

Project coordinator organisation: **CIRAD**

# 1. Project execution

## 1.1 summary description of project objectives

The shea tree, *karité* in French, (*Vitellaria paradoxa*) is a tree species growing extensively in the agroforestry parklands of semi-arid Africa in a 6,000 km x 500km zone from Senegal to Uganda, where it is protected and managed. Shea butter has been traditionally extracted by women from the dried kernels of the shea tree for many millennia. Total production potential is estimated to reach over 2.5 million metric tonnes of raw kernels.
People living in the semi-arid zone of sub-Saharan Africa (SSA) have traditionally used shea butter in large quantities. It is estimated that at least 150,000 t of kernels are consumed annually for frying, adding to sauces, as a skin pomade, for medicinal applications, to make soap, for lanterns, and for cultural purposes at ceremonies, including births and weddings.

The demand for vegetable fat in the western marketplace continues to grow as shea butter is now commonly used in the production of cocoa butter equivalents or improvers for use in chocolate (up to 5% non-cocoa fat content by weight is allowed under European Union (EU) regulations in products labelled as such), other confectionaries and margarines. Shea exports from Africa are now estimated to have grown to an annual maximum of 150,000 t of dry kernel with a current market value of approximately US$30 million with prices around US$200 t f.o.b. West African port. Processed in Europe and India, this is used for the preparation of ca. 18,000 t of stearin (the high melting point 'fat' fraction) with an estimated value of US$36 million. It is unknown what volume is used in the United States (US) for edible or cosmetic products since the US does not permit non-cocoa vegetable fat in chocolate products and does not have a specific import category for shea.

Countries strongly involved in the shea butter tree trade have developed for many years research and studies to improve the management of parkland, the valorisation of genetic resources, the processing of shea butter. This is the case for the African countries involved in the project such as Senegal, Mali, Ghana, Burkina Faso and Uganda, which recognise that the species makes a significant contribution to the livelihoods of millions and the economics of a number of African nations.

There have been some considerable advances in scientific knowledge and in implementation of techniques on the shea tree during the last ten years. However, there are still many threats and constraints in need of urgent attention:

### Threats to the natural resource:

- For the West African Sahel, the last twenty-five years has seen a significant decline in rainfall (30% less than the average calculated before 1960) threatening the shea parklands (negative impact on natural regeneration),
- Shea tree populations are severely threatened as a result of agricultural mechanisation which reduces the shea tree density; furthermore there is no information available as to the consequence of this on the species diversity and population quality in either the medium or long term.

Market constraints:

- The non-traditional market is continuing to evolve rapidly, as demand from the international personal care industry increases, although the potential of shea butter remains poorly understood and documented, with very low levels of consumer awareness;
- Since 1st January 2005, new EU regulations require the traceability from 'source to shelf' for all agricultural products. There have been few attempts, however, to improve traceability of shea products;
- Strong vertical integration of all steps in the value-added chain beyond West Africa and the dominance of a few players make it a buyers' market with narrowly limited bargaining power of the producers;
- Lack of knowledge amongst producers and middlemen regarding prevailing rules and regulations in the EU; as a result, African players in the value added chain do not actively shape the market themselves;
- The lack of knowledge of variation of the most economically important traits (e.g. fat content and profile) currently constrains the implementation of any effective quality label of the product;

Need to improve quality in production:

- Initial results on the chemical characteristics of shea kernels among farmer varieties are not sufficiently well understood. For any tree-breeding program to successfully capture gains in economic traits, it is important to determine whether this variation results from "nurture or nature", i.e. environmental (edaphic or climatic) variation, or genetic differentiation between populations.
- The impact of climate variation and of mechanisation of agriculture on the production of shea tree is not assessed. Prediction of this potential impact is needed to organise future production.
- A major 'quality control' constraint has now been identified at the initial post-harvest stages of production. It is important that research is quickly focused on how to provide appropriate technology at this socially and economically fragile step in the industry.

Need to develop new product

- Although the demand of edible fat is very important, the non-traditional market for shea butter in personal care products is growing remarkably and represents the highest potential to add value at source. The potential of the species and especially the variation according to tree and origins is still not well known.

As a result, **the overall objective** of INNOVKAR is to improve the production and marketing of shea tree products based on a sustainable, fully functioning, agroforestry system environment.

The specific objectives corresponding to the work packages of the project can be defined by:

**1 - To promote the conservation of germplasm facing climatic change by the characterisation of shea tree parklands on response to drought** using new ecophysiological approaches and modelling distribution on mid and long term;
**2 - To improve the sustainable management of parkland by analysing the impact of**

**global change on the natural regeneration and the production of fruits** through the field analyses and the use of integrated ecological models and software platforms;

**3 - To promote the delineation of provenances (and in consequence certified origin) across the natural range** by combining classical morphological traits, chemical property of kernel and new functional molecular markers coming from innovative genomic tools;

**4 - To facilitate domestication by farmers and elaborate a pre-breeding programme** by characterising farmer varieties of shea tree based on morphologycal and chemical traits (fatty acid, unsaponifiable content …), by developing basic horticultural techniques and mobilising material for a pre-breeding programme;

**5 – To provide efficient and low cost method for chemical analyses** by implementing near infrared spectrometry of the shea kernels for screening large sample and improving the analysis capacity;

**6 - To encourage and promote traceability initiatives of shea product from fruit to shea butter** through innovative research on methodology (genomic and metabolic approaches);

**7 – To promote new applications of shea butter** that utilise some of the other beneficial properties of shea butter for cosmetic and pharmaceutical application, e.g. the anti-oxidant properties of catechins….

**8 – To improve quality control during the initial production stages of shea butter**, to develop socially and economically acceptable protocols for quality shea kernel or butter at the quantities internationally demanded for both the edible and personal care markets;

**9 – To identify market opportunity by** analysing the value chain of shea products with a view to framing a trade and policy environment which can assist poor producers and poor countries to participate more effectively in the global shea economy;

**10 - To synthesize, consolidate, share and disseminate information** on innovative technologies for sustainable management and trade of shea tree using scientific results and operational strategy in connection with the main stakeholders: formers, economic operators and policy and decision makers.

## 1.2 - Contractors involved

To provide significant research results, with national, regional and international impact, Senegal, Mali, Ghana, Burkina Faso and Uganda, which are strongly involved in the production and sustainable management of the shea tree, have decided to elaborate an integrated project. Those countries are also strongly concerned by the numerous constraints cited above and have participated in past research programmes on shea tree.

In collaboration with 5 European partners, based on the state of the art and the constraint analysis, it has been decided to define research priorities in line with those of the specific INCO measures. The topic research implemented by the partners have been guided by:

- innovative tools and techniques for the characterisation, development and use of origins with enhanced tolerance to drought and heat,
- development and dissemination of sustainable management of genetic resources taking into account traditional knowledge and innovative methods, and
- the development of innovative, efficient, environment-friendly, post harvest, storage, processing and marketing methods.

| | **Participant n°** | **Participant organisation name** | **Participant organisation short name** | **Country** | **Date enter the project** | **Date exit the project** |
|---|---|---|---|---|---|---|
| CO | 1 coordinator | International Cooperation Centre in Agronomic Research for Development | CIRAD | France | 1 | 60 |
| CR | 2 | University of York | UOY | United Kingdom | 1 | 60 |
| CR | 3(11) | Royal Veterinary and Agricultural University | KVL | Denmark | 1 | 60 |
| CR | 4 | AarhusKarlshamn Sweden AB | AAK | Sweden | 1 | 24 |
| CR | 5 | UNIQUE Forestry Consultants GmbH | UNIQUE | Germany | 1 | 60 |
| CR | 6 | Institut d'Economie Rurale | IER | Mali | 1 | 60 |
| CR | 7 | Institut Sénégalais de Recherches Agricoles | ISRA | Senegal | 1 | 60 |
| CR | 8 | Centre National de la Recherche Scientifique et Technologique | CNRST | Burkina Faso | 1 | 60 |
| CR | 9 | Makerere University | UNIMAK | Uganda | 1 | 60 |
| CR | 10 | University for Development Studies | UDS | Ghana | 1 | 60 |
| CR | 12 | University of Montpellier Faculy of pharrmacy | UM1 | France | 38 | 60 |

# 1.3 - Work performed and end results

## 131 - Work Package 1: Shea tree parkland dynamics and production: characterisation of natural regeneration, prediction of recovery using modelling and quantification of fruit production

**General objective**
To provide recommendations for management of parkland in connection with climatic zones and farmer practices by analysing natural regeneration, by modelling its dynamics on mid and long term and by quantifying fruit production.

**Specific objectives**
1 – To assess the impact of climate change and farmer practises of the natural regeneration of shea tree.
2 – To assess the impact of climate change and farmer practises on fruit production
3 – To predict shea tree parkland dynamics on long term using a predictive model of Shea regeneration and production under different management scenarios

**Scientists involved**
Mahesh Poudyal (UoY, England)
Brigitte Bastide, Diallo B. Ousmane & Kaboré Sibiry Albert (INERA, Burkina Faso)
Dahl Kjær Erik (KVL, Denmark)
George Nyarko, Joshua A. Yidana, Felix K. Abagale & Francis A. Chimsah (UDS, Ghana)
Bokary Kelly & Haby Sanou (IER, Mali)
G Eilu, JBL Okullo, Patrick Byakagaba & Edward Mwavu (Makerere University, Uganda); Massamba Thiam (Senegal)

### 1.3.1.1 WP1 - Methodologies and approaches employed

*Establish permanent plots to study natural regeneration and fruit production according to a gradient of mean annual rainfalls (Task 1.1)*
To characterise the relationship between the natural regeneration and the climatic zones three sites in each African country involved in the project were selected according to climatic gradient. In each site two types of land use (fields and fallows) were considered. For each type of land use, three land use history (New: 0-5 years, Medium: 6-10 years, Old: more than 10 years) based on the number of years that particular land use type was in place continuously. For each land use history, three permanent plots, of 50m x 50m (0.25 ha), were established, giving 18 plots per site and 54 plots for a country.

*Measure the spatial distribution of trees and to assess mortality and growth within different cohort (Task 1.2)*
All adult trees (circumference at 1.30 m above the ground higher than 10 cm) in all permanent plots were measured to characterized stands. Variables measured were: total height, height at the first branch, circumference at 1.30 m, crown size).
To study the shea natural regeneration, each plot was sub-divided into 16 sub-plots of 12.5 x 12.5 m. Five (5) sub-plots were selected randomly for regeneration count and measurement. In each subplot, seedlings (height = 0 - 50 cm) were counted. For shea plants having 51 - 100 cm of height, measured variables were: height and collar circumference. For saplings (101 -

150 cm of height) measured variables were: height, collar circumference and circumference at 1,30 m.

*Conduct phenology studies and assess fruit production (Task 1.3)*
For each land use history of each land use type, 10 shea trees were monitored. For flowering monitoring, parameters like the 'length of flowering' i.e. the number of days between the 'start and the end' of flowering, the 'length of active phase' were determined in each site. The quantity of flowers was estimated by class variables using percentage of crown covered.
Fruit abundance was measured on each tree using class variables, and adequate techniques to quantify fruiting in terms of fruit flesh and nut production. The fruits of monitored trees in each land use history were collected by the women of household to whom the land belonged. For each tree, fruits number and fresh nuts weight were determined.

*Use model to predict the dynamics of parkland according to various management scenarios (Task 1.5)*
Using the data from the permanent plots (Task 1.2), a count-data modelling approach was used to estimate shea regeneration (seedling count) in different classes of land use types and land use histories. The initial modelling was performed on the data from Burkina Faso and Mali, with a view to extend the model to other countries – on a per-country basis as well as at an integrated level.

Table 1.3.1-1 summarises the activities carried out under work package 1 over the entire duration of the project.

**Table 1.3.1-1: Summary of the WP1 project activities over the entire project period (from Year 1-5)**

| Country | Permanent Plots | Data Collection | | | | | Data Analysis | |
|---|---|---|---|---|---|---|---|---|
| | *1st Period* | *1st Period* | *2nd Period* | *3rd Period* | *4th Period* | *5th Period* | *2nd, 3rd & 4th Periods* | *5th Period* |
| Burkina Faso | Established on all sites | Tree measurements, fruit production, phenology | Continuation from Year I + Soil and Stand characteristics | Regeneration, phenology, fruit production, soil sampling to do | Phenology, fruit production, soil sampling to do | Phenology | In-country analysis of Year I, II & III data + part of integrated data analysis. | In-country analysis of data (regeneration, phenology, fruit quantification) + modelling shea regeneration |
| Ghana | Established on all sites | Tree measurements | Continuation from Year I (but no phenology or fruit production assessments) | Tree and regeneration monitoring at the end of the rainy season (Dec 2008). No data collection since January 2009 due to lack of funds. | No fieldwork during this period due to lack of funds, and due to changes in INNOVKAR-UDS team[1] | Plots assessment for diversity, adult tree measurement in May/June 2011. Shea regeneration monitoring in Jan 2011 and May/June 2011. Soil sampling from the plots. | Preliminary analysis of tree and regeneration data from 2008. Adult tree data used in integrated data analysis (preliminary) | In-country analysis of data (species diversity in the plots, and size-class distribution of shea trees) |
| Mali | Established on all sites | Tree measurements, fruit production, phenology | Continuation from Year I + Soil and Stand characteristics (qualitative) | Regeneration, phenology, fruit production | Regeneration (end of rainy season only), phenology, fruit production. | Species diversity in the permanent plots, regeneration | In-country analysis of Year I, II & III data + part of integrated data analysis | In-country analysis of data (regeneration, phenology, fruit quantification) + modelling shea regeneration |
| Senegal | Established on all sites | Data collected without correct protocol | Data collection re-started following the correct protocol[2] | Continuation from Year II (Phenology monitoring stopped in June 2009 due to the lack of funds) | No fieldwork during this period due to lack of funds. | Species diversity in the permanent plots, regeneration | Preliminary analysis of Year II data on Tree and regeneration + Adult tree data from previous periods used in integrated data analysis (preliminary) | In-country analysis of data (size-class distribution, regeneration, species diversity) |
| Uganda | Established on all sites | Tree measurements | Continuation from year I + phenology, fruit production, soil samples taken | Continuation from Year II | Continuation from Year III – soil samples yet to be analysed | Species diversity in the permanent plots, regeneration, phenology, fruit production. | In-country analysis of data + preparation of manuscripts | In-country analysis of data (regeneration, size-class, species diversity) |

[1] This situation was rectified at the end of 2010, and there have been two data collection from the plots in 2011 since.

[2] In Senegal, the first assessment was carried out without following the correct protocol. After the Second Meeting in Tamale in 2008, this was rectified and the data collection was restarted following the correct protocol since the end of the rainy season plots assessments in 2008.

### 1.3.1.2 WP1 - Achievements of the project to the state-of-the-art.

Due to this project, we now have a cluster of 50m x 50m (0.25 ha) permanent plots covering three climatic gradients in each of the five countries for shea tree monitoring (adult trees, regeneration, phenology, fruit production) based on a standard study protocol (Task 1.1). These plots have generated a rich set of data for this project and will continue to do so in future if the same sites are used for shea tree monitoring in the future. Furthermore, some partners have extended their permanent plots to new sites, such as two new sites in Mali's extreme north. Table 1.3.2-1 summarises the study sites in all five countries where permanent plots are located.

**Table 1.3.2-1: Study sites where permanent plots have been established for the project.**

| **Country** | **Study Sites (# permanent plots)** | | | |
|---|---|---|---|---|
| | *South* | *Central* | *North* | *Others* |
| Burkina Faso | Niangoloko (18) | Sobaka (18) | Guibaré (18) | |
| Ghana | Kawumpe (18) | Nyankpala (18) | Paga (18) | |
| Mali | Daelan (18) | Mperesso (18) | Naféqué (18) | Sassambourou, Tori (both in extreme north) |
| Senegal | Salemata (18) | Samecouta (18) | Saraya (18) | |
| Uganda* | Nakasongola (8) | Katakwi (12), Lira (12) | Moyo (12) | |

* Not all classes of land use history was available in Uganda, hence the lower number of permanent plots.

From all these permanent plots, data on adult shea trees, shea regeneration and plot diversity was collected. Furthermore, using a standard sampling procedure adult shea trees were selected to monitor phenology and fruit production. From each permanent plot, soil samples were also taken for analysis, and using the nearest weather station climatic data for each study site was collected. Table 1.3.2-2 provides a summary of the data collected from all the study sites, and a list of variables considered. The actual database forms a part of the WP1 deliverables, which has already been submitted.

The data gathered from the study sites and permanent plots were used in a series of analyses, primarily at an individual country level, looking at stand characteristics, regeneration, and plot diversity across fields and fallows. Some of these analyses have already resulted in published papers, which are submitted as part of the WP1 deliverables and publishable results. Furthermore, an integrated analysis of shea regeneration dynamics in field and fallows was done using the data from Burkina Faso and Mali as a starting point. A count data model has been developed to analyse the shea regeneration in different land use types, which can be either used at an individual country level or can be used to analyse regeneration at an integrated level if comparable data are available. Box 1 provides some basic information about the shea regeneration modelling used in this work package.

The biggest contribution and achievement of this work package, however, has been the collection of the standard set of data on shea trees across the climatic gradients and across the five countries in the Sudano-sahelian zone. These data on various aspects of shea trees in the agroforestry parklands will not only help in a better understanding of the shea parkland dynamics but they will also contribute towards informed policy decisions regarding the sustainable management of shea parklands.

**Table 1.3.2-2: Summary of the data collected from all the study sites between 2007-2011. The actual data collected is submitted as part of the WP1 deliverables.**

| **Country (Partner)** | **Study sites** | **Land Use (Field, Fallow)** | **History (New, Medium, Old)** | **Total no of plots** | **Data Collection** | | | | | | | **Remarks** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *Tree* | *Regeneration* | *Plot diversity* | *Phenology* | *Fruit production* | *Soil* | *Climate* | |
| Burkina Faso (INERA) | Guibaré | Both | All | 18 | 2 | 4 | Yes | Apr 2008 – June 2011 | 2008 – 2011 | - | All | Not all land use types containing shea trees was available in Uganda, for example, no fields in Nakasongola. |
| | Sobaka | Both | All | 18 | 2 | 4 | Yes | | | Collected | All | |
| | Niangoloko | Both | All | 18 | 2 | 4 | Yes | | | - | All | |
| Ghana (UDS) | Paga | Both | All | 18 | 3 | 4 | Yes | - | - | Yes | | |
| | Nyankpala | Both | All | 18 | 3 | 4 | Yes | - | - | Yes | | |
| | Kawumpe | Both | All | 18 | 3 | 4 | Yes | - | - | Yes | | |
| Mali (IER) | Nafégué | Both | All | 18 | 2 | 6 | Yes | Feb 2008 – Feb 2010 | 2008 – 2010 | - | Rainfall, rainy days | |
| | Mperesso | Both | All | 18 | 2 | 6 | Yes | | | - | Rainfall, Temp, Humidity | |
| | Daelan | Both | All | 18 | 2 | 6 | Yes | | | - | Rainfall, rainy days, temp, humidity | |
| Senegal (ISRA) | Saraya | Both | All | 18 | 3 | 3 | Yes | 2007 – 2011 | 2008 – 2009 | Collected | Planned | |
| | Samecouta | Both | All | 18 | 3 | 3 | Yes | | | Collected | Planned | |
| | Salemata | Both | All | 18 | 3 | 3 | Yes | | | Collected | Planned | |
| Uganda (UNIMAK) | Moyo | Both | Partial | 12 | 1 | 2 | Yes | Nov 2009 – Nov 2011 | 2009 – 2010 | Yes | All | |
| | Katakwe | Both | Partial | 12 | 1 | 2 | Yes | | | Yes | All | |
| | Lira | Both | Partial | 12 | 1 | 2 | Yes | | | Yes | All | |
| | Nakasongola | Fallow | Partial | 8 | 1 | 2 | Yes | | | Yes | All | |
| | | | | | | | | | | | | |

| **Variables** | | | | | | |
|---|---|---|---|---|---|---|
| *Tree* | *Regeneration* | *Phenology* | *Fruiting* | *Soil* | *Climate* | *Plot history, plot diversity, and land use pattern* |
| DBH, Height, Crown diameter, Location (GPS) | Seedling count, Height, Base diameter, DBH (for class 3) | Flowering, Fruiting, Leafing | Fruit count, Nuts weight | Soil characteristics and composition | Temperature, Rainfall, Humidity | Ownership, farming practices, fire, plot diversity (number of species and composition), use of tractor, chemical fertilisers, pesticides etc. |

**Box WP1-1: A general description of the shea regeneration modelling in field and fallows.**

***Modelling shea regeneration (D1.5)***

We examine the influence of land use practices, and other factors (adult shea density, for example) on the regeneration of shea in the two countries, separately and combined, through a regression model. Shea regeneration is measured as number of seedlings in each randomly selected subplot and is hence a count data. Due to the nature of the data, we use count data models (Poisson, Negative Binomial) using generalised linear modelling in R to estimate total regeneration. We start by using Poisson regression model, which is commonly used for count data models. The density function of a discrete random variable, $Y$, with a Poisson distribution is:

$$\Pr[Y = y] = \frac{e^{-\mu}\mu^{y}}{y!}, y = 0, 1, 2, \ldots \qquad (1)$$

The Poisson distribution has a single parameter, $\mu$, where $E[Y] = V[Y] = \mu$. Assuming $y_i$ as the number of occurrences of the event of interest, in our case the number of shea seedlings in a randomly selected subplot, and $\mathbf{x}_i$ as the vector of independent regressors that are assumed to determine $y_i$ (for example, land use type, land use history, adult shea density, etc.), $y_i$ given $\mathbf{x}_i$ is Poisson-distributed with parameter $u_i$ as:

$$f(y_i|\mathbf{x}_i) = \frac{e^{-\mu_i}\mu_i^{y_i}}{y_i!}, y = 0, 1, 2, \ldots \qquad (2)$$

Given the characteristics of the distribution, a Poisson regression function can be specified (and estimated) in log-linear form as:

$$\mu_i = \exp(\mathbf{x}_i'\beta) \qquad \text{OR} \qquad ln\mu_i = \mathbf{x}_i'\beta \qquad (3)$$

Equation (3) shows the most common form a Poisson regression equation is estimated, with log-link, but it also ensures that $\mu > 0$. However, it also indicates that due to the multiplicative nature of the function while estimating the parameter, $\mu_i$, we have to take into account the level of other regressors, especially the continuous variables, while estimating the changes in dependent variable (shea regeneration count in our case) for a change in the selected regressor. For each country, we estimate shea regeneration with the following variables:

**Dependent: $y$ = Total shea regeneration in randomly selected subplots**

Independent: $\mathbf{x}$ = Gradient, Season, LandUse (3 categorical variables + their relevant interactions), shea_density (density of adult shea trees in the permanent plot where the randomly selected subplots are located), LandUse:History (interaction of LandUse and History variables, giving rise to a categorical variable with Field and Fallow of various age class as categories), LandUse:shea_density (interaction of land use categories and adult shea density)

For the combined data from Burkina Faso and Mali, we use following regressors for our shea seedling count model:

**x = Country, Season, LandUse, shea_density, LandUse:History, LandUse:shea_density**

Poisson model assumes the equality of conditional mean and conditional variance for $y$ (i.e., $E[y_i|\mathbf{x}_i] = V[y_i|\mathbf{x}_i]$), however, it is clear from the basic descriptive statistics that variance is many times larger than the mean for $y$ (i.e., number of seedlings). This is termed overdispersion in Poisson (which is also verified from the estimated Poisson model). Although overdispersion, like heteroskedasticity, does not affect the consistency of the parameter estimates, it results in inconsistent estimates of standard errors, and hence produces unreliable tests of significance (Cameron and Trivedi 1998). Negative Binomial model adjusts for the overdispersion and produces consistent estimates for standard errors. We therefore use Negative Binomial model to estimate the proposed model for regeneration.

### 1.3.1.3 WP1 - impact of the project on its industry or research sector

The results from WP1 are primarily beneficial for the research community, and government and non-government agencies working in agroforestry in Sudano-Sahel zone in general, and shea trees in particular. We highlight some of the preliminary results from Work Package 1 through figures in this section.

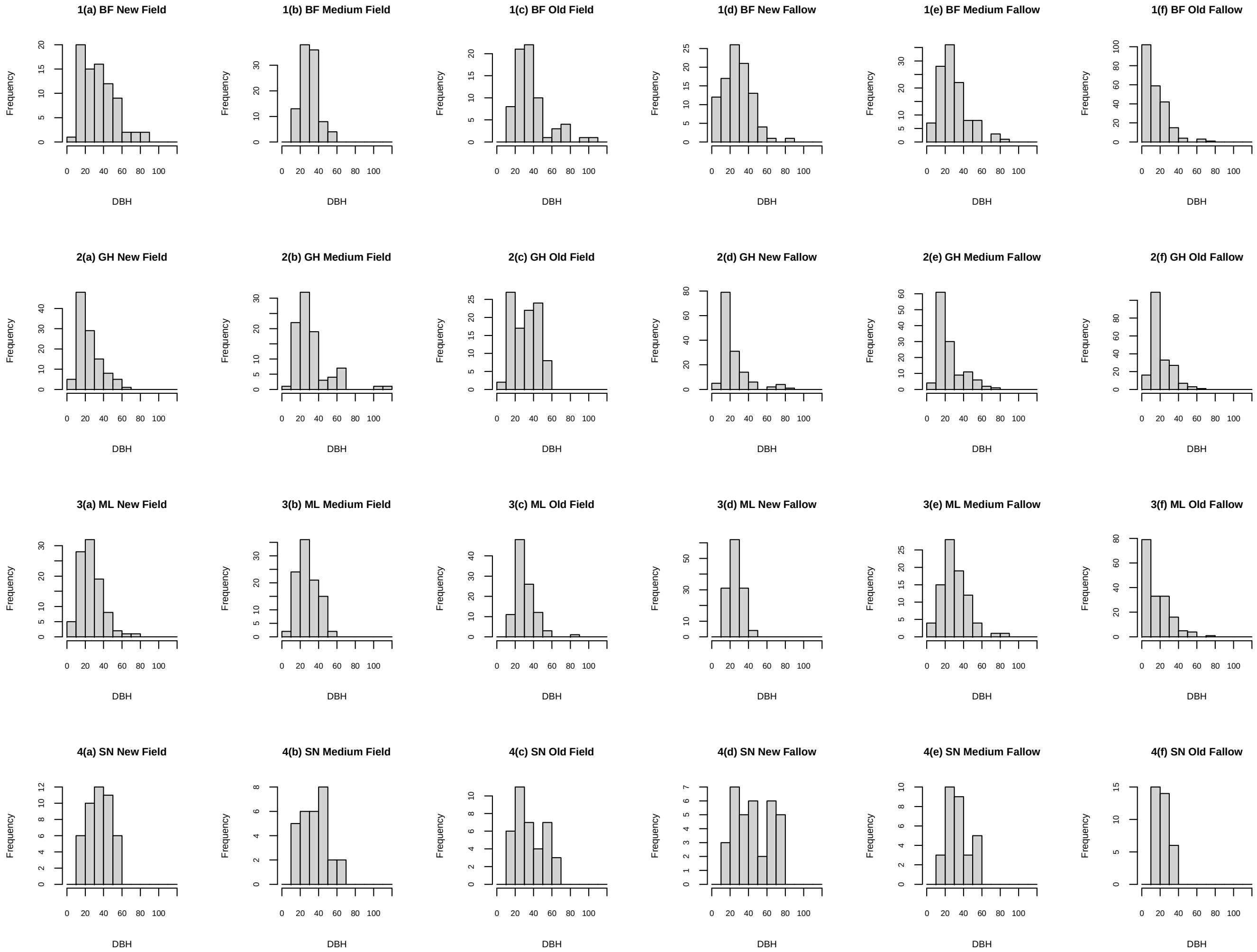



**Figure WP1-1: Diameter at breast height (DBH) of adult shea trees in three classes of field and fallow in Burkina Faso, Ghana, Senegal, and Mali. The histograms highlight that generally old fallows have higher number of shea trees with smaller DBH, while medium and old fields tend to have shea trees with larger DBH.**

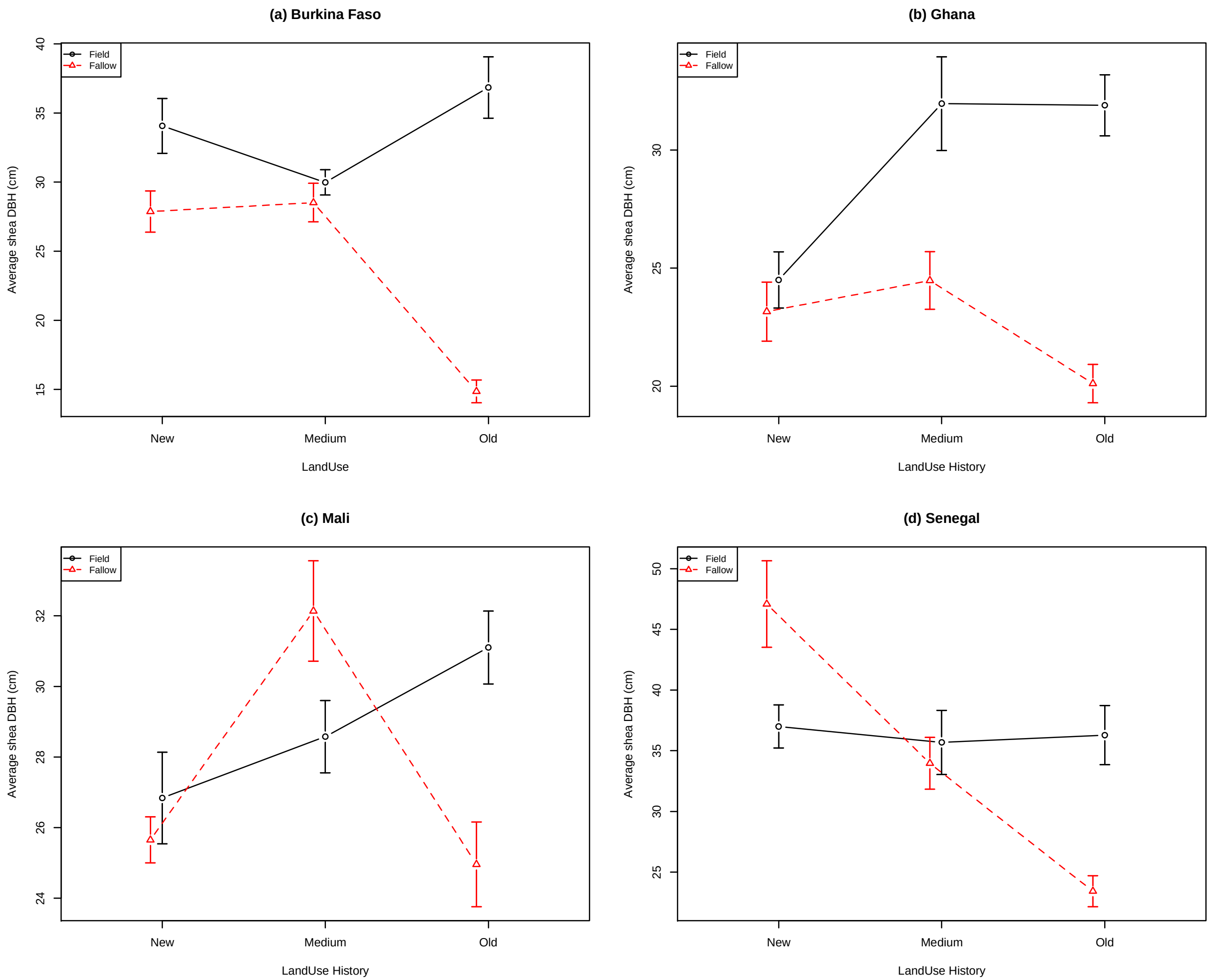



**Figure WP1-2: Average DBH of adult shea trees in Field and Fallows of three classes (New, Medium and Old) in Burkina Faso, Ghana, Mali and Senegal. This figure further highlights the difference between shea tree sizes in various land use types and land use histories. The difference in shea tree size between old field and fallow is particularly striking, showing not just natural competition for resource in shea growth (old field) but also benefits of intensive management and less competition on its growth (old field).**

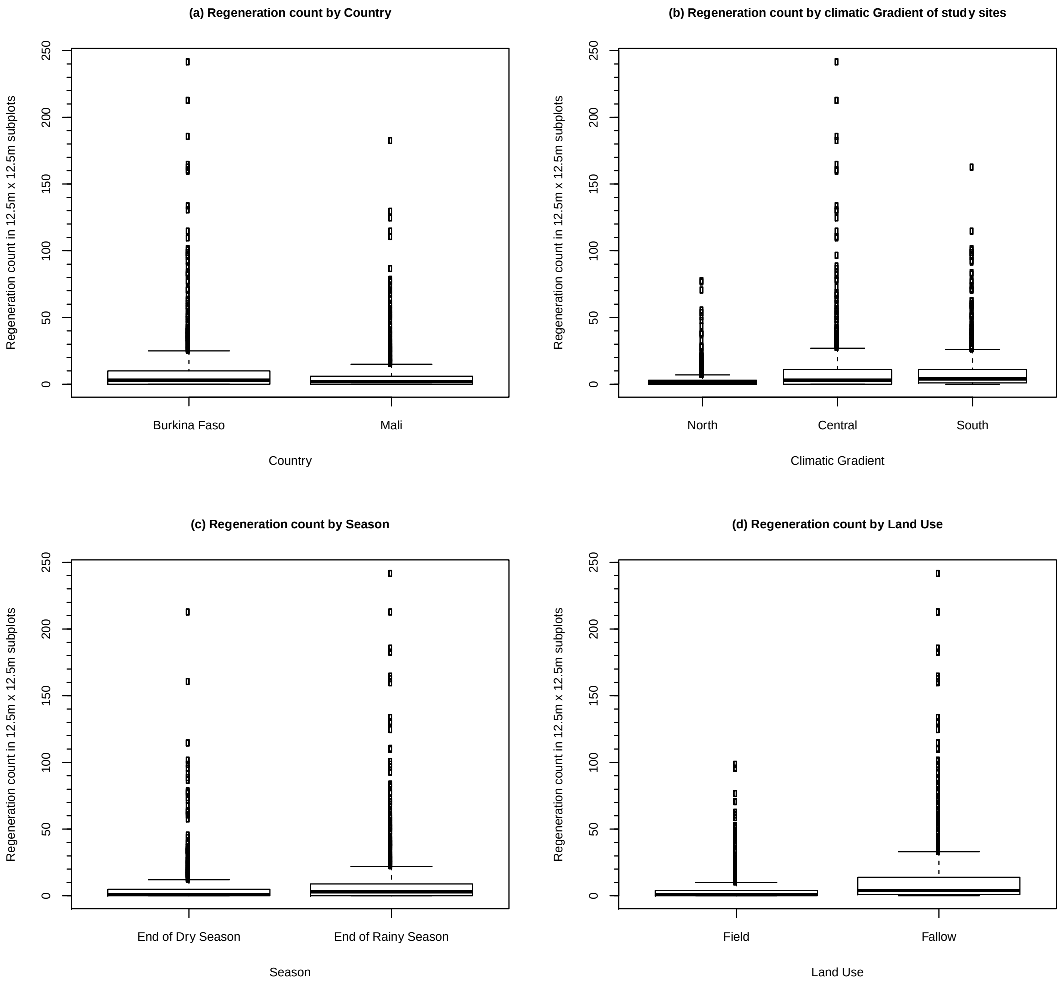



**Figure WP1-3: Regeneration count in Burkina Faso and Mali. (a) Generally shea regeneration was found to be higher in Burkina Faso than in Mali. (b) In terms of climatic gradient, central and southern sites had higher shea regeneration than northern site. (c) Number of shea seedlings at the end of rainy season was higher than that at the end of dry season. (d) Fallow plots generally had significantly higher shea regeneration than field plots.**

## 132 - Work package 2: Adaptation and resilience of shea tree facing climate change and drought using ecophysiological and modelling approaches

**General Objectives**

The main objective is to define management recommendation of shea tree parkland based on their ability to face drought and on the prediction of the distribution of shea tree under future climatic scenarios.

**Specific objectives**

- To build a climatic index of the sites studied;
- To characterise germplasm adaptation to rainfall gradient by studying the variation of tree phenology, of tree morphological and ecophysiological traits in the natural populations of the Sudano-Sahelian zones;
- To model changes in the distribution of the Shea tree under future climate change scenarios.

**Scientists involved**

For the studies on parkland : Bastide Brigitte, Bayala Jules, Roupsard Olivier, Jourdan Christophe, Bazié Paulin

For the study related to climate change scenari and the distribution of shea tree : Platts P.J., Poudyal M. and Mc Clean C.J.

### 1.3.2.1 WP2 - Methodologies and approaches employed

The methodology is described with the achievements of the project

### 1.3.2.2 WP2 - Achievements of the project to the state-of-the-art.

The tasks in this WP2 are carried out by CNRST-INERA (Burkina Faso), IER (Mali), ISRA (Senegal), UNIMAK (Uganda), UDS (Ghana), CIRAD (France) and UOY (UK). The first year was devoted to selecting sites for tasks 2.1, 2.2, 2.3, 2.4 and 2.7 in one hand and in another hand collecting seeds in African countries and shipping the collected seeds to the partner of Burkina Faso in charge of conducted the nursery experiment (tasks 2.5 and 2.6).

Data on shea populations across its natural range, tree phenology on natural populations and $^{13}C$ discrimination are collected and processed since 2008 (tasks 2.2, 2.3, 2.4 and 2.7). The species distribution maps for shea distribution under current and future scenarios are obtained for all the models tested.

From 2009, WP2 consists of one nursery experiment (CNRST-INERA, Burkina Faso), one another experiment conducted in parkland for carbon budget estimation (CNRST-INERA, Burkina Faso) and the bioclimatic modeling of shea (UOY, UK).

Due to the little amount of replicates that survived pet treatment, it has been decided to abandon the nursery experiment from October 2010. The two activities were the experiment conducted in parkland for carbon budget estimation (CNRST-INERA, Burkina Faso) and the bioclimatic modeling of shea (UOY, UK). In 2010, the images of the roots from September 2008 to November 2010 were digitalized on a table A0 format with RhizoDigit 1_3 (a software developed by CIRAD). The analysis of present day distribution Shea was conducted and the first results of the impacts of 21 st century climate change on Shea distribution were available.

The data of leaf phenology using a camera equipped with a fisheye and images of the roots were analyzed in 2011. One manuscript is in preparation. Shea distribution model and analysis from the previous period were refining.

*Tasks 2.1, 2.2, 2.3, 2.4 and 2.7. Study of shea populations across its natural range, characterisation of abiotic variables, tree phenology on natural populations and $^{13}C$ discrimination*

Due to overlaps with some activities in WP1, the tasks 2.1, 2.2, 2.3, 2.4 and 2.7 of WP2 are carried out within the framework of WP1.

*Task 2.5. and 2.6. Study of morphological traits, tree structure, ecophysiological traits and analyse of the variation of these variables*

These tasks involved a nursery experiment and a parkland experiment for carbon budget estimation of shea butter species. The protocols of these two experiments have been developed in close collaboration with Olivier Roupsard and Chrstophe Jourdan of CIRAD.

*Nursery experiment*

The main objective of the nursery experiment was to delineate provenances on the basis of their strategy and their ability to face drought by determining their water use efficiency at different soil water regimes and its relationship with carbon isotope discrimination ($\delta^{13}C$).

*Methodology*

We examined the changes in morphological traits as well in ecophysiological parameters, i.e. nutrient uptake, photosynthesis and dry matter accumulation at different soil water regimes. To this end, seed were received from two locations in Mali (EIR partner 6), one in Uganda (UNIMAK partner 9), one from Senegal (ISRA partner 7) and one in Ghana (UDS partner 10). In addition seeds were collected in 3 villages in Burkina Faso (CNRST/INERA partner 8) giving a total of 8 provenances instead of 7 as planned. The seeds were sown in pots (50 cm diameter, 20 cm high) filled with sand for germination between the end of June and the beginning August 2007. After 5 months single seedlings were transplanted to 15 L pots containing 7 kg of substrate composed sand (1v), arable soil (2v) and manure (1v). Due to poor germination of some provenances, only one lifespan is now planned instead of the 3 previously planned. The application of different water regimes started on 31 October 2008, i.e about 16 months after sowing.

*Results*

For growth variables, the analysis revealed significant difference between provenances before and after the application of different water regimes. Ghana (12 and 12.85 cm, respectively) and Karaba (11 and 12. 12 cm, respectively) displayed the highest numbers of leaves and heights whereas the trend was unclear for the collar diameter even though there was significant difference between provenances for this variable. The water regimes did not differ significantly at two measurement dates for any of growth variables except at the last measurement date for the number of leaves. At this date (roughly one month and half after the application of the stress), the number of leaves significantly decreased from 100% ET regime (7 leaves) to 75% ET (6 leaves) and 50% ET (5 leaves) regimes. All provenances seem to react to water stress by shading leaves in the first instance and this may ultimately result in reduced growth for height, diameter and biomass accumulation in the long run. The results obtained before the application of the stress showed that transpiration and net photosynthesis differed from one provenance to another. Similar trends were observed for dendrometric parameters and morphological parameters of the leaf. With regard to water regime effect, the results showed that a prolonged water stress strongly affected the transpiration, photosynthesis and the stomatal conductance. The poor physiological functioning of the seedlings of regimes 2 and 3 due to the stress resulted in a poor development of leaves and a reduction of the growth in height and diameter of the seedlings. All provenances were severely affected by the water stress.

Due to the little amount of replicates that survived pet treatment, it has been decided to abandon the nursery experiment from October 2010.

*Conclusions*
This study did permit to separate the provenances between the most resistant and the most vulnerable to water stress. However the provenance of Uganda displayed the lowest mean values whatever is the variable and the water regime, thus showing its poor adaptation to the climate of West Africa. This type of investigation, which gives an idea of the effect of a possible climatic change which would result in a dryness, must be continue and expand to other species.

*Parkland experiment*
The parkland experiment aims at estimating the carbon budget (incoming C by photosynthesis, use of this C for growth, autotrophic respiration, leaves, roots and fruits production and for reserve dynamics) of the trees in Sobaka (Burkina Faso) with detailed investigations and equipments. Objectives are : (i) to explore aerial and root phenology of shea trees over 2 contrasted growing seasons ; to relate both compartiments dynamics in farmer field or fallow conditions and the age of the trees.

*Methodology*
This experiment was conducted in Burkina Faso. 10 trees of different sizes and levels of fruit production were selected in farmed fields and in an old fallow. Data on climate, tree growth, tree physiological functioning and nutritional status are being collected. In addition to these variables, a set of trees, representative of the age structure were excavated in every plot, concurrently with soil profile description, down to the maximum rooting depth. The root biomass was estimated by classes of root diameter (> or < 2 mm at least). Fine root turnover was assessed with the help of rhizotrons installed under each tree at various distances from tree trunk (100 cm for big trees and 50 cm for small trees). Tree growth reserves were measured and the carbon balance estimated.
Measurements related to characteristics of the leaves, root density, stomatal conductance, transpiration and photosynthesis were done from November 2008 to February 2009 (for the results see previous reports). The study continued in 2009 and 2010 in order to take into account others parameters as canopy phenology with being assessed by a photo hemispheric and root dynamics using rhizotrons in order to obtain a better understanding of the physiological functioning of the shea tree.

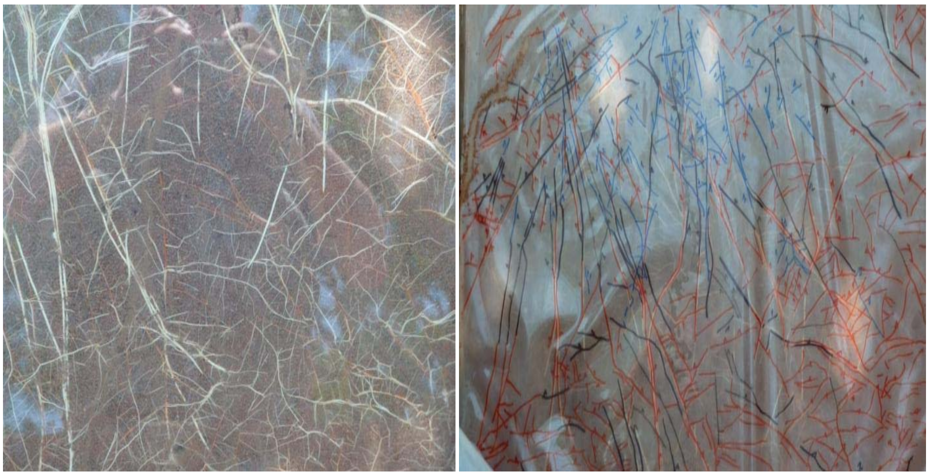


**Figure WP2-1. Rhizotron installed at vertical position under a tree in Sobaka, Burkina Faso**

In 2010, the images of the roots from September 2008 to November 2010 were digitalized on a table A0 format with RhizoDigit 1_3 (a software developed by CIRAD). The data were analyzed in 2011.

Canopy phenology using a camera equipped with a fisheye was assessed every month from September 2008 to November 2010

The hemiphotos have been performed (from July 2008 to Mach 2011), every month at the same dates as for the observation of roots, using 4 photos per tree and per date (4 azimuths). Data were analyzed by PhD student Paulin Bazie (from 07/2008 to 03/2011), using the software GAP LIGHT ANALYZER (Frazer *et al.*, 1999), then summarized on a look-up table, according to Roupsard *et al*., 2008.

**Figure WP2-2 : Hemiphotos of canopy tree in the fallow in Sobaka, Burkina Faso**

Most of the following up activities of Sobaka were partly possible in 2010 with the support of an International Foundation of Science (IFS) grant obtained by the PhD student who is conducting this experiment.

*Results*
Canopy openness showed seasonally variations with a maximum fall of leaves (70% of canopy gap) during the dry season (March-April) and a maximum leaf production (28% of canopy gap) at the beginning of rainy season (July-August). The location of the shea trees (Field or Fallow) and the tree growth stage (Young or Adult) have also effects on the canopy phenology : the shea trees located in the fallow begin earlier to lose their leaves (November) than those in the field (January) ; the interaction land use type* tree growth stage was more significant for adults trees than for young ones. There is a very large variation in leaf loss (40% in August to nearly 90% in April) in adults located in the fallow compared to those in the field (20% in August to 50% in April). No significant variation was observed between young trees according to land use type even though the change was more pronounced in the field compared to the fallow.
Like canopy openness root dynamic showed seasonally variations : root production and emission was high in July-September (rainy season) and less in March-April (dry season). The minimum growth rates were observed in April for coarse and fine roots.

Land use type as also an effect on the root production that was higher in the field than in the fallow.The coarse and fine root growth rates are higher in the Field than in Fallow except in August where coarse and fine root growth rates amounted 0.23 $cm.j^{-1}$ and 0.11 $cm.j^{-1}$ resp. Adults located in the field had a higher root growth rate than those in the fallow. The same trend was observed with young trees.

*Conclusions*
Our observations showed that the dynamics of leaves and roots of Shea tree (*Vitellaria paradoxa*) follow the same trends :

- The roots were produced when the leaves were issued from July to September during the wet (rainy) season
- The roots ceased to grow in October (end of raining season) and began to degenerate in November as the leaves began to fall.

The maximum root production was observed in August while the light interception was also maximal (min. canopy openness). At that time, the production of C-photoassimilate is optimal. We can conclude that there is an immediate C partitioning through belowground parts.

*Task 2.7. To model changes in the distribution of the Shea tree under climate change scenarios*
*Analysis of present day distribution*

*Methodology*

- Shea presence localities

Data describing the current Shea range were obtained from John Hall (University of Wales, Bangor), which formed the basis of shea distribution map in *Vitellaria paradoxa* as the initial distribution data for shea. In 2010, these records were supplemented with Shea plot data from Burkina Faso, Mali and Ghana, the collection localities of herbarium specimens held at the Royal Botanic Gardens, Kew (UK) and the Missouri Botanical Garden, Missouri, and three outliers in the distribution data described above, identified by Dr. Peter Lovett. A total of 241 records remained across the Sahel of Africa. At the continental scale, the distribution is constrained by the Sahara Desert to the north and tropical rainforests to the south. Collation of these data within 10 arc-minute grid cells (squares of side *c*. 18 km) resulted in a final modeling subset of 188 distinct presence localities.

- Environmental datasets

Predictors variables were climate (WorldClim), topography (CGIAR-SRTM), agro-ecological zone data for soils constraints (FAO-IIASA) and fire radiative power for fire intensity (MODIS, 2001-2009). Other variables were considered for modeling but were omitted due to high levels of colinearity.

- Modeling approach

Generalised Additive Models (GAMs) were used to predict the present distribution of shea. The present day shea distribution was correlated with climate, topography, agro-ecological zone data and fire radiative power. Predictor variables were chosen using two contrasting stepwise selection procedures: beginning with a null/full model, variables were added/removed according to Akaike/Bayesian Information Criterion.
Models were calibrated under two spatial extents: between 0°-15°N (latitudinal limits of the current shea range) and between 10°S-25°N (these limits ±10°).
At each step, model performance was assessed by cross-validating the area under the ROC curve. The model with the highest cross-validation score was retained. A multimodel was then calculated using a performance-weighted average of these 'best-model' predictions.

*Results*

The moisture index, defined as the ratio of annual rainfall and potential evapotranspiration (EPT), was found to be the best environmental predictor of shea distribution. The response curve for this variable indicates that shea is most likely to occur in relatively dry climates, but not too dry (i.e., desert). The second best predictor was fire radiative power: an intermediate degree of burning is beneficial; however, this might be attributed to a correlation between shea parklands and the frequency of managed fires.

***Shea under climate change scenarios***

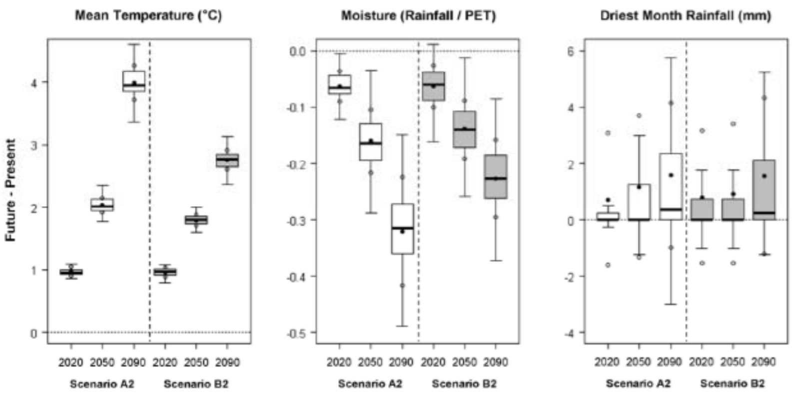
*Methodology*

In order to investigate the impacts of $21^{st}$ century climate change on shea distribution, the predictor set was reduced to three uncorrelated (Pearson's $r \leq 0.7$) climate variables: mean annual temperature, moisture index and driest month rainfall.
Climate projections for the $21^{st}$ century were obtained from CIAT . Three time windows were investigated: 2020, 2050 and 2080.
Model extrapolations were contrasted for two IPCC emissions scenarios (A2 (higher emissions) and B2 (median emissions)), using multimodel averages derived from four General Circulation Models (CCCMA-CGCM2, CSIRO-MK2, NEIS99 and UKMO-HADCM3).

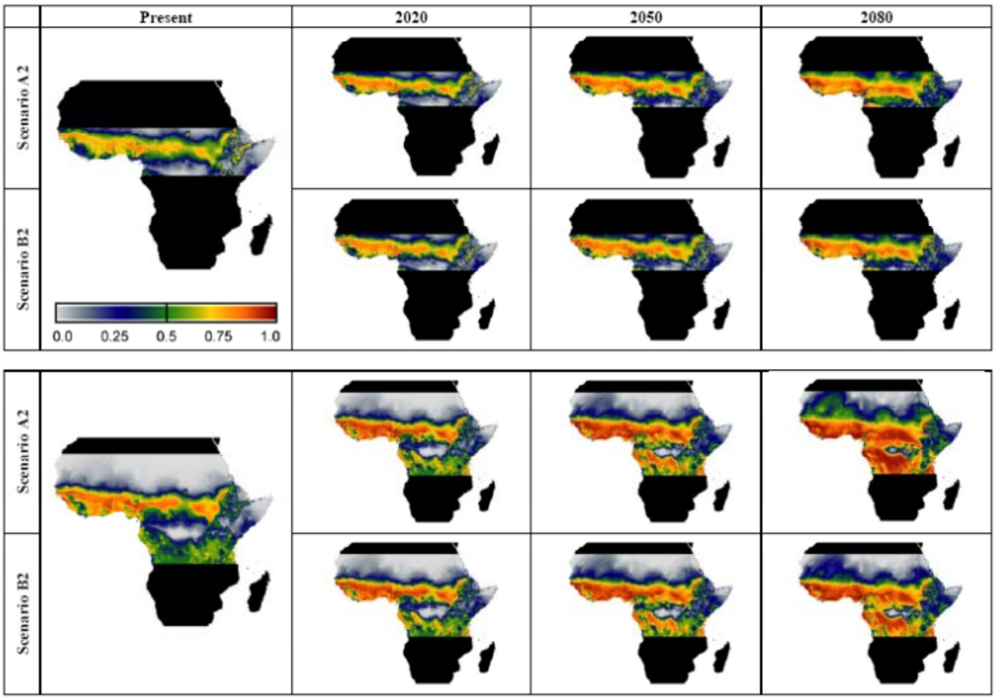
Results

Distribution models predict an increase in the number of climatically suitable areas in the 21st century. The two  scenarios (A2 and B2) give similar results for the first half of the 21st century, but thereafter the A2 scenario resulted in a more rapid increase in the amount of climate-space suitable for Shea, particularly within its current latitudinal range (0°-15°N). Areas already drier than the moisture optimum (i.e., moisture index < 0.5), such as those at the northern limits of the Shea range, are predicted to remain marginal.

# Climate scenarios




Boxplots summarising 21st century climate anomalies forecast for two SRES emissions scenarios, focussing on the three climate variables used in multiple regression. The plots summarise change across 188 distinct 10 arc-minute grid cells, each of which is documented to support shea trees under contemporary climate. Box whiskers extend up to 1.5 times the interquartile range from each box. Filled and open circles show means ± standard deviations.




Maps showing modelled climatic suitability for shea under current and future climate scenarios (extrapolating the GAM predictions)

**Figure WP2-3 : characteristics of scenarios and impact on the prediction of the distribution of shea tree**

*Conclusions*

These predictions are subject to high uncertainty. So, the health and establishment of shea trees, particularly in marginal areas, has to be monitored carefully in relation to climate. Further investigations (beyond INNOVKAR) are needed:

1. Development of a mechanistic (as opposed to correlative) model for shea distribution could provide information on relationship between shea presence/absence and moisture ;
2. Inclusion of fire radiative power in future analyses, as and when data permit ;
3. Use of a combination of topography and climate to represent more directly the seasonally flooded areas that constitute one obstacle to the shea distribution.

### 1.3.2.3 WP2 - impact of the project on its industry or research sector

The results of the WP2 related to ecophysiology do not have a direct impact of industry and research sector. They are just preliminary results that need to be confirmed by further experiments and research.

The results related to the impact of climate change on shea tree distribution need also to be confirmed by additional investigation. However they can be used by administration, forest services, or policy makers to define long term strategy to address environmental issues.

### 1.3.3 Work package 3 – Genetic resources characterisation and provenance delineation using morphological, molecular and chemical traits

A better characterisation of the genetic resources of a species is a prerequisite to implement a sustainable strategy at the local, regional and across the wide natural range and to develop certified origin. Within their natural range tree species exhibit generally strong variation due to genetic and environmental factors. This genetic diversity is the consequences of evolutionary forces such as drift, mutation, migration and natural or man selection. Within the natural range the evolutionary forces have created different provenances that can be different for their adaptive traits but also for the economical traits. The differentiation of these provenances is a prerequisite to the definition of certified origin in shea tree.

General objective
To delineate provenances on the basis of morphological, molecular and chemical traits for promoting the certification of origins

Specific objectives
1 – To improve the understanding of the pattern of variation of shea tree within the natural range
2 – To give the scientific basis to delineate origin in the perspective of certification

Scientists involved
Senegal : Massamba Thiam, Momar Wade, Ablaye Sylla, Malick Dione, Therence
Makerere : John Bosco Lamoirs Okullo, John Bosco Acot Okello and Vincent Muwanika
Cirad : JM Bouvet, A Vaillant, F Allal

#### 1.3.3.1 WP3 - Methodologies and approaches employed

To achieve these objectives the methodology was based on different tasks
Task 3.1 : to sample the populations of shea tree across the natural range : two main sampling transects were implemented. The first concerns the distribution of the species according to the West-East gradient in the Soudano-Sahelian climatic zone. The second transect concerns the North-South gradient..

Task 3.2 : to measure morphological traits : twenty fruit and forty leaves per tree will be collected in each population. Traits measured on the trees will concern different organs: vegetative traits (height, circumference, presence of tapinanthus), leaves (length and width lamina, petiole length), floral (size of the organs of the flowers) and fruit and nut size (length and width).

Task 3.3 : to measure chemical constituent: Chemical composition of the kernel will be conducted on a sub-sample of 10 fruits per tree, 30 tree per population will be randomly selected. Using conventional technics and NIRS (WP5) the main analyses will be : shea butter quality titrations--free fatty acids, peroxide value, iodine value—plus humidity and impurities. Fatty acid profile, unsaponifiable percentage, tocopherols and catechins. triglyceride analyses, including stearic-oleic-stearic (SOS) which is of central interest to the chocolate industry

Task 3.4 : to measure molecular variation:New molecular markers will be defined using targeted genes involved in the lipid pathway. We will use the single nucleotide polymorphism (SNPs) with a targeted gene approach. The trees sampled in the different populations will be genotyped using the set of primers and the SNP markers.

Task 3.5 : To analyse the variation combining the morphological, molecular and chemical traits :Quantitative variation between and within populations for the various traits will be analysed with classical mixed models and theory of quantitative genetics (variance components, repeatability, correlation ….) : the relation between population means and abiotic variable (rainfall, dry season, temperature, altitude, soil condition, etc..) will be analysed through classical linear regression method in order to assess the impact of these variable on phenotypic variation. Delineation of provenances in relation with the WP (functional molecular markers) will be define using multivariate techniques such as principal components analyses or tree methods

### 1.3.3.2 WP3 - Achievements of the project to the state-of-the-art.

The project has improved our knowledge in the following domains :

**The phylogeography of *Vitellaria paradoxa* over Africa**

In this project, we investigated the impact of historical events such as the last glacial maximum (LGM) and other more recent factors such as human agricultural practises, on the present genetic pattern of *Vitellaria paradoxa* (shea tree) by adopting two sampling strategies: the first concerned all the natural range and the second more specifically the West Africa range with a very dense sampling.

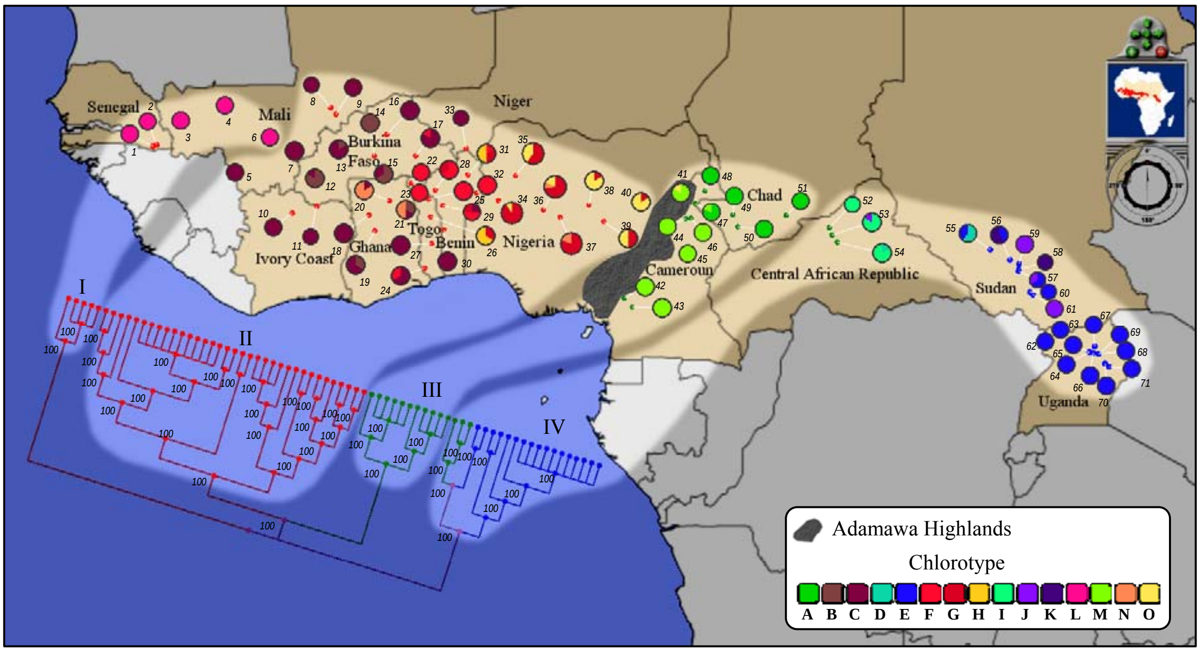
A range-wide sampling of the species enabled us to sample 374 individuals from 71 populations distributed throughout sub-Sahelian Africa. Trees were genotyped using 3 chloroplasts and 12 nuclear microsatellites, and were sequenced for 2 polymorphic chloroplast intergenic spacers. Analyses of genetic diversity and structure were based on frequency-based and Bayesian methods. Potential distributions of *V. paradoxa* at present, during the LGM and the last interglacial period, were examined using DIVA-GIS ecological niche modelling (ENM). Haplotypic and allelic richness varied significantly across the range according to chloroplast and nuclear microsatellites, which pointed to higher diversity in West Africa. A high but contrasted level of differentiation was revealed among populations with a clear phylogeographic signal, with both nuclear ($F_{ST}$=0.21; $R_{ST}$=0.28; $R_{ST}$>$R_{ST}$ (permuted)) and chloroplast simple sequence repeats (SSRs) ($G_{ST}$=0.81; $N_{ST}$=0.90; $N_{ST}$>$N_{ST}$ (permuted)). We identified a strong geographically related structure separating western and eastern populations, and a substructure in the eastern part of the area consistent with subspecies distinction (this result is illustrated with Fig WP3.1). Using ENM, we deduced that perturbations during the LGM fragmented the potential eastern distribution of shea tree, but not its distribution in West Africa. Our main results suggest that climate variations are the major factor explaining the genetic pattern of *V. paradoxa*.

In Western Africa, eleven nuclear microsatellites (nuc) were used to genotype 673 trees selected in 38 populations. They revealed moderate to high within-population diversity: allelic richness ranged from $R_{nuc}$=3.99 to 5.63. This diversity was evenly distributed across West Africa. Populations were weakly differentiated ($F_{STnuc}$=0.085; $P < 0.0001$) and a pattern of isolation by distance was noted. No phylogeographic signal could be detected across the studied sample (this result is illustrated with Fig WP3.2). Additionally, two chloroplast microsatellite loci, leading to 11 chlorotypes, were used to analyse a sub-set of 370 individuals. Some variation in chloroplast allelic richness among populations could be detected ($R_{cp}$ =0.00 to 4.36), but these differences were not significant. No trend with latitude and longitude were observed. Differentiation was marked ($G_{STcp}$=0.553; $P < 0.0001$), but without a significant phylogeographical signal. Population expansion was detected considering the total population using approximate Bayesian computation (nuclear microsatellites) and mismatch distribution (chloroplast microsatellites) methods. This expansion signal and the isolation by distance pattern could be linked to the past climatic conditions in West Africa during the Pleistocene and Holocene which should have been favourable to shea tree development. In addition, human activities through agroforestry and domestication (started 10 000 BP) have probably enhanced gene flow and population expansion.

These complementary samplings and analyses stressed the factors explaining the evolutionary divergence among sub species and populations in the different regions of the natural range. These studies should be associated with agromorphological and ecophysiological analyses to understand other factors explaining diversity for example the impact of local conditions (soil, climatic, human, etc.).

***Figure WP3.1:*** (a) geographic distribution of the 15 chlorotypes of *V. paradoxa*. Unrooted neighbour-joining tree drawn with GENGIS software (Parks *et al.*, 2009) using Cavalli-Sforza genetic distances. Numbers at the base of the branches are percentages corresponding to the bootstrap values after 1000 replications. (b) Median-Joining network computed using NETWORK (http://www.fluxus-engineering.com/), with 1 = West African Chlorotypes; 2 = East African chlorotypes; 3 and 4= Central African chlorotypes.

(a)




1: Bandafassi; 2: Samecouta; 3: Mahina; 4: Torodo; 5: Badougou; 6: Kodougouni; 7: Mperesso; 8: Songo; 9: Tenndeli; 10: Napieoledougou; 11: Korhogo; 12: Peni; 13: Karo; 14: Bonogo; 15: Pô; 16: Konia; 17: Fada N'Gourma; 18: Larabanga; 19: Kawampe; 20: Kadia; 21: Yendi; 22: Tabango; 23: Payô; 24: Koudassi; 25: Kante; 26: Kolina; 27: Djangbassou; 28: Cocota; 29: Kpale; 30: Pira; 31: Kandi; 32: Kalale; 33: Boumba; 34: Wawa; 35: Rijau; 36: Paiko; 37: Abuja; 38: Kuru; 39: Shendam; 40: Kaltungo; 41: Mubi; 42: Bangangte; 43: Foumban; 44: Ngong; 45: Ngaoundere; 46: Mafa-Kilda; 47: Figuil; 48: Mendeo; 49: Kelo; 50: Mondou; 51: Sarh; 52: North; 53: Central; 54: South; 55: Kuajena; 56: Cuebet; 57: Wulu; 58: Kuel Kuac; 59: Matengai; 60: Mbara; 61: Mundri; 62: Arua; 63: Eria; 64: Adumara; 65: Otongere; 66: Okwongo; 67: Lacankweri ; 68: Olwoko; 69: Otuke ss; 70: Abarilela; 71: Akoboi

(b)

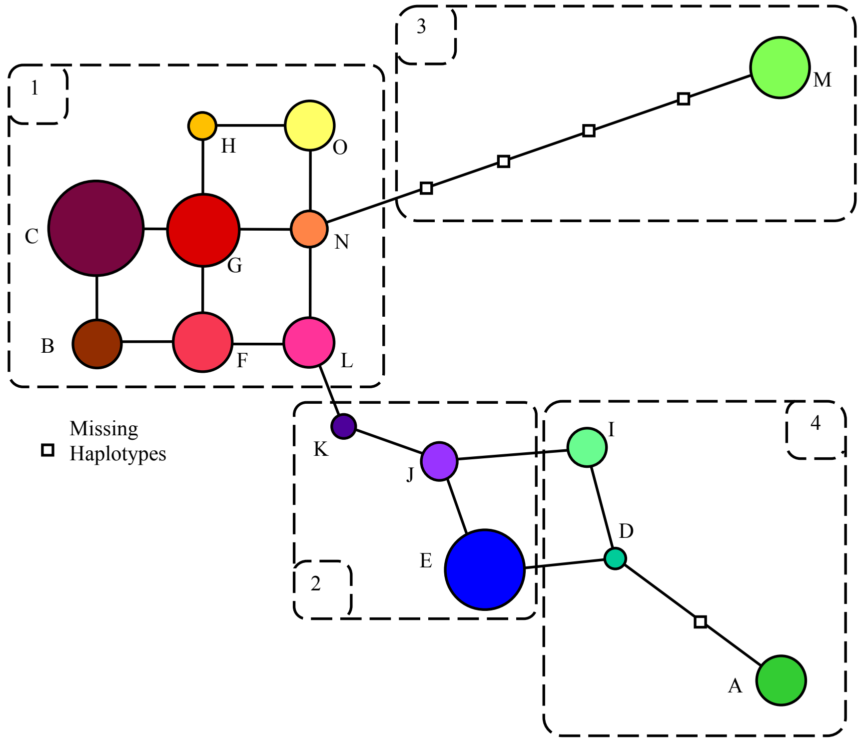
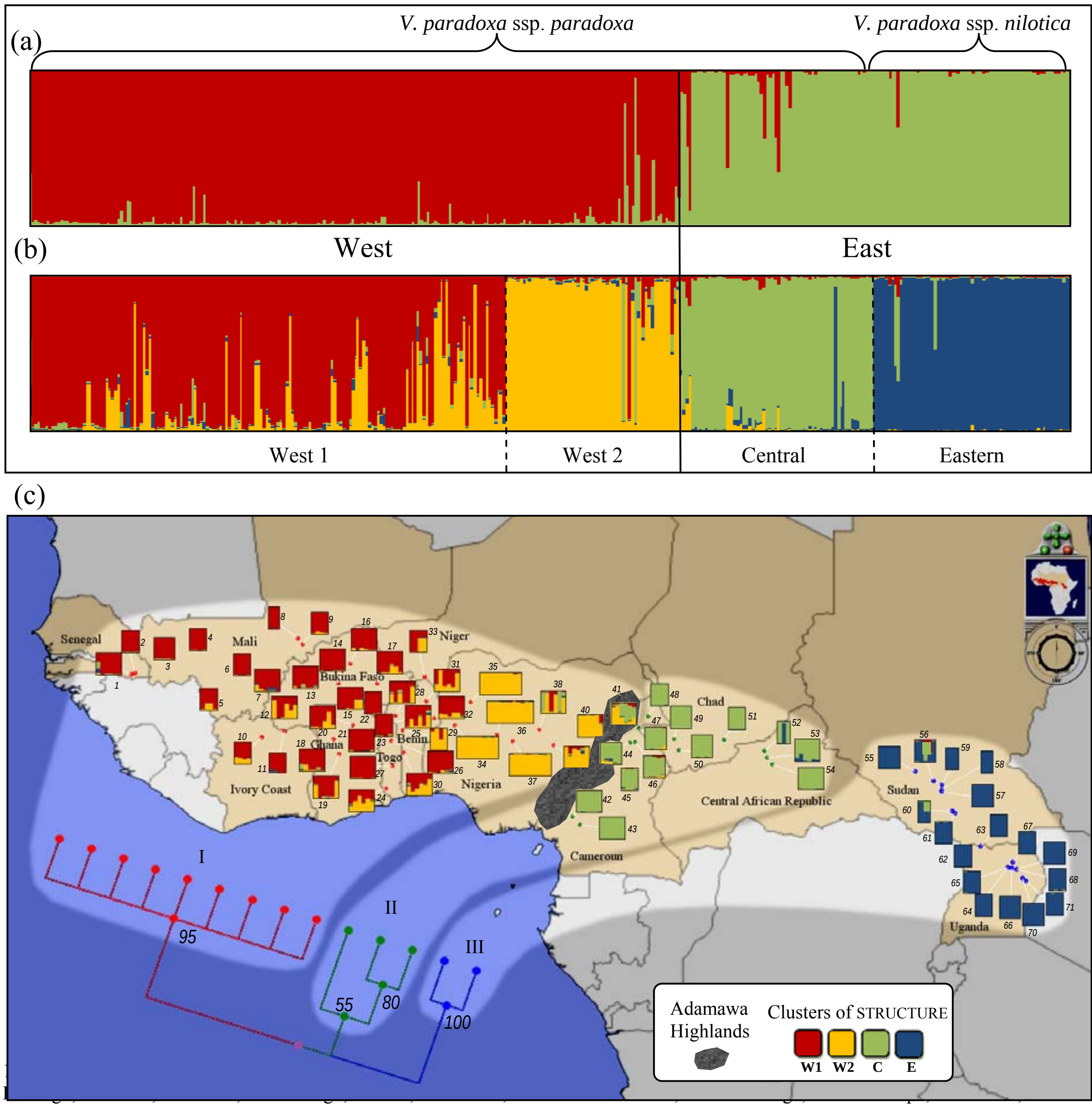


***Figure WP3.2:*** (a) Bar plot of clustering of individuals made by STRUCTURE K = 2 (Pritchard *et al.*, 2000). (b) Bar plot of clustering of individuals made by STRUCTURE K =4. (c) Clustering of individuals based on geographical locations; Cavalli-Sforza distance unrooted neighbour-joining tree drawn with GENGIS software (Parks *et al.*, 2009). Numbers at the base of the branches are percentages corresponding to the bootstrap values after 1000 replications. W1: “West 1”; W2: “West 2”; C: “Central”; E: “Eastern”;

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Tabango; 23: Payô; 24: Koudassi; 25: Kante; 26: Kolina; 27: Djangbassou; 28: Cocota; 29: Kpale; 30: Pira; 31: Kandi; 32: Kalale; 33: Boumba; 34: Wawa; 35: Rijau; 36: Paiko; 37: Abuja; 38: Kuru; 39: Shendam; 40: Kaltungo; 41: Mubi; 42: Bangangte; 43: Foumban; 44: Ngong; 45: Ngaoundere; 46: Mafa-Kilda; 47: Figuil; 48: Mendeo; 49: Kelo; 50: Mondou; 51: Sarh; 52: North; 53: Central; 54: South; 55: Kuajena; 56: Cuebet; 57: Wulu; 58: Kuel Kuac; 59: Matengai; 60: Mbara; 61: Mundri; 62: Arua; 63: Eria; 64: Adumara; 65: Otongere; 66: Okwongo; 67: Lacankweri ; 68: Olwoko; 69: Otuke ss; 70: Abarilela; 71: Akoboi

**Pattern of molecular and chemical variation in shea tree (*Vitellaria paradoxa*) - Consequences for the management of genetic resources.**

In this project, we investigated the delineation of provenances of this species using two approaches. The first use molecular markers and try to understand the historical factors that shaped the genetic diversity within the shea tree natural range. (see the paragraph above).

The second study uses the chemical variation of fatty acid and tocopherol to infer the relationship between those constituents and the abiotic variables such as climate characteristics. Based on samples from 456 trees distributed in 17 locations across the species natural range from Senegal to Uganda, the fatty acid and tocopherol variation, and its relationship with geographic and climatic variables, was assessed in order to address the pattern and the origin of this variation across the natural range.

Significant differences between Western and Eastern regions for oleic, stearic acid, saturated-unsaturated acid ratio and γ-tocopherol were identified that it is postulated maybe a result of genetic drift due to the evolutionary history of shea tree populations.

Within regions the difference among stands was significant for most constituents; however the major part of the variation was observed among trees within stand (53-90%). Relationships with climatic variables were not verified, weakening evidence for clinal variation hypotheses suggested by previous studies. (these results are illustrated in figures WP3.3 and WP3.4).

Combining both studies shows that the evolutionary process of western and eastern populations during the last twenty thousand years and the fragmentation due to last glacial maximum could explain the difference of seed chemical contents in the two sub varieties *V. Paradoxa var paradoxa* and *V. Paradoxa var nilotica*.

These two studies contribute to a delineation of provenances based on different markers and traits. Our results reinforce the major separation between West Africa populations and Eastern African origins. Within each region, both approaches did not detect important variation and did not conduct to the delineation of clear new provenances. The central zone of the natural range, Cameroun, Chad, East Nigeria, Central African Republic presented some specificity but our data could not lead to a clear identification of a major central provenance. Complementary samplings should be conducted to analyse more precisely this zone.

**Figure WP3-3.** Box plots comparison of stearic and oleic acid within (A) Mali and (B) Ghana-Burkina transect. Crosses are mean values; black bars represent median values; upper and lower side of rectangles give 1st and 3rd quartiles and horizontal grey bars represent extreme values

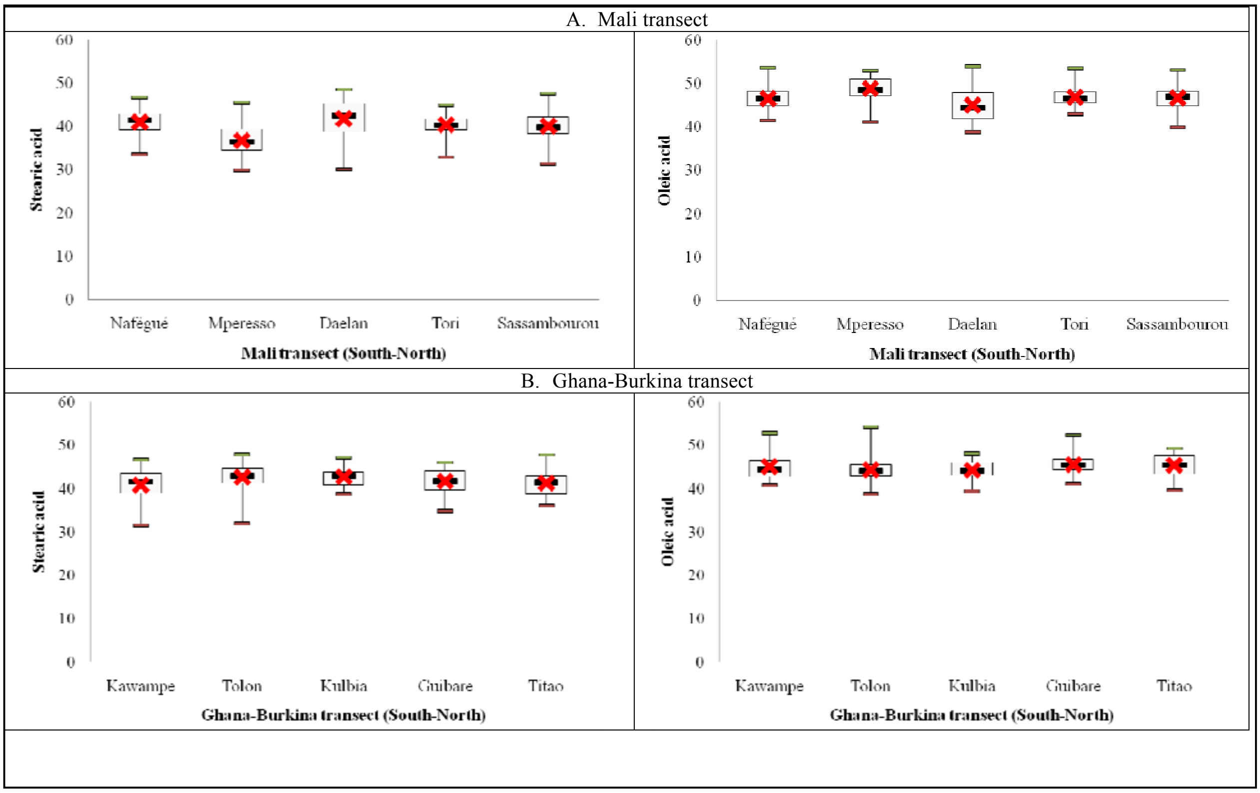
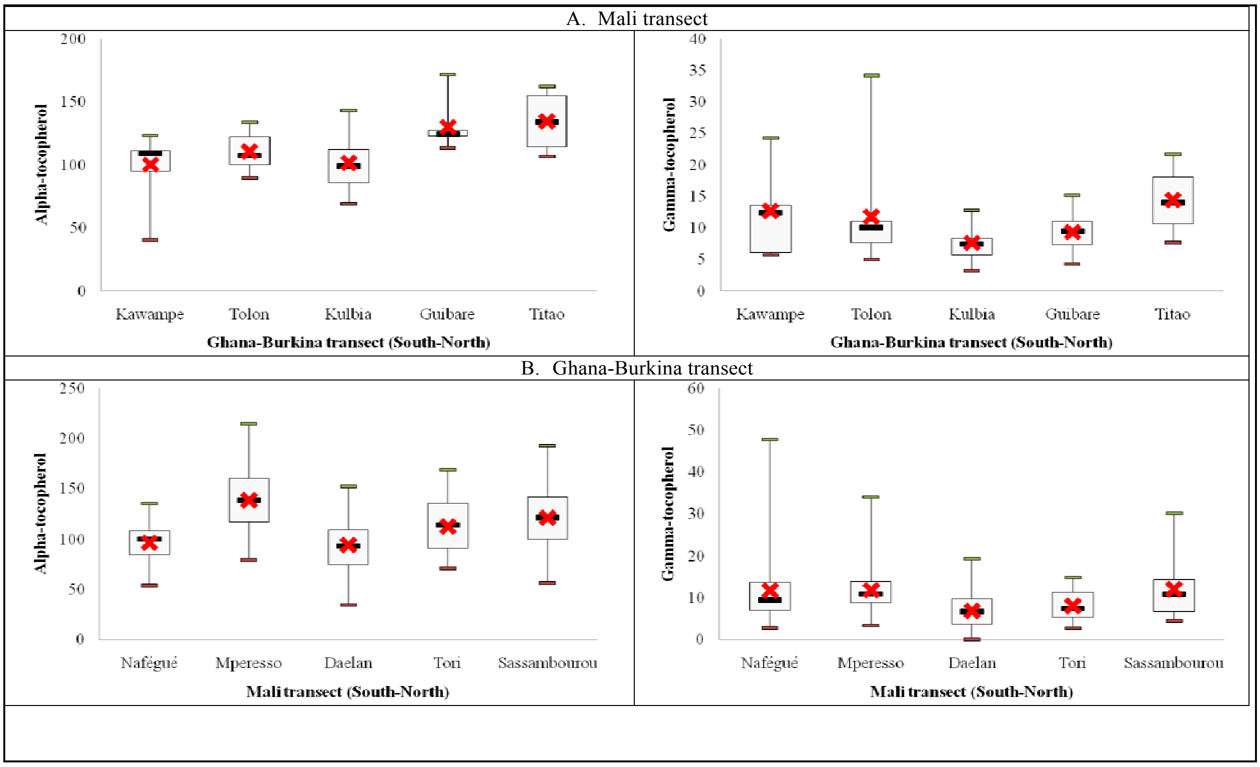



Figure WP3.4. Box plots comparison of α- and γ-tocopherols within (A) Mali and (B) Ghana-Burkina transect. Crosses are mean values; black bars represent median values; upper and lower side of rectangles give 1st and 3rd quartiles and horizontal grey bars represent extreme values



**Polymorphism of fatty acid candidate gene *in Vitellaria paradoxa***

In plants, and particularly in the shea tree, the saturated vs. unsaturated fatty acids ratio depends on the activity of a key enzyme in the fatty acids biosynthesis pathway. This plastidial enzyme, called SAD (Stearoyl Acyl-carrier-protein Desaturase), specifically catalyzes the conversion of stearic acid (C18:0) into oleic acid (C18:1).

In the present study we explore the polymorphism of *SAD* gene in 40 trees from the whole natural area. The sequencing of *SAD* gene resulted in the identification of an unexpected gene duplication. This duplication displays in *Vitellaria paradoxa* a chemically-related pattern with, in easterner populations of shea trees two functional gene copies (*SAD1* and *SAD2*), and in West African trees a functional ancestral copy (*SAD1*) and a truncated gene copy (*SAD2*). This result is the major explanation of the diverging chemical pattern of western and eastern shea trees. In addition, a strong directional selection was detected in the West African sampling for both copies, while no evidence for positive selection was detected in the easterner region. Such selection pattern could result from adaptation or human selection, but can also be a consequence of the recently identified demographic expansion of *Vitellaria paradoxa* in West Africa. In this region, the comparison of *SAD* copies polymorphisms shows that *SAD2* accumulates 3-times more mutations than *SAD1*, due to *SAD2* un-functionality. We therefore conclude in the future pseudogenization of *SAD2* in West African shea trees, while not in easterner populations. Due to the strong genetic structure between West African and easterner populations, this gene could appears as a marker of the ongoing speciation.

### 1.3.3.3 WP3 - impact of the project on its industry or research sector

The results obtained by the WP3 confirm that two main provenances can be defined in shea tree, the West African and east African provenances. No clear pattern of variation can be identified among the populations within these two main provenances.

This result can be used by traders involved in shea tree to better define their trade strategy.

### 1.3.4 **Work package 4 – *domestication and Pre-breeding of shea tree : characterisation, mobilisation of local varieties by a multitrait and participative approach and horticultural research***

Farmers have identified different varieties in the parklands on the basis of precocity in flowering and fruiting, sweetness of pulp fruit, size and colour of leaves and fruits. These varieties are predominantly described by local people on the basis of subjective criteria. The identification, characterisation, evaluation and propagation of these putative superior shea tree varieties are important pre-breeding activities that can form a strong genetic foundation for initiation of improvement programmes that can lead to improved production of parklands. Such breeding programmes can be established according to different strategies. Establishment of key genetic parameters are important in order to allow development of guidelines for effective domestication based on sound genetic parameters. Existing clonal trials should therefore be evaluated in order to estimate broad sense heritabilities for different traits, whereas new clonal trials will serve the purpose of improving this knowledge and lead to estimation of breeding values of the identified putative varieties.

General objective
To promote domestication of shea tree by characterising farmer varieties on the basis of morphological, fruit quality, molecular and chemical traits, by mobilising clones and by developing guidelines for breeding strategies

Specific objectives
1 – To characterise local varieties using morphological, molecular and chemical traits,
2 – To mobilise the gene pool for future domestication, establish knowledge on genetic parameters
3 - To develop guidelines for effective domestication based on sound genetic principles.

Scientist involved

#### 1.3.4.1 WP4 - Methodologies and approaches employed

Task 4.1 : to collect information on variety using farmer interview
In each African country involved in the project, we will assess the indigenous knowledge on the local varieties in region where shea tree is significantly produced. Targeting harvesters/producers, we will carry semi-structured interviews focusing on the horticultural and agroforestry practices associated with the shea tree.

Task 4.2 : To characterise the variety combining a multitrait approach .
The varieties were characterised using morphological traits of fruit, leaves and trees, using chemical constituents of the kernel, using molecular markers. Characterisation of the varieties on the basis of multitrait approach will be performed using multivariate techniques such as principal components analyses or tree methods. The performance of the farmers' varieties will be compared to the performance of the trees sampled to characterise the provenances of shea tree in connection with the WP3.

Task 4.3 : development of an improved gene pool
In each African country involved in the project, we develop an improved gene pool by selection in five steps.
First step involves identifying a single region/area that is candidate for mobilisation based on phenotypic ecological reasoning in line what already described in WP3. The second step involved 'with farmer' identification of 150 superior trees in the region based on existing knowledge as described in 4.1 above. The number of clones are narrowed down in the third step form 150 to 50

based on the qualitative and quantitative assessments (task 4.1-4.2.) combined with farmers experiences. The fourth step draws upon the results of activity 4.3, allowing final selection of 15 superior trees/varieties for the region based on their fruit quality. The fifth step involves grafting of the selected trees in clonal trials/germplasm bank (one per African country for demonstration).

Task 4.4 assessing genetic gain.
Existing clonal trials in Burkina Faso and Mali will be assessed as described in task 3 above. Based on the findings, broad sense heritabilities and genetic variance components will be estimated in order to assess the genetic of the different traits. The analysis of the data will be done in a joint statistical Workshop held in the region

Task 4.5 : guidelines for development of breeding programmes
The clonal trials/germplasm banks are an important pre-breeding delivery developed within the project. Due to the time frame of the projects, assessment of the trials cannot be carried out within the project. However, development of plans for future assessment and potential applications of the findings will be discussed among the partners during the assessment workshops (held in connection with task 7) and in the frame of the WP10

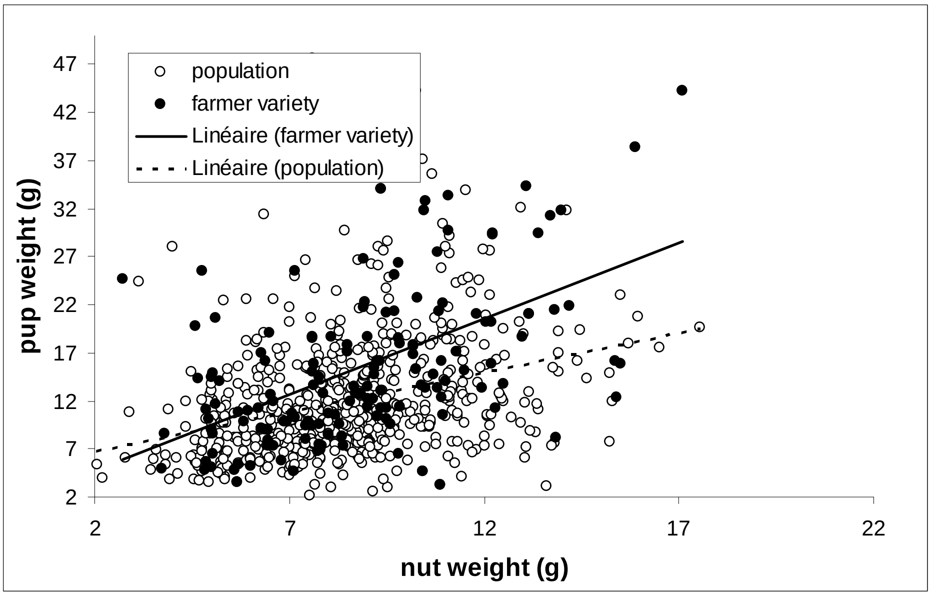
Task 4.6 : horticultural research
Experiments will be conducted to improve the technique of propagation by seed and the vegetative propagation by grafting and by cutting using robust technology easily transferable to farmers

### 1.3.4.2 WP4 - Achievements of the project to the state-of-the-art.

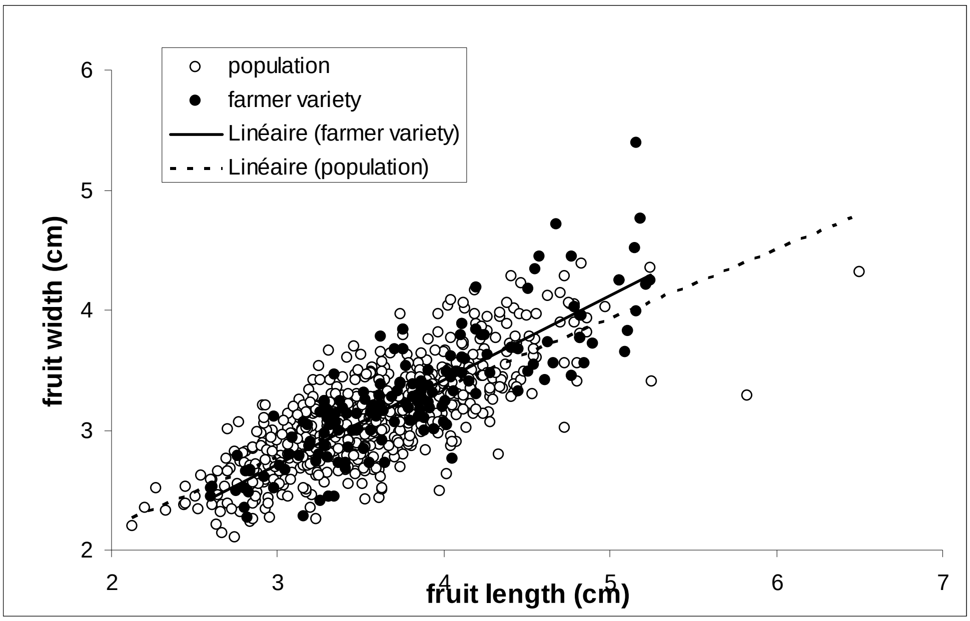
Here, through the Mali and Uganda results we give an illustration of what was conducted by each African country involved in the project.
To domesticate shea butter tree, an important species of agro-forestry parklands, it is important to survey farmers´ knowledges, to identify and characterize the morphotypes; and to propagate superior genotypes in order to bring species into wider cultivation. Surveys conducted in Mali have shown that farmers have knowledges on shea which vary according to the abundance and to the socio-economic importance of the species in the region. Farmers´ preferences and selection criteria were identified The criteria varied according to ethnic groups. Based on these criteria, 145 accessions were identified which were clustered in 15 groups. Some of the morphotypes are the same but have different names du to the difference in ethnic groups. When comparing the morphotypes to the global population of shea in Mali,
significant difference was observed for all main fruit traits except for the nut weight (F=2.67 and Prob=0.128. The distribution pattern showed that the morphotypes are distributed throughout the country. Chemical analyses of the nuts showed the same trend. Plus trees were identified according to fat content; some of them were propagated on farm forestablishment an improved gene pool which was supposed to provide regularly material for propagation to farmers.

**Figure WP4-1: Comparison of fruit characteristics between the farmers' varieties and the representative sample of the Malian population of shea tree (a) for fruit and nut weight relationship, (b) for fruit length and width relationship**

a




b



The Morphological variation among shea tree 'Ethno-varieties' in Uganda". In this country local folk classification by farmers recognises the presence of 44 ethno-varieties on the basis of fruit and nut characters.
Morphological variation was analyzed in shea tree ethno-varieties in West Nile, Northern and Teso farming systems to (1) assess the patterns of morphological variation among shea tree ethno-varieties and (2) establish whether there is morphological evidence for shea tree folk classification in Uganda. Knowledge of fixed variation is important for any breeding or conservation programme. A total of 176 shea trees representing all the 44 farmer ethno-varieties were analysed for 14 fruit, nut, leaf and tree traits. Morphological measurements were made on 40-50 leaves, 20-30 fruits, crown and stem diameters for a maximum of 5 trees of each ethno-variety in the three farming systems. Pearson's correlation coefficients, Principal Component and Hierarchical Cluster Analysis were utilised to explore and reveal patterns in morphological variation among the ethno-varieties. High variation was found in pulp weight (CoefVar =35.9%), DBH (CoefVar = 28.48%), fruit weight (CoefVar = 27.81%) and canopy diameter (CoefVar = 26.69%).
Apocopoco (soft pulp variety) had the heaviest fruits, nuts and pulp while Acula (oval fruit variety) had the longest fruits. There was 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 ($r = 0.788$, $p < 0.001$). Principal component analysis showed no underlying quantitative morphological structure among the 44 ethno-varieties. Hierarchical clusteranalysis revealed the presence of five groups with no clear aggregation based on ethnographic or geographic separation.
From morphological evidence alone, there are no discrete forms of V. paradoxa related to folk classification Uganda. However, the high variation in fruit characteristics offers great opportunities for cultivar selection for improvement and domestication programmes.

With the data collected in each country, it was decided to make a meta data analysis combining the data of the different countries. The final objective is to make a publication with the title: Farmers varieties - A resources for improved Shea? This work can be done by comparing the farmers varieties with the random selected samples:

In this project numerous debates and reflection have concernend the way to improve shea tree : "Domestication: a route to better Shea? A framework and experience from domestication of Shea and other Sudano-Sahelian fruit trees has been presented duting the final conference. We highlight knowledge gained in INNOVKAR and outline likely new routes towards improvement that results from the project open for future activities. The INOVKAR project has moved the present level of understanding substantially forward in relation to basic pre-breeding knowledge,and has thereby put the West African countries in a position where they can now develop country specific or regionalbreeding programmes. But what should such programmes include, what are the likely effects and how can they be organised?

#### 1.3.4.3 WP4-1 - impact of the project on its industry or research sector

At this stage of the research no impact on industry can be defined.

**However these firstCaractérisations moléculaire, morphologique et anatomique des champignons du genre *Ganoderma* associés au dépérissement du palmier à huile pour déterminer l'origine phylogénétique des *Ganoderma* pathogènes et comprendre leur émergence en tant que pathogène.**

Au cours des travaux de 2009 à 2012, le développement et l'optimisation des techniques d'extraction de l'ADN ont été réalisés en vue de l'obtention de séquences sur des matériels d'origines différentes et parfois difficiles à amplifier (Rapport GANODIV de 2009 à 2012).
Aujourd'hui, cette optimisation permet d'établir au sein du laboratoire un protocole fiable pour les études portant sur la diversité des champignons du genre *Ganoderma*. Grâce au protocole mis au

point, la caractérisation moléculaire a pu être réalisée sur une centaine d'échantillons de *Ganoderma,* d'Indonésie, de Guyane, de Nouvelle Calédonie et d'Europe. Après une sélection des séquences obtenues expérimentalement ou issues de la banque de données NCBI, la construction d'arbres phylogénétiques a permis de mettre en évidence des groupes taxonomiques de *Ganoderma* d'Europe et d'Indonésie.

## 4. Variabilité génétique de *Ganoderma boninense* agent de la pourriture basale chez le palmier à huile

L'étude de génétique des populations utilisera des marqueurs anonymes (microsatellites) pour caractériser un échantillon d'isolats prélevés sur différentes plantations d'Asie du Sud-Est (Nord Sumatra, Thailande, Malaisie péninsulaire & insulaire (Borneo-Sabah) et dans une moindre mesure, d'Afrique et Amérique du Sud. Les plantations seront choisies de sorte à représenter au mieux la diversité des plantations présentes dans la région (âge des arbres, nombre de générations de palmier plantés, précédents, sol…) et un dizaine d'isolats sera prélevé par plantation. Des isolats de G. boninense provenant de la forêt ou de cocotier seront rajoutés à l'échantillonnage et nous chercherons à constituer une collection d'environ 200 isolats. Le niveau de diversité, le déséquilibre de liaison et la structure génétique seront étudiés en premier lieu afin 1) d'identifier d'éventuels grands groupes historiques et 2) de rechercher des phénomènes d'isolation par la distance révélateur des capacités de dispersion du champignon. Ces informations obtenues, nous proposerons des scénarios démographiques pertinents pour expliquer la distribution actuelle de l'espèce et nous les testerons à l'aide de méthodes basées sur le calcul approché bayésien (Approximate Baysien Computation).

## 5. Etude de la production des laccases et des lignine-peroxydase fongique : relation avec l'agressivité des isolats de *Ganoderma boninense*

Cette étude devrait permettre 1) mieux comprendre l'importance des capacités à dégrader le bois dans l'attaque du palmier et la réalisation du phénotype agressif de *G. boninense* 2) de déterminer s'il existe plusieurs profils d'attaque en relation avec les caractéristiques enzymatiques des isolats et 3) de déterminer les éventuelles cibles biochimiques préférentielles de ces attaques. Ce dernier point pourra avoir des retombées directe en termes d'amélioration du palmier, les cibles biochimiques préférentielles pouvant être à terme utilisées comme marqueur de la tolérance/sensibilité au *Ganoderma boninense* des cultivars de palmier à huile.

research results can help the decision makers to define a strategy to start a breeding programme on shea tree in each of the African country participating to the project.

## 1.3.5 Work package 5 – *chemical analyses using traditional and high throughput methods : the near infrared spectrometry method (NIRS)*

Conventional analytical methods for determination of chemical constituents are highly time-consuming, labor-intensive and very expensive especially when a high number of sample are required for relevant analyses. In addition large volumes of solvents are considered as environmentally problematic.These disadvantages are the reason for testing the technique of Near Infra Red Spectrometry as a potential and alternative method for analyzing chemical constituent of shea tree nuts. Chemometric NIRS-methods are now widely accepted. They are used in numerous applications for qualitative and quantitative analyses in various fields, e.g. food, agriculture, medicine, chemicals, pharmaceuticals, polymers and cosmetics. Its popularity comes from its three major advantages as an analytical technique: speed, calculable accuracy and little sample preparation.

**General objective**
To provide efficient and low cost tools for analysing chemical constituent of shea tree kernel

**Specific objectives**
1 – to develop the NIRS method on shea tree kernel for chemical analyses
2 – to test the applicability on various situations : screening of origins and screening of varieties
3 – to transfer the method to partners

**Scientist involved**
Fabrice Davrieux, Georges Piombo
Bokary Kelly
Massamba Thiam
Boukary Diallo
John Bosco Okullo
George Nyarko

### 1.3.5.1 WP5 - Methodologies and approaches employed

The methodologies used were based on the following tasks.

Task 5.1 : definition of the reference sample and elaboration of a spectral data base
For this development, a set of calibration samples was collected. These samples were collected so that it can represent all the chemical variations to be expected in the shea tree population where future predictions have to be conducted. The type of sample (kernel, kernel powder, butter oil) was defined before starting the NIRS analyses. The sample strategy was conducted in connection with the WP3, WP4, WP6 and WP7.

Task 5.2 : conventional analyses and calibration
The purpose of the calibration was to establish a mathematical relationship between the NIR spectra and the chemical parameters of the analysed sample set.Chemical analyses using traditional techniques were conducted with a subset of samples selected in task 5.1 on the basis of a spectral variation. The chemical analyses will concern the following main constituents: free fatty acids, peroxide value, iodine value—plus humidity and impurities.
Fatty acid profile, unsaponifiable percentage, tocopherols and catechins.
Triglyceride analyses, including stearic-oleic-stearic (SOS)

Task 5.3 : analysis of the phenotypic variation using mathematical model elaborated with the NIRS method
Once the mathematical model was established, the analysis of the unknown sample can be performed. The NIRS will be used to screen the individuals trees from different origins (200 to

400 samples) of the WP3, to screen the farmers' varieties in the WP4 (around 500 samples are to be analysed).

Task 5.4 : transfer of method

The use of these technologies by the partners of developing country is one the objective of this work package. The researcher involved in the work package will participate to the analyses of their sample in the European laboratory in charge of the implementation of the technique. Training session to the researchers will be conducted by mission. The transfer will consist in : protocol transfer, standardisation of devices etc…

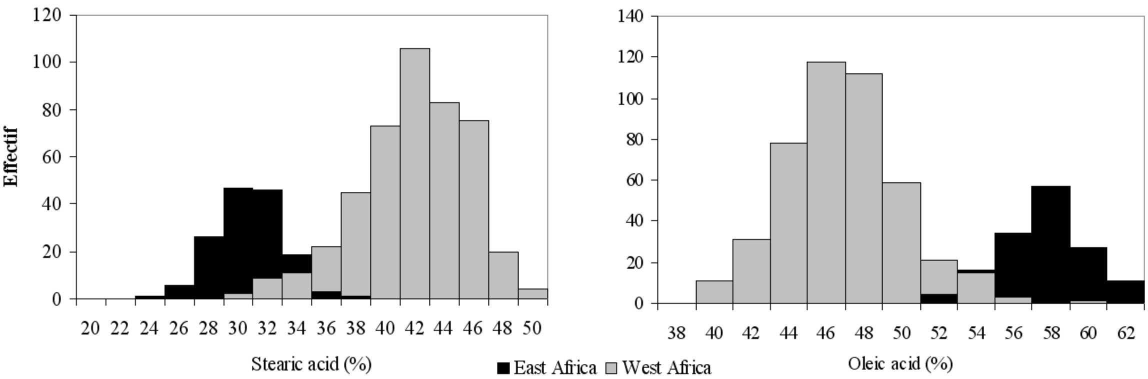
### 1.3.5.2 WP5 - Achievements of the project to the state-of-the-art.

Chemical analyses were conducted to establish the calibration data set and the prediction model.
Nuts samples were collected over two years (2007 and 2008) in homogeneous conditions in four West African countries (Subsp. *paradoxa*): Senegal, Mali, Burkina and Ghana; and 1 East African country (Subsp. *nilotica*): Uganda. Within each country different sites according to a north-south gradient were sampled. Sampling represented a total of 624 trees (GPS located) collected in 17 sites: Senegal (Kenioto, Samecouta and Saraya), Mali (Nafégué, Mperesso, Daelan, Tori and Sassambourou), Burkina (Titao and Guibare), Ghana (Kawampe, Tolon and Kulbia) and Uganda (Katakwi, Pader, Moyo and Uleppi-Arua). An average of 30 mature fruits was collected per tree. Post-harvest treatment including depulping and drying (3 days at 60°C), has been done onsite. The dried nuts were sent to CIRAD laboratory Montpellier (France). At receipt nuts were oven-dried two days at 60°C in order to stabilize moisture content, then stored at room temperature before analyzes.
Powders prepared from collected nuts were analyzed for moisture content, fat content using solvent extraction, fatty acid (FA) profiles using gas chromatography, tocopherols content using HPLC with fluorimetric detector, Triacylglycerols (TAG) profiles using HPLC and free fatty acids (FFA) using potassium hydroxide neutralization.
Fat content was on average equal to 49.66%, the values ranged from 29.96% to 59.66%. Oleic acid relative percentage was systematically higher than stearic acid. However East African shea butters were richer in Oleic acid (56.64%) than West African butters (45.91%). At the opposite, Stearic acid relative percentage was higher in West Africa (40.91%) than in East Africa (29.72%). According to oleic and stearic acids relative proportions, 2 groups corresponding to East and West Africa, were defined.
Only α-tocophérol and γ-tocophérol were detected in the samples, α-tocophérol was preponderant with an average content equal to 112 mg/kg while γ-tocophérol was ten times less concentrate. In Uganda γ-tocophérol was three times more concentrate than in West African shea nuts. TAG profiles were fully described including isomeric position by Liquid Chromatography and Mass Spectroscopy. TAG profiles revealed SOO and SOS were preponderant. SOS was higher in West Africa than in East. The trioleine TAG (OOO) relative percentage was systematically higher in the Ugandan shea butters.
These results based on a large collection of samples, confirm the differentiation in fat shea nut composition due to geographical origins.

The purpose of this study was to develop near infrared spectroscopy (NIRS) calibrations to characterize Shea nut fat profiles. Powders prepared from nuts collected from 624 trees in five African countries (Senegal, Mali, Burkina Faso, Ghana and Uganda) were analyzed for moisture content, fat content using solvent extraction, and fatty acid profiles using gas chromatography.
A subset of 156 samples were analysed for free fatty acids (FFA) content. The FFA range was increased using 53 samples enriched by natural evolution during storage in climatic oven (28°C, 60%RH).
Results confirmed the differences between East and West African Shea nut fat composition: eastern nuts had significantly higher fat and oleic acid contents. Near infrared reflectance spectra were recorded for each sample. Ten percent of the samples were randomly selected for validation

and the remaining samples used for calibration. For each constituent, calibration equations were developed using Modified Partial Least Squares (MPLS) regression. The equation performances were evaluated using the Ratio Performance to Deviation ($RPD_p$) and $R_p^2$ parameters, obtained by comparison of the validation set NIR predictions and corresponding laboratory values. Moisture ($RPD_p$=4.45; $R_p^2$=0.95) and fat ($RPD_p$=5.6; $R_p^2$=0.97) calibrations enabled accurate determination of these traits. NIR models for stearic ($RPD_p$=6.26; $R_p^2$=0.98) and oleic ($RPD_p$=7.91; $R_p^2$=0.99) acids were highly efficient, and enabled sharp characterization of these two major Shea butter fatty acids. High FFA samples obtained by incubation were beneficial for calibration set up, FFA calibration allowed accurate prediction of natural range of shea nuts FFA content ($RPD_p$=8.52, $R^2_p$=0,98).
This study demonstrated the ability of near-infrared spectroscopy for high-throughput phenotyping of Shea nuts. NIRS can be used for Shea nuts fat quality assessment. Transferring this technology to producing countries will enable high-throughput shea nut quality control and a traceability survey. Moreover, our NIR models make it possible to carry out further quantitative genetic investigations.

**Figure WP5.1.** Histograms of stearic and oleic acids relative percentages.




**Figur
e WP5-2.** Scatter plot of the 2 first PCs extracted from PCA done on the 602 shea nuts spectra set (95% confidence ellipses).

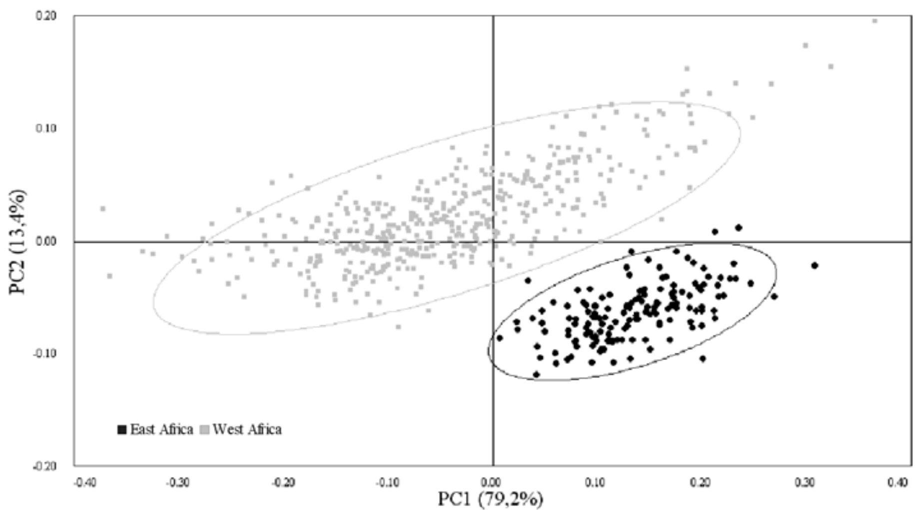
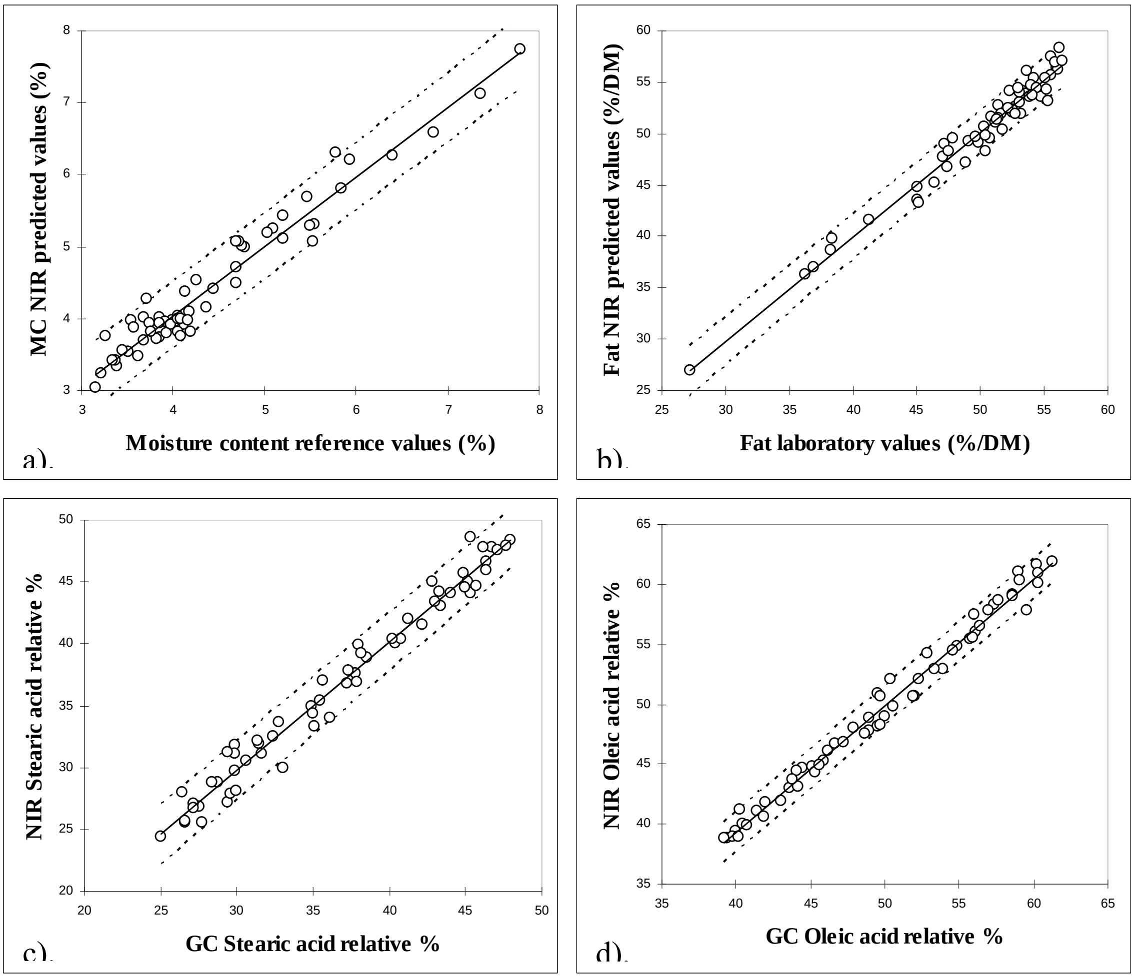



**Figure WP5-3.** Scatter plots of laboratory values versus NIR predicted values for the 60 validation. samples (95% confidence interval). **a).** Moisture Content in %; **b).** Fat content in % of dry matter (DM); **c).** Stearic acid relative composition in %; **d).** Oleic acid relative composition in %.



#### 1.3.5.3 WP5 – Impact of the project on its industry and research sector

Under the European Innovkar project, a near-infrared spectral data base was developed, this database involves 624 samples of shea nuts from 17 production sites across 5 countries: Mali, Senegal, Burkina Faso, Uganda and Ghana. The database was established over three crops (2007, 2008 and 2009). Together with the spectral analysis, samples were analyzed in the laboratory for their water, fat, free fatty acids (FFA) contents and their fatty acids and triglycerides profiles. Calibration equations to predict the moisture fat and FFA contents and fatty acid profiles of almonds were set up and validated. The performances of the models allow the use of near infrared technology for non-destructive and high-throughput routine quality control of almonds.

The spectral database also separates, with a classification rate of 100%, almonds according to geographical origin between West Africa and East Africa.

According to these high performances, the main results of the WP5 were presented during the Global Shea Conference (Bamako, Mali 14-20 March, 2010, http://www.globalshea.com/). This annual conference brings together all stakeholders (300 participants) of the shea sector: from the producer to the end user, through government agencies and non-governmental organizations. Following the presentations, appointments “business-to-business” have been organized. Meetings were organized with the Purchasing Managers and Quality Managers of the four main exporters of nuts and shea butters: Loders Croklaan (Malaysian IOI Group), Ghana Speciality Fats Industries (Group Wimar), Aarhus Karlshamm Sweden (AAK), Burkarina SARL (Olvea Group HRIS) and The Body Shop (L'Oréal). These companies represent 90% of the volume of nuts and shea butter exported.

The main demand for nuts quality control concerns:

- High Stearin content.
- High fat content
- Low FFA content (<7%)
- Low moisture content (< 9%)
- No impurity.

Prospects and interesting contacts concern the Dutch company Loders Croklaan based in the Netherlands and and aprt of the Malaysian company IOI Group, a global leader in the production of palm oil and the global leader in the supply of oils, fats and nutritional ingredients. The company has plans to build two laboratories (in Ghana and Burkina) for monitoring free fatty acids (FFA), fat and water content of almonds. The cost and time of analysis were clearly mentioned as important factors of almonds lots quality control. The added value of NIRS tool was obvious to them.

Two missions (2011) were realized, by CIRAD researchers and CIRAD jurists, in order to discuss the calibrations commercialization in the name of all the Innovkar project partners. To date, no contract was signed with Loders Croklaan Company. The company decided to develop its own models using the project knowledge and methodology.

**Research Sector:**

We demonstrated that NIR allows rapid (less than one minute per sample) non-destructive and reliable determination in one shot of moisture, fat, stearic and oleic acid and FFA contents of Shea nuts. Thus our models can be applied for high throughput characterization of Shea nut quality. Transferring this technology will enable a Shea nut quality control and traceability survey. This could be applied to better understanding and amelioration of butter extraction process.

Moreover, our NIR models make it possible to carry out further quantitative genetics investigations. Indeed the management of thousands of samples in very short time will support breeding programs and high throughput phenotyping.

### 1.3.6 Work package 6 – *Development of new scientific tools to assure the determination of foodstuff origin : promoting the traceability of shea tree products*

Commercial exchanges are more and more intensive and spread all over the world. Consumers are more and more exigent and interested in food product quality and origin. Mad cows, antibiotics, dioxine, GMO, chicken flu have developed a non-trust feeling in consumer opinions about food quality and safety.

To guarantee trust in commercial exchanges, valorisation of trademark food products : origin, protected areas… food product nature and origin must be known and guarantee. EU rules are more and more strict for certification and origin named of food products (ACTA-ACTIA, 1998).
The new European law (European regulation CE 178/2002) about safety and traceability which was published in 2002, was applicable in January 2005. This law will ask to industry to trace their product and assure the safety of the consumer.
For traceability and safety, documents permit only to know package place or the exporter identity. Production place, food additives or ingredients origins are unknown. For example it is not easy to know if the name of a processed product is right or wrong, if it comes from a Mali or a Ghanean farm.

This project will permit to test or to create new analytical methods for the identification of the processed shea tree product origin by analytical methods.

The WP6 will use the vegetal material collected in the countries involved in the project and will benefit from the collection made in the other WPs (WP3 and WP4).

General objective
To encourage and promote traceability initiatives through innovative research on methodology (genomic and metabolic approaches)

#### 1.3.6.1 WP6 - Methodologies and approaches employed

Task 6.1 : Distinction of origins based on fruit micro organisms using molecular biology method (DGGE)
In connection with the WP3 which analyses the variation between origins using fruit morphology and chemical composition, five fruits per tree will be collected in each population. The micro organisms present on fruits will be measured by a global method consisting of extracting the full DNA of all the bacteria in one step, then analysing them on a denaturant gel including a gradient. It permit to analyse in one step all the bacteria present of the fruit and obtain a bar code of the contaminants which could be linked to the origin of the fruit. We will use essentially a new technique develop for other projects : Denaturant Gel Gradient Electrophoresis (DGGE) This molecular biology method permit to analyze in one spot the global contain in bacteria and conduct to a biological bar code. It gives the ecology of bacteria present on the product and could be link to the ecology of bacteria at the geographical origin of the product.

Task 6.2 : Distinction of origins by analysis of the solid content of the oil against temperature by Large band Mass spectrophotometry
The objective of this task is to draw the curve relating solid content of shea tree oil and temperatures and to compare the form of the curves obtained for the different origins of shea tree with the one obtained on cocoa oil.  Oil samples will be collected by researchers involved in other WPs and send to be analysed by Large band NMR. This technology gives information on the solid content of the oil. These data could be used for further valorisation in industry or cosmetics.

Task 6.3 : Distinction of origins by DNA extracted from shea butter : extraction and purification technique from shea butter
To apply available techniques to extract and purify DNA from shea butter at the different stages of the production chain and to evaluate whether the DNA collected is quantitatively and qualitatively applicable for genomic analysis using PCR technology. To test these methods for differentiation of origins and experiment will be carried out with butter extract from the different origins of the project.

Task 6.4 : To define traceability methods based on the results obtained from the different WP
The objective of this task is to make the synthesis of the different methods used to differentiate the origins of shea tree : methods implemented in the task 6.1-6.3 and methods from other WPs such as WP3, WP4 and WP5. The different approaches to differentiate the origins will be statistically analysed and their potential to implement traceability methods will be discussed.

Remark : the method of NIRS developed in the WP5 will be analysed in connection with the task 6.4. Its interest as a method for improving traceability of shea tree will be studied in this work-package

### 1.3.6.2 WP6 - Achievements of the project to the state-of-the-art.

**Choices of the biological markers**
The micro-organisms present on the surface of the fruits of shea tree must be characterized by a method utilizing molecular biology, which avoids heavy methods of culture of microorganisms. It is not question in this project to determine the name of the microbial species but it is still possible to do that by sequencing bands. The totality of the micro-organisms must be highlighted and it was thus necessary to choose universal PCR markers.
The knowledge published on the shea tree is very weak, and those on the micro-organisms present on the fruits even weaker. Research thus relates to all the microorganisms likely to develop on the surface of the fruit which are bacteria, yeasts and moulds. CIRAD already developed for this project a technique of PCR/DGGE for the bacterial species which was never applied to the fruits and developed during this year two novel methods of PCR-DGGE for the determination of yeast and fungi ecology. This last method will be published very soon (accepted for publication).

**Principal of PCR/DGGE (Denaturing Gradient Gel Electrophoresis)**
The principle of PCR-DGGE rests on the separation of same size DNA sequences produced by a PCR. The variations of nucleic acid sequences in the genome of the various microbial species give to the DNA molecules different properties of fusion. Indeed, the GC% of a sequence and the sequence of the pairs of bases are determining factors of the melting point. Between the two strands of DNA, the bases Guanine and Cytosine are bound by three hydrogen bonds, while the bases Thymine and Adenosine are bound by two connections. So it is more difficult to break G-C connections than AT connections. The molecules having the most important GC% will be more difficult to denature. The separation of the DNA having different properties of fusion can be carried out thanks to an electrophoresis of DNA in a polyacrylamide gel containing a denaturing gradient (DGGE) based on urea and formamide (Ampe *et al.*, 1999).
The amplified double strand DNA molecules, linked by a GC clamp, are deposited on the gel. Progressively with their progression, the denaturing conditions are increasingly strong. PCR products with the distinct sequences start to open with different denaturing concentrations, in other words with different positions on the gel. To prevent total dissociation of the molecule, and thus to be able to observe the threshold of migration, a sequence of 40 nucleotides rich in GC (GC Clamp) is added at end 5' of the forward starter. The speed of migration decreases with the opening of the molecule, a double strand molecule completely closed moving more quickly in the polyacrylamide meshes than a partially degraded molecule. The simple strand molecule then will stop **(Fig. WP2-2)** (Ercolini, 2003; Muyzer *et al.*, 1993).

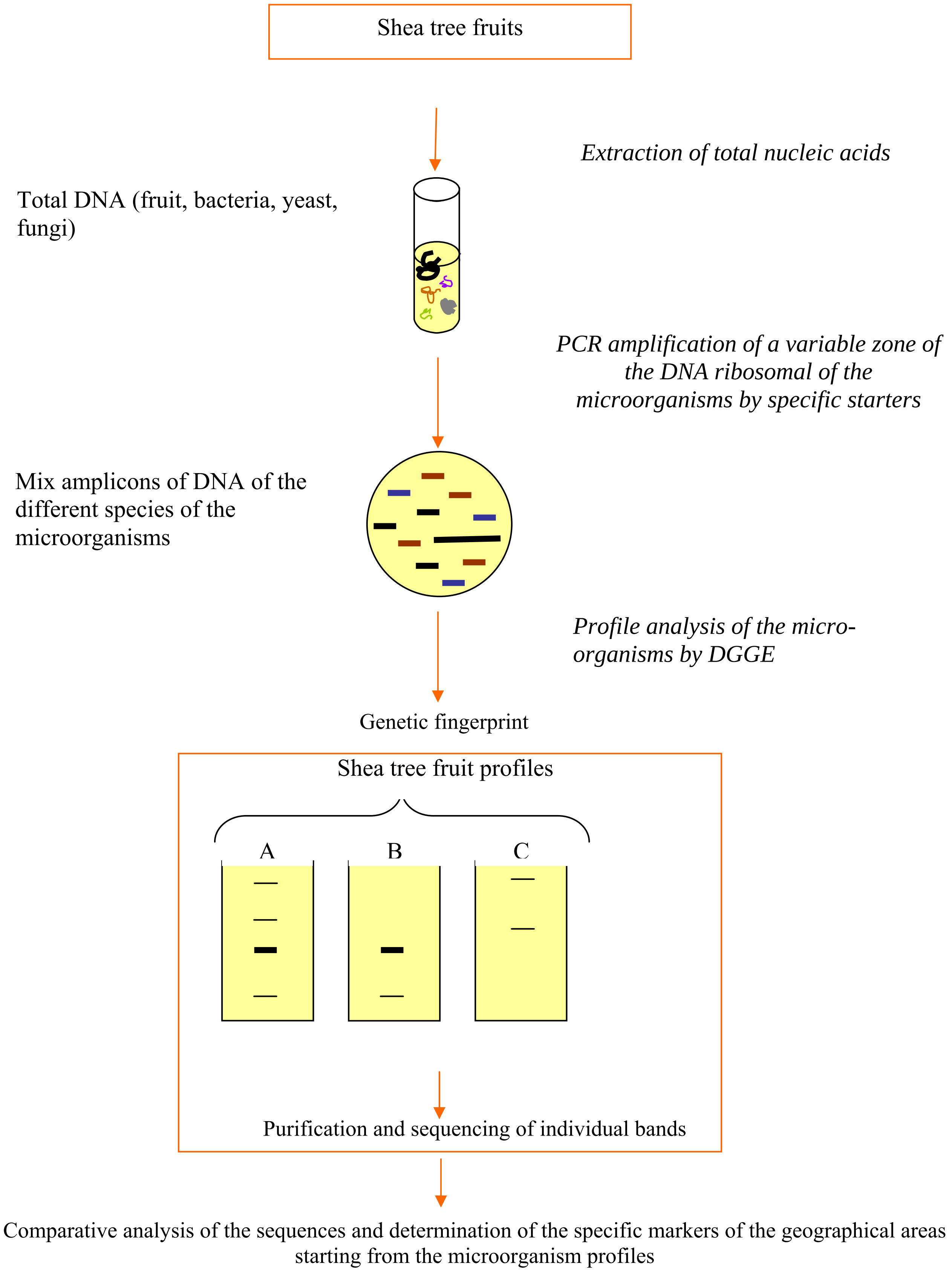



**Fig. WP6-1. Diagram of the analytical procedure in PCR/DGGE used for the fruit samples of Shea tree**

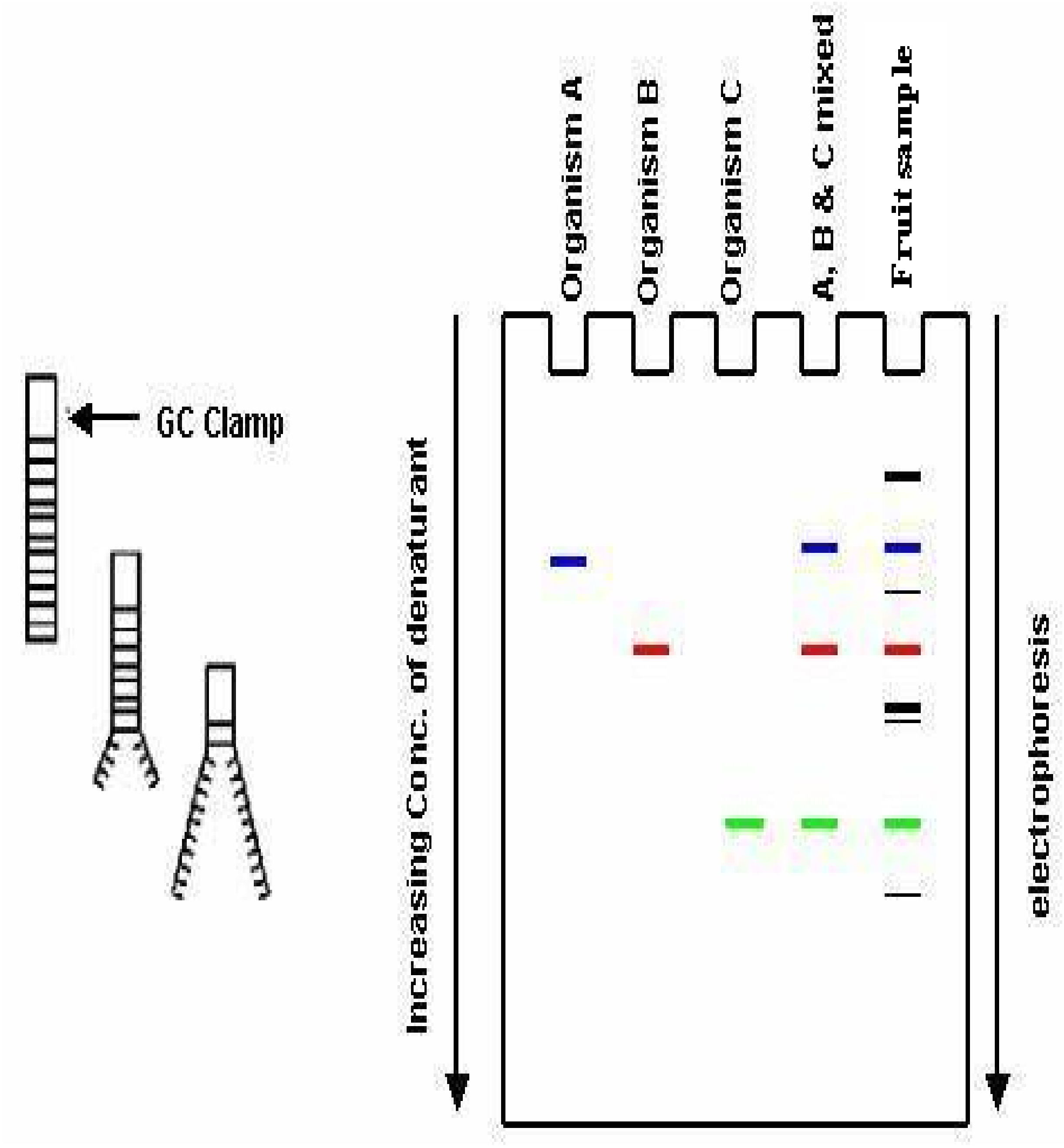



**Fig. WP6-2. Principle of DGGE:** PCR amplicons sharing the same length are separated electrophoretically in a sequence-dependent manner based on their GC-content. The increasing gradient of denaturing components along the gel confers the double stranded amplicons into single stranded DNA through melting domains which will decrease their mobility (and thus their position in the gel). A GC-clamp attached to the 5' end of one of the PCR primers prevents the amplicons from complete denaturation. Different sequences will result in different origins of melting domains and consequently in different positions in the gel where the DNA fragments halts.

**Sampling**

The quality of the method is directly linked to the quality of samples. Moreover, for this study, the samples must be harvested in order to preserve their initial flora. However the fruits of shea tree have to be transported on long distance between Africa and France. Lastly, the choice of the places of taking away must be precise and indexed. For this reason, it was important to set up a protocol of sampling which will ensure the uniformity of the fruits received at the laboratory in the shortest possible times.

The collection of fruits was made by none contaminate method to preserve their initial flora. The fruits were picked directly on tree with gloves and put in sterile sachets. These sachets were closed hermetically and were introduced into an ice box containing frozen water bottles then kept in a refrigerator. The tree received a code of reference. The samples were transferred in the refrigerators but without ice. The airline companies and times of way are controlled by the partners.

The samples were harvested in- 3 countries: Senegal; Mali; Uganda and on 3 sites per country for Senegal & Uganda and 5 sites per country for Mali.

| Date of harvest | Harvesting technique | Mali | Senegal | Uganda |
|---|---|---|---|---|
| June 2008, Juillet 2009 | Number of sites | 5 | 3 | 3 |
| | Number of trees | 3 | 3 | 3 |
| | Number of harvested samples | 5 | 5 | 5 |

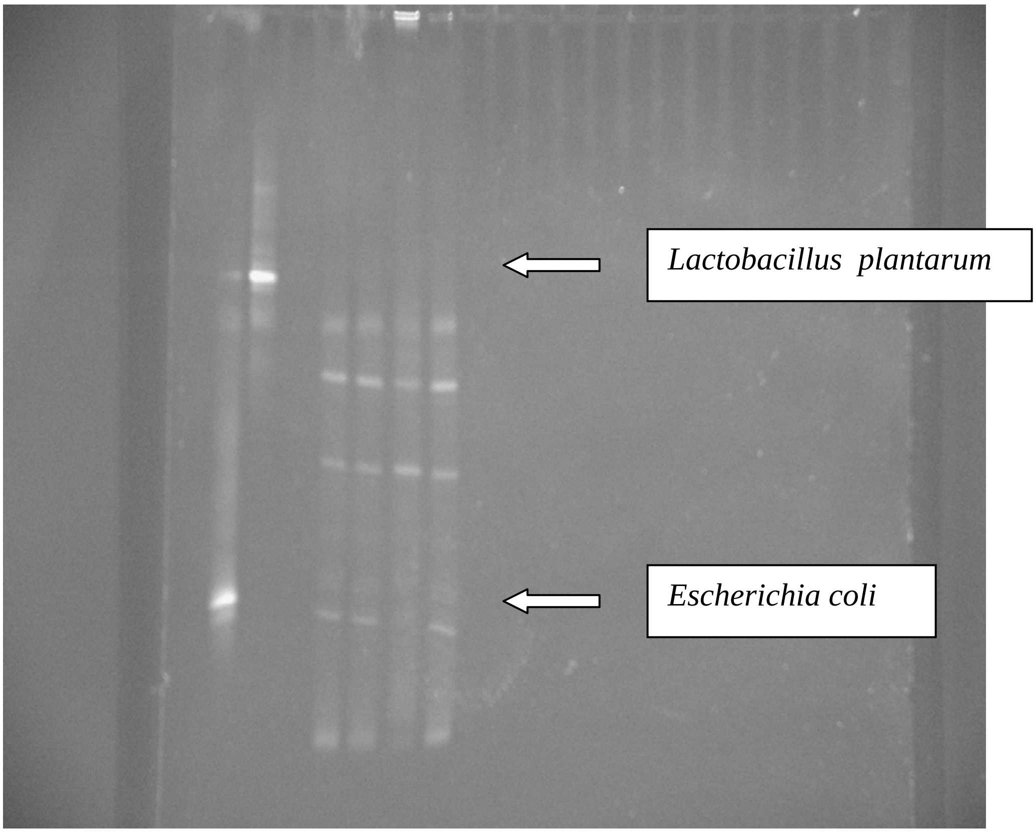
This protocol was sent to the African teams involved in the project. Once in the European laboratories, the samples were stored at -20°C until to be used.

**Results achieved -Validation of the method for bacteria presents on the shea tree fruits**

The first shea tree fruits were harvested during the starting mission of the project in Mali and then some other fruits were sent also by Mali team to CIRAD (see WP3). Initially, we used the protocol of PCR- DGGE of Leesing (2005). It was developed and standardized by CIRAD Qualisud team to study the microbial flora of Tilapias as markers of geographical origin. A first extraction of the total DNA was thus carried out starting from these fruits.

We obtained two types of DNA, those with high molecular weight (higher than 1kb) corresponding to genomic DNA, coming from the shea tree fruit as well as from the microorganisms; and those with weaker molecular weight corresponding to plasmidic DNA (microorganisms). The DNA extraction is thus validated. The DNA fragments obtained were then passed in electrophoresis on a denaturing gradient gel (DGGE). Gel revealed well the presence of several bands corresponding to the DNA of various bacterial species that we will not seek to determine the name of the species within the framework of this study. The DNA extracted from pure strains of *E. coli* and *Lactobacillus* A6 was used as positive references for the DGGE. The intensity of these two bands and their respective positions make it possible to check all the steps of DGGE, but also to be able to compare the gels between them. The 4 tracks did not present any differences in terms of quality and quantity. The fruits came from the same site and it was interesting to note that DGGE profiles were identical. The protocol used here was thus validated for the study of the total bacteria colony count. It could thus be taken again during the analysis of the samples of shea tree coming from different farms and trees from sub-Saharan Africa (Fig. 3).

**Fig. WP6-3. Photo of a DGGE gel representing bacteria resulting from 4 shea tree fruits of the same origin**




**Result – extension of the method to yeast and moulds**

The 2 nd year of the project consisted in developing the same analytical method of molecular biology called PCR/DGGE to follow the population of the two other families of microorganisms: yeasts and moulds, and to link this measurement of ecology to the geographical origin of shea tree fruit. Aly El Sheikha, PhD student advised by Didier Montet, developed two novel methods of analysis of yeasts and moulds, whereas never published by other teams.

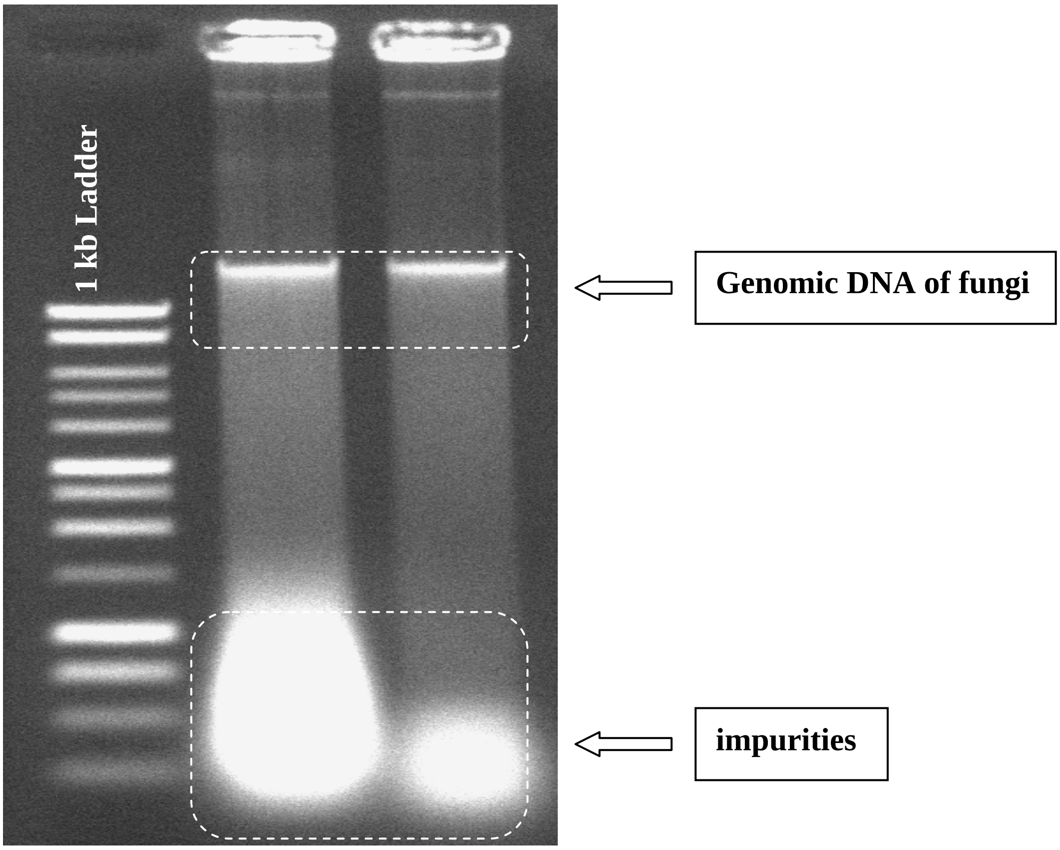
<u>Liquid nitrogen method for DNA extraction of Fungi</u>

*Protocol principle:* The principle of protocol relies on using liquid nitrogen for breaking the cell wall of fungi in order to facilitate DNA extraction by enzymatic and biochemical reagents.
*Protocol advantages:* The protocol for fungi was developed on pure strains of fungi. The extraction was initially tested on a pure strain of *Aspergillus niger*. The protocol of Leesing cannot thus be applied to all the microorganisms. This was explained by the difference of the structure as well as composition of the cell walls of these microorganisms. Indeed, the walls of fungi are made up mainly of glucanes in a conformation in triple helices. These complex chains are then associated with other parietal polymers (covalent bond with chitin and the galactomanane) to constitute the rigidity of the fungi wall. The glucanes represent 60 % of the dry weight of the fungi wall (Bex *et al*., 2006). The efficiency of the protocol was checked on

agarose gel (0.8%), it clearly appeared bands of mould DNA comparing with 1 kb ladder (Fig. 4).

*Remarks on this protocol:* This method was very powerful but we wanted to *develop an inexpensive and a field applicable extraction method for African teams.* For avoiding unfavourable sides of this protocol (expensive, danger and non applicable), we proposed to create another protocol without using liquid nitrogen.

**Fig. WP6-4. Photo of agarose gel representing genomic DNA of fungi comparing with 1 kb ladder**



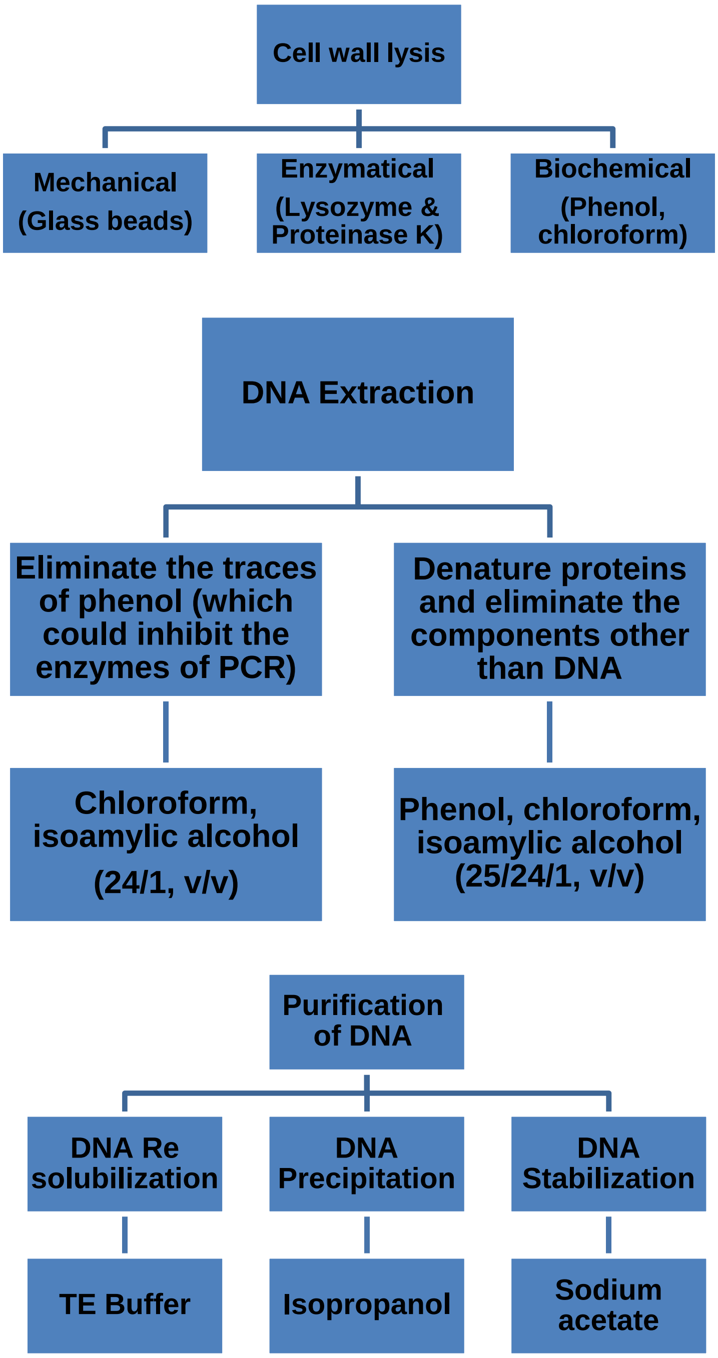
Mechanical, enzymatic and biochemical method for DNA extraction of Fungi and Yeast

It already exist many kits of extraction for fungi and yeast but we wanted to develop a cheap and practical protocol which could be valid for DNA extraction for moulds and yeasts but also which permit to obtain a high level of purity avoiding the use of purification kits for PCR reaction. This protocol is then very new for all necessary three stages: extraction, PCR and DGGE.

*Protocol principle*

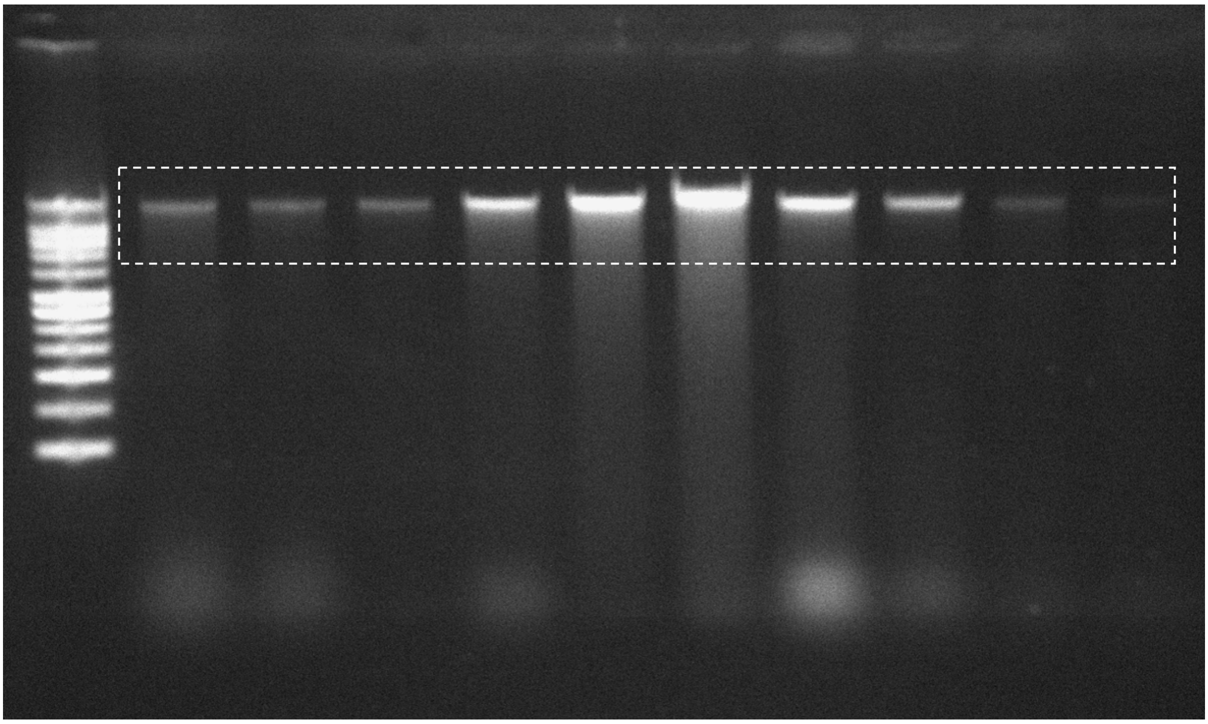
1. DNA Extraction of yeast and fungi: The principle of DNA extraction in given in Figure 5. The efficiency of the protocol was checked on agarose gel (0.8%) and it clearly appeared bands of pure strains of yeast and moulds genomic DNA comparing with Supercoiled ladder (16.21 kb) (Fig. 6).

**Fig. WP6-5. Diagram of the strategy for DNA extraction of Fungi and Yeast**

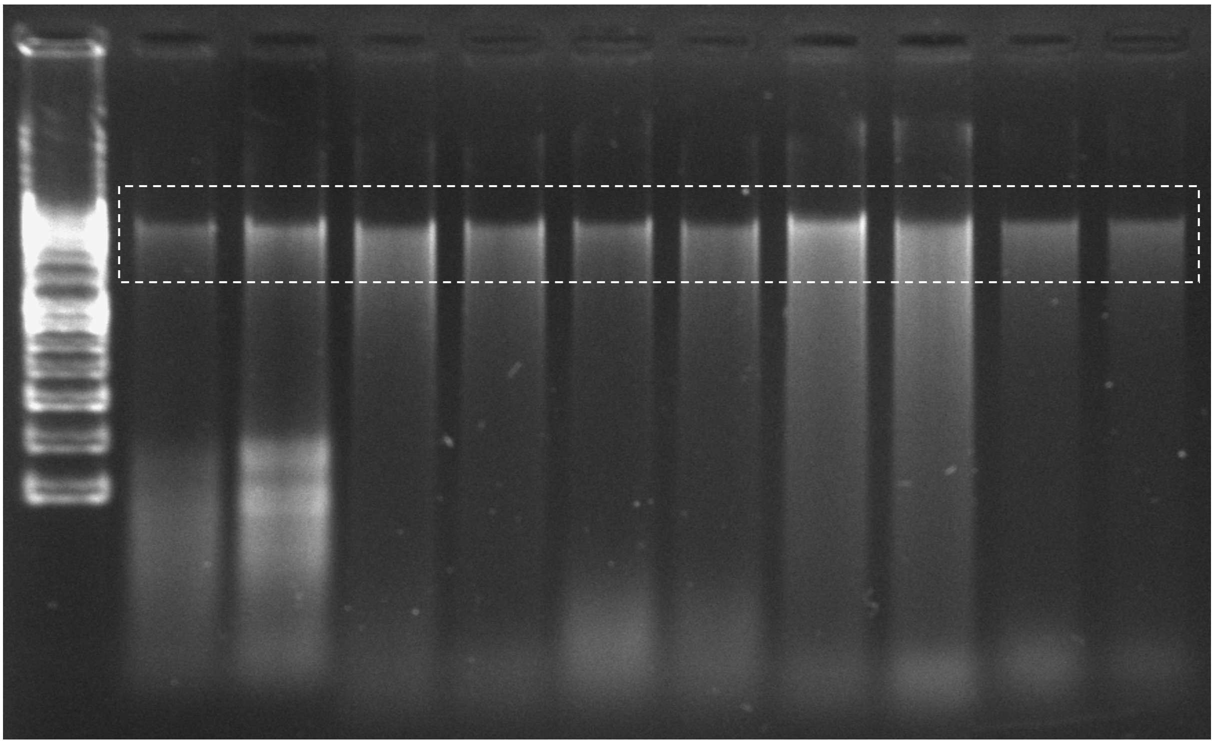


**WP6-6. Photo of agarose gel representing genomic DNA of yeast and fungi compared with Supercoiled ladder**

**A. Yeast**



**B. Fungi**



Genomic DNA

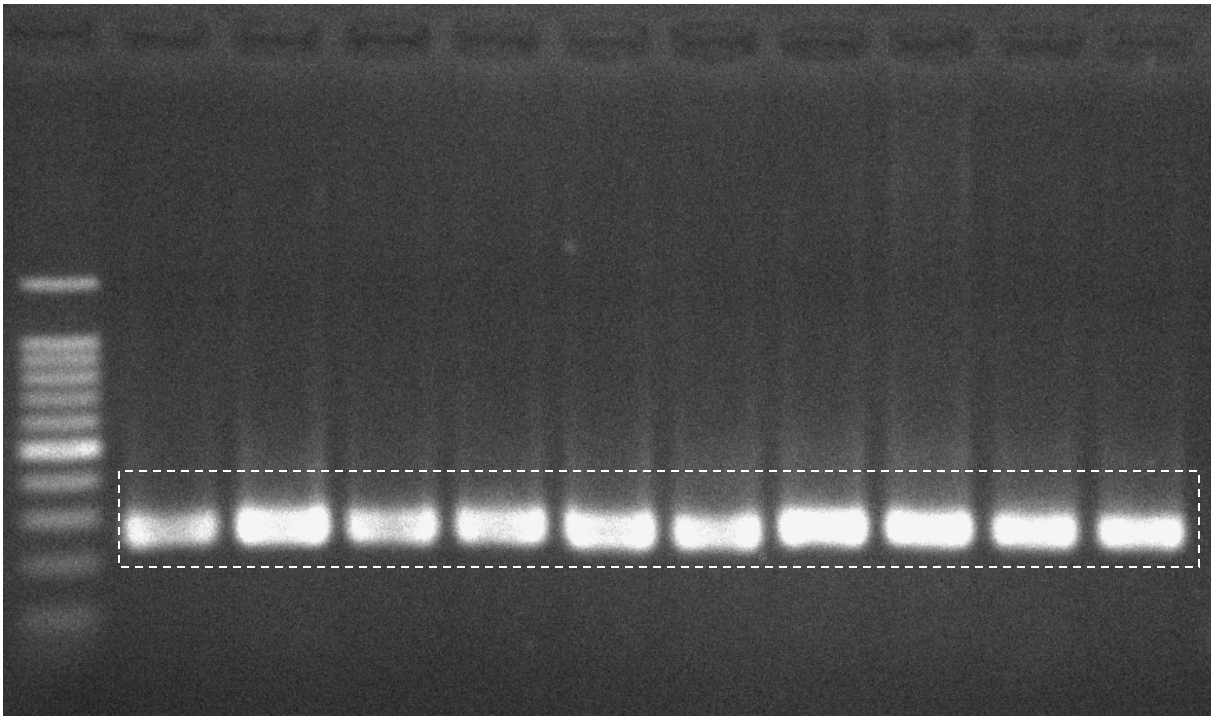
PCR of yeast and fungi universal fragments:

*For Yeast*, A fragment of the D1/D2 region of the 26S rRNA gene was amplified using eukaryotic universal primers NL1GC (5`-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCC ATA TCA ATA AGC GGA GGA AAA G-3`, Sigma, France) and the a reverse primer LS2 (5`-ATT CCC AAA CAA CTC GAC TC-3`, Sigma, France) amplifying an approximately 250 bp fragment (Kurtzann and Robnett, 1998; Cocolin *et al.*, 2000). A 30-bp GC-clamp (Sigma, France) was added to the forward primer (the GC-clamp is underlined) in order to insure that the fragment of DNA will remain partially double-stranded and that the region screened is in the lowest melting domain (Sheffield, Beck, Stone & Myers, 1989).

*For Fungi*, A fragment of region of the 28S rRNA gene was amplified using eukaryotic universal primers U1GC (5`-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GTG AAA TTG TTG AAA GGG AA-3`, Sigma, France) and a reverse primer U2 (5`- GAC TCC TTG GTC CGTGTT -3`, Sigma, France) amplifying an approximately 260 bp fragment (Sandhu *et al.*, 1995). A 30-bp GC-clamp (Sigma, France) was added to the forward primer (the GC-clamp is underlined) in order to insure that the fragment of DNA will remain partially double-stranded and that the region screened is in the lowest melting domain (Sheffield, Beck, Stone & Myers, 1989). The PCR products were checked on agarose gel (2%) comparing with 100 bp ladder (Fig. 7).

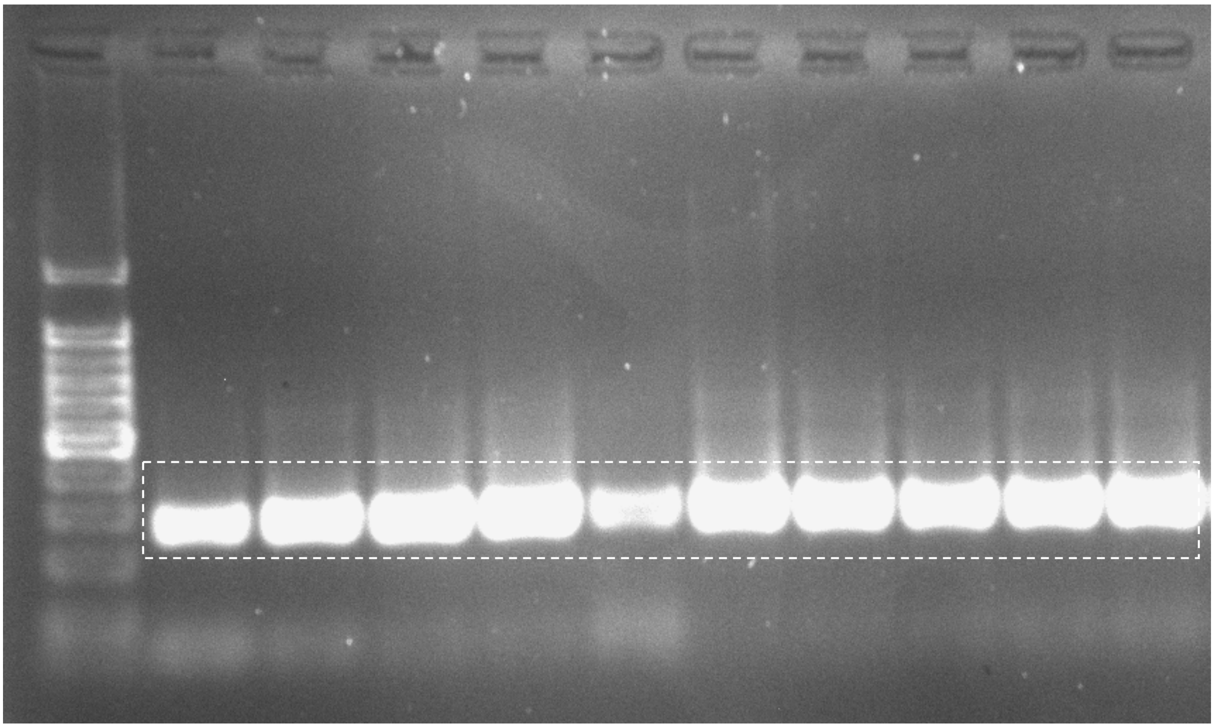
**Fig. WP6-7. Photo of agarose gel for verification of PCR reaction of yeast and moulds DNA**

**A. Yeast**



250 bp fragment

**B. Fungi**





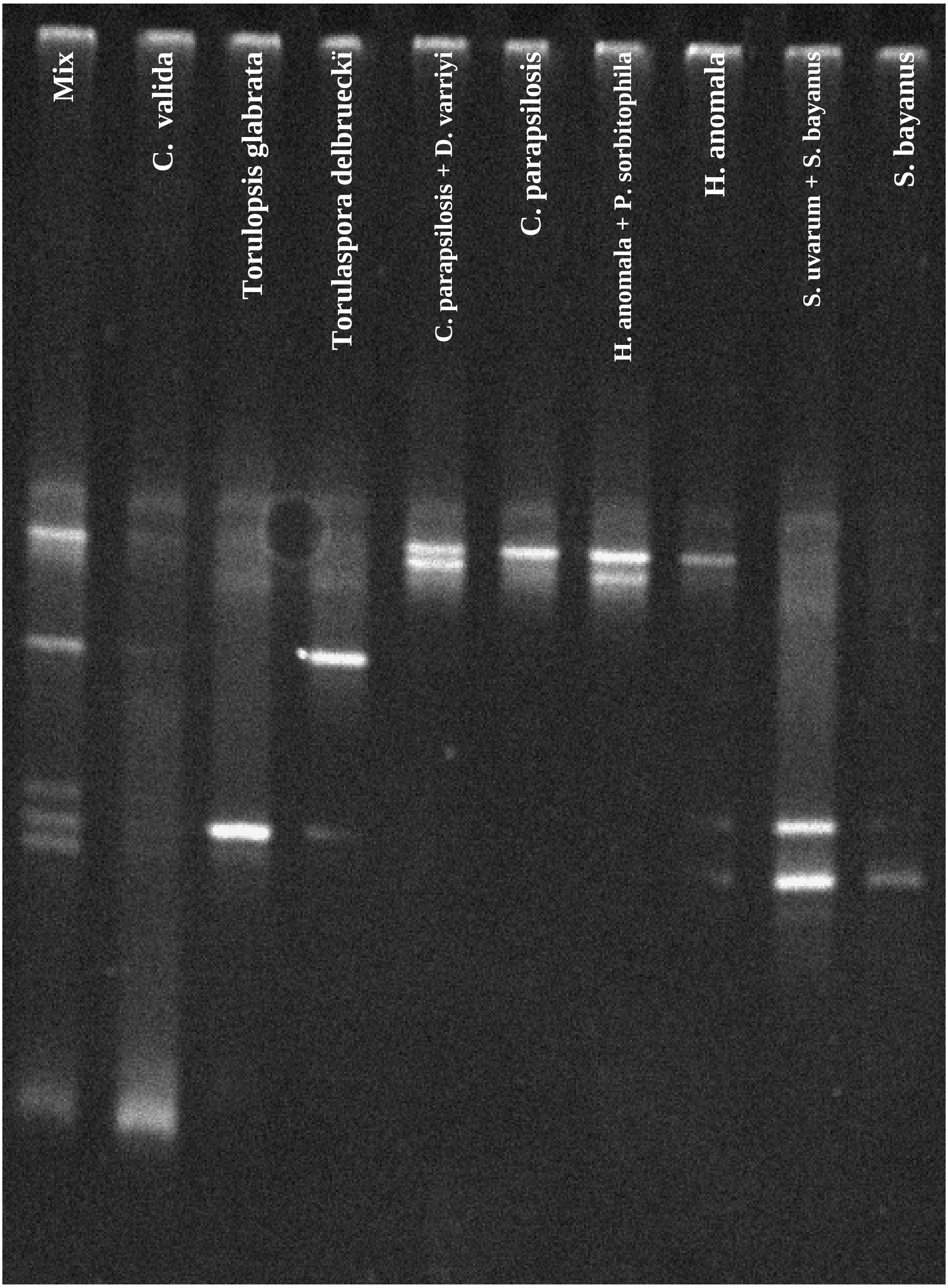
<u>Denaturing Gradient Gel Electrophoresis (DGGE) analysis:</u>

*For Yeast*, The PCR products were analyzed by Denaturing Gradient Gel Electrophoresis (DGGE) by using a Bio-Rad DcodeTM universal mutation detection system (Bio-Rad Laboratories, Hercules, USA). All electrophoresis were performed at 60°C using a denaturing gradient ranging from 30% to 60% (100% corresponded to 7M urea and 40% [v/v] formamide, Promega, France). The gels were electrophoresed at 20 V for 10 min and then at 80 V for 8 h.

*For Fungi*, The PCR products were analyzed by DGGE by using a Bio-Rad DcodeTM universal mutation detection system. All electrophoresis were performed at 60°C using a denaturing gradient ranging from 40% to 70% (100% Corresponded to 7 M urea and 40% [v/v] formamide, Promega, France). The gels were electrophoresed at 20 V for 10 min and then at 80 V for 16 h. After electrophoresis, the gels were stained for 30 min with ethidium bromide and rinsed for 20 min in distilled water and then photographed on a UV transilluminator using black and white camera (Scion Company, USA) with the Gel Smart 7.3 system software (Clara Vision, Les Ulis, France) (Fig. 8).

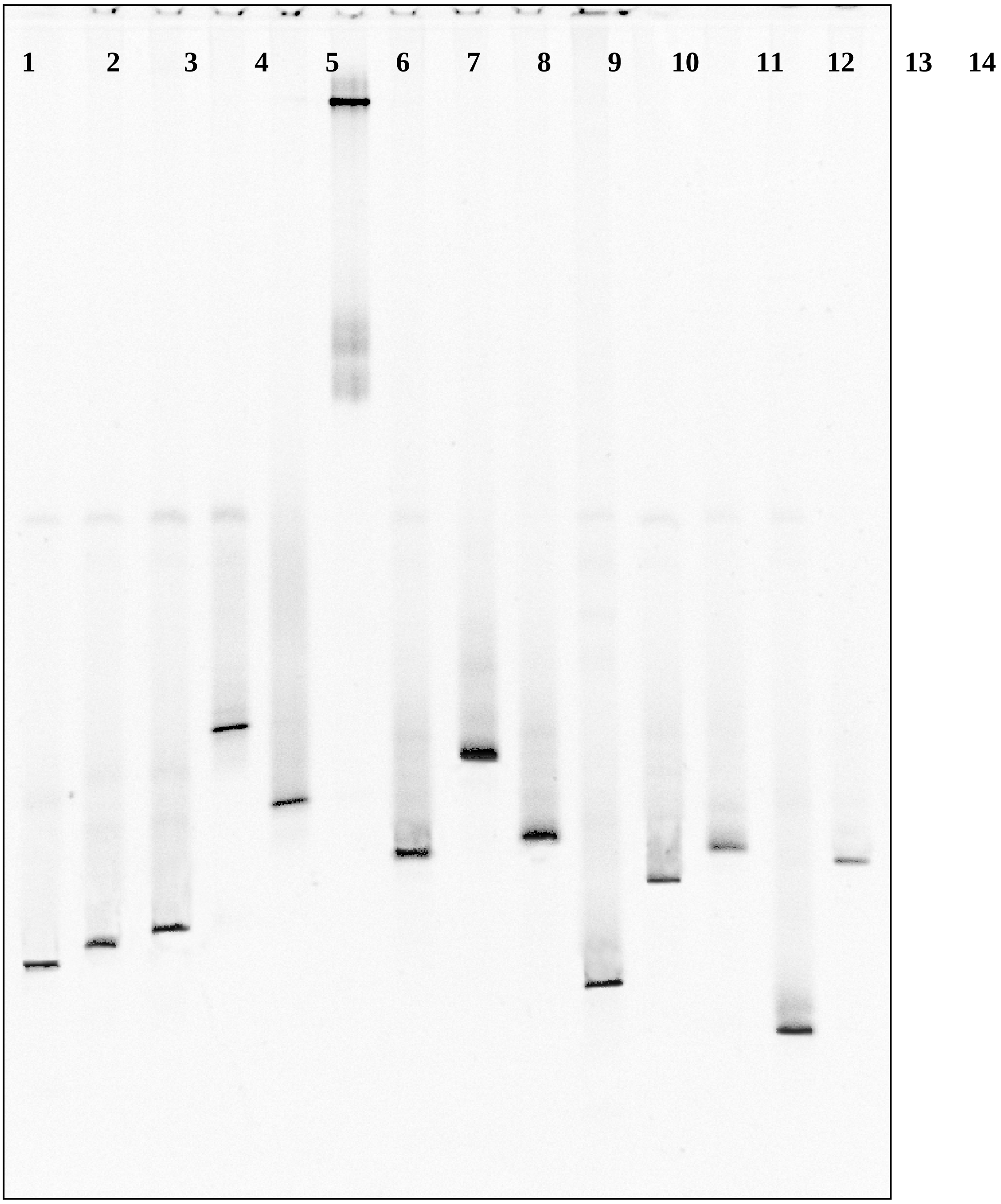
**Fig. WP6-8. PCR-DGGE band profiles of yeast and moulds**

**A. PCR-DGGE 26S rDNA band profiles of pure yeast strains**



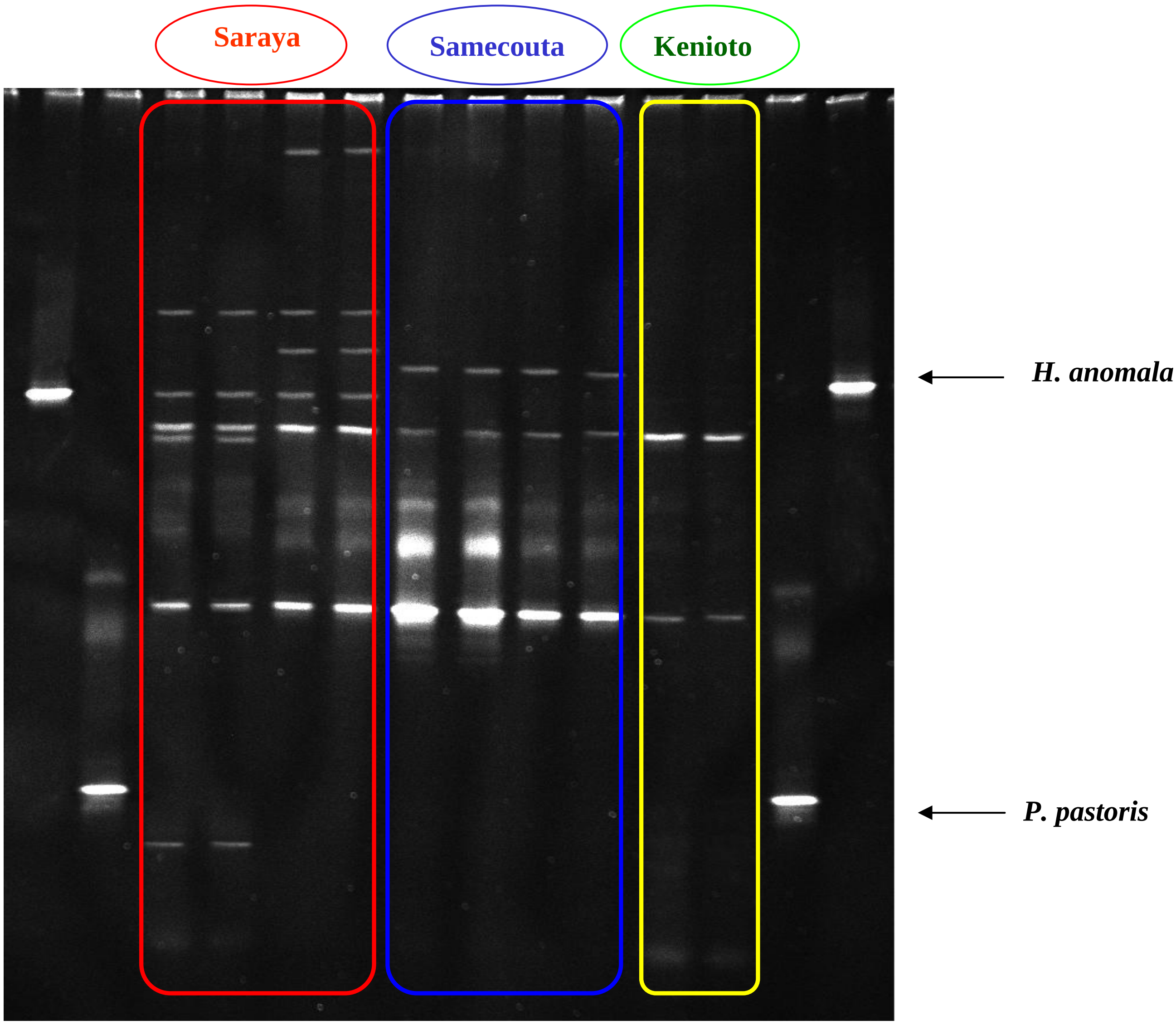
**B. PCR-DGGE 28S rDNA band profiles of pure fungi strains**

(1) *Penicillium roqueforti*. (2) *Cladosporium musae* CMR 55. (3) *Cladosporium musae* C62. (4) *Aspergillus tubigensis* CBS 161.79. (5) *Fusarium osyporum*. (6) *Mucor racemosus*. (7) *Aspergillus ochraceus*. (8) *Aspergillus carbonarius*. (9) *Aspergillus bombycis*. (10) *Aspergillus nomius* (11) *Aspergillus nomius* (12) *Aspergillus niger* CET 2091. (13) *Trichoderma harzianum* CBS 819. 68. (14) *Aspergillus parvisclerotigenus*.

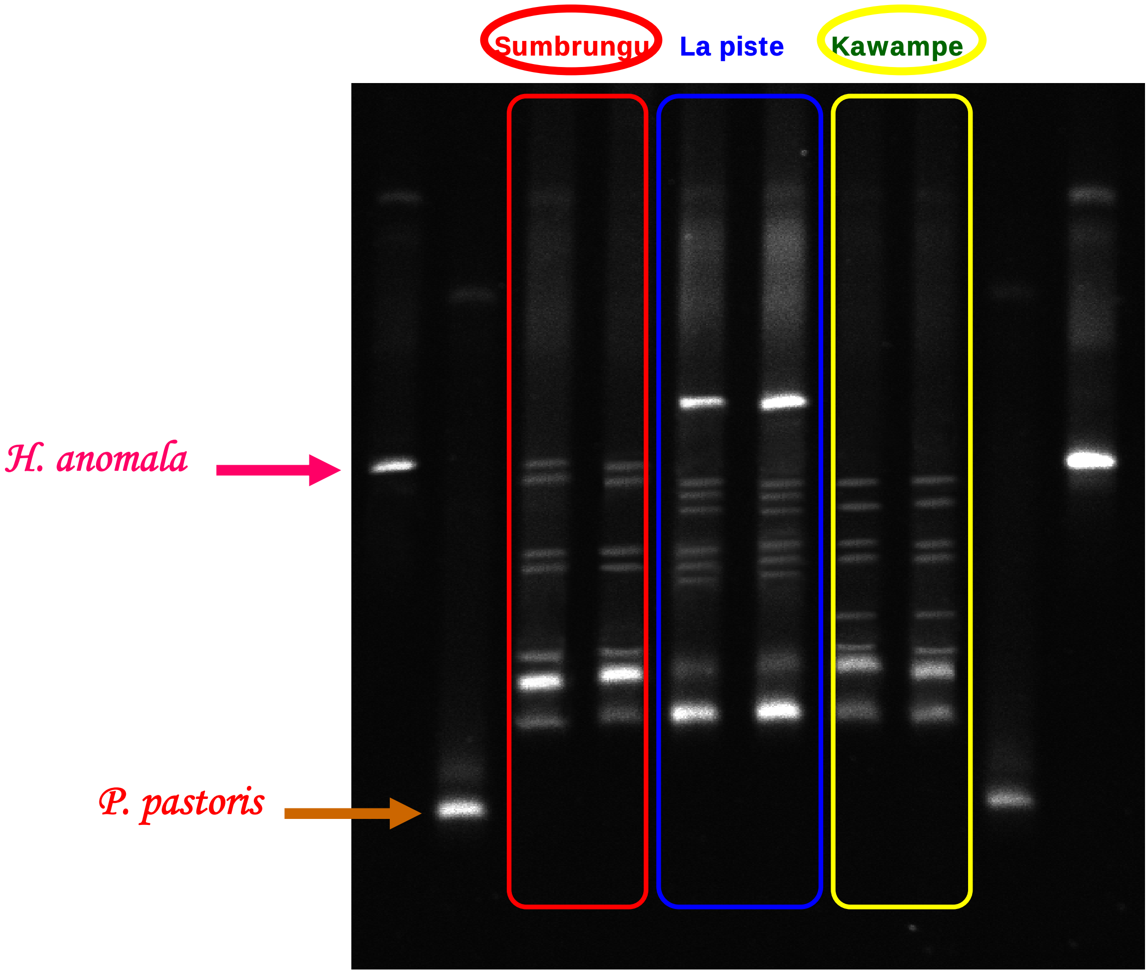



**Result - Validation the novel method of analysis of yeasts on Shea tree fruits**

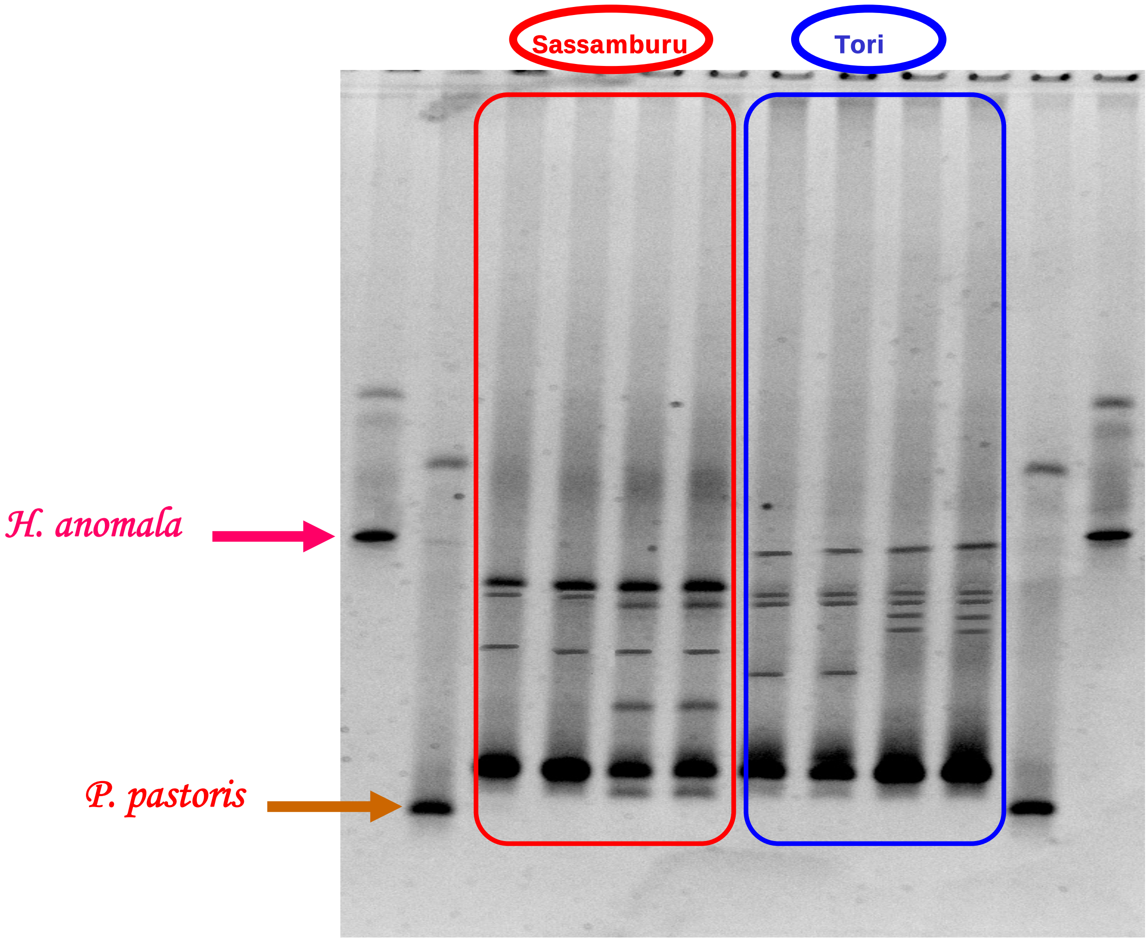
**(A) DGGE profile of yeast on Shea tree fruits of the 3 different areas of the Kédougou region in Senegal.**




**(B) DGGE profile of yeast on Shea tree fruits of the 3 different regions in Ghana.**

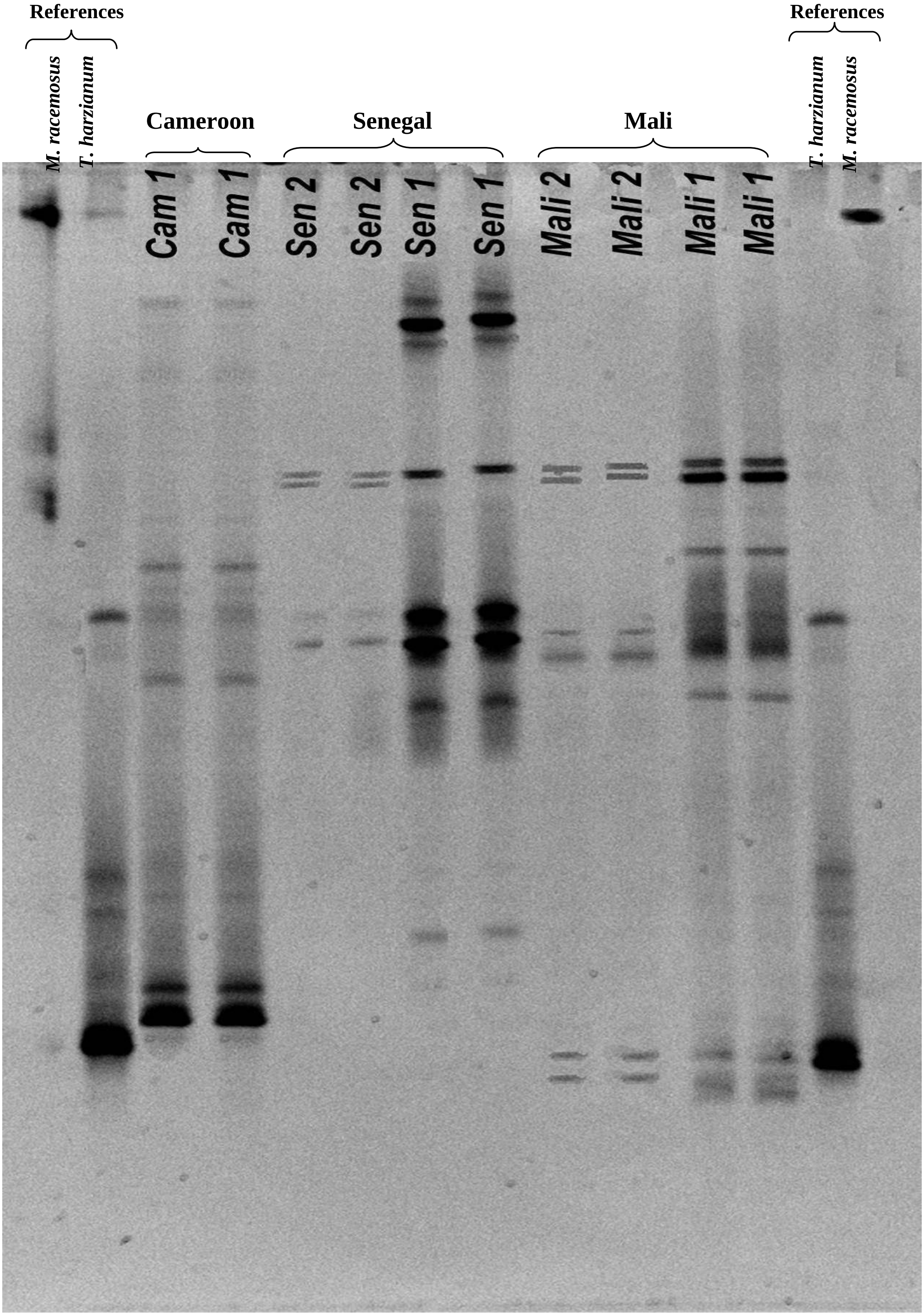



**(C) DGGE profile of yeast on Shea tree fruits of the 2 different regions in Mali.**




**6.3 *<u>In the 4th year of the project: 2010</u>***

**6.3.1. Validation the novel method of analysis of moulds on Shea tree fruits**




**Result – evaluation/conclusion related to the main results**

The PCR-DGGE approach has been profitably applied to study fungal communities that very often play an important role in food fermentations. Three papers were published by our team that described the linkage between bacterial, yeast and fungi communities and the geographical origin of fruits (Le Nguyen et al., 2008; Montet et al., 2008; El Sheikha et al., 2009, 2010; El Sheikha, 2010). But we think that we publish the first paper which introduces a unique 'biological bar code' of the Shea tree fruit using 28S rDNA fingerprinting of fungi. In our study, we proved that the DGGE pattern Cluster analysis of 28S rDNA band profiles of Shea tree fruit from three countries Mali, Senegal and Cameroon.

Fingerprint of fungal communities on Shea fruits of the DNA fungal communities from Shea tree fruit was strongly linked to the microbial environment of the fruit.
The analysis of Shea tree fruit samples from different locations showed some significant differences in the migration patterns on the DGGE gel. However, the duplicates for each sampling location gave statistically similar DGGE patterns throughout the study. The differences in the band profiles can be attributed to the differences in environment between districts. In the gel some common bands appeared
in all of the samples independently to the location. These bands could be common fungi for all of the Shea tree fruit samples. The presence of fungi on the fruit is the good reflection of the environment of the sampling areas thus they could serve as markers of the geographical origin of the fruit. These results can give an idea of the biodiversity of the fungi according to the geographical origin.
In fact, when comparing the different locations of fruits sampling with the statistical analysis of DGGE pattern throughout the study, we could noted that we obtained a complete statistical correspondence between the geographical areas and the fungal communities. We could conclude that there were enough environmental differences between the districts where the Shea tree fruits were harvested to obtain a major effect on the fungal ecology, whereupon we could create a statistical link between the fungi populations and the geographical area.
In conclusion, the analysis of Shea tree fruit fungi communities by PCR-DGGE could be applied to differentiate geographical locations. We showed that the biological markers for the specific locations were sufficient statistically to discriminate regions. This global technique is quicker (less than 24 h) than all of the classical microbial techniques and avoids the precise analysis of fungi by biochemistry or molecular biology (sequencing). This method can thus be proposed as a rapid analytical traceability tool for fruits and could be considered as a provider of a unique biological bar
code for each country.

#### 1.3.6.3 WP6 - impact of the project on its industry or research sector

WP6 developed analytical tools that permit to trace shea tree nuts all over the world. This tool could be useful in case of geographical origin confusion and origin mistake conducting to economical disagreements. These cases are rare but the quality and the fusion point of shea tree oil being very different from a region to another, and taking in account the fact that shea tree butter could replace a part of cocoa butter in the industry of chocolate (UE regulation 5%), this tool could have an importance in the future. At this level of research, the molecular analytical is ready to discriminate geographical origin of shea tree production.
Some other analytical techniques of traceability were also developed concerning micro lipid components analyzed by classical methods but also by Near infrared spectroscopy (NIRS). This usual tool of food industry was used as a global analysis and not as usually as molecule determination. NIRS permitted also to discriminate geographical origin simply by comparing full infrared spectra.

This project allows our team to propose two new analytical tools for food traceability whereas only one exists on the market (isotope analysis of wine).

## 1.3.7- Work package 7: – *Evaluating the suitability of different shea butters as raw material sources for cosmetic and pharmaceutical applications*

Shea butter unsaponifiables and extractables constitute important sources of raw materials for skin care actives, cosmeceutical actives, dietary supplements and pharmaceutical actives. Such raw materials can be based on triterpenoids such as lupeol, amyrins and butyrospermol and their esters with cinnamic or fatty acids. Other important constituents from derived shea products are based on water soluble polyphenolic components such as catechins. Concentrations of these constituents may vary with the geographical origin of the shea butter but also with the extraction method used to obtain the shea butter. The suitability of the shea butter to serve as a raw material for the subsequent production of high value added ingredients will be investigated in this work-package using previously developed and validated methods.

**General objective**

Evaluate the suitability of shea butters and other derived materials from different locations and with different processing pre-histories to serve as raw materials for the development of high value added ingredients for skin care and cosmeceutical uses.

**Specific objectives**

- Determination of quality parameters separating shea butters and derived materials for that are suited/non-suited for further processing into skin care and cosmeceutical actives
- Classification of shea butters and derived materials from different locations in terms of their suitability to serve as raw materials for subsequent processing
- Development of prototype skin care/cosmeceutical products from selected shea butters from different locations and processing histories

### 1.3.7.1 WP7 - Methodologies and approaches employed

The methodology was implemented through three different tasks.

*Task 7.1 : Determination of relevant quality parameters:* Samples were selected for fractionation experiments using previously developed standard conditions to evaluate the suitability to produce high value added ingredients for skin care/cosmeceutical use.

The fractions obtained were evaluated for their physicochemical properties (solid fat content, crystallisation behaviour, compatibility with other ingredients, rheology etc) and their unsaponifiable content and the composition of triterpenes and other actives.

*Task 7.2 : Comparison of the properties of shea butters from two varieties:* The screening methods were used to select samples from the two main varieties for standard fractionation experiments. The ingredients obtained by fractionation were characterised and evaluated with respect to their attractiveness for applications in skin care/cosmeceuticals by a comparison of the triterpene content and composition as well as the physicochemical properties as described above (7.1). Selected ingredients can also be evaluated for their moisturising properties in vivo or anti-inflammatory properties in vitro using skin models.

*Task 7.3 :  Development of new product prototypes:* The best candidates from 7.2 were tested for their functionality in skin care/cosmeceutical applications. Anhydrous and emulsified formulations were characterised in terms of stability and attractiveness for end-users using a combination of instrumental techniques such as rheology and sensory analysis.

### 1.3.7.2 WP7 - Achievements of the project to the state-of-the-art.

A preliminary task consisted in extraction of powder seed shea with different solvants. The objectives were a standardization of extraction. For this, the Soxhlet method extraction was used. It allows a continuous extraction of a powder. Several parameters were checked to obtain the best extraction method; temperature, extraction time and solvent: hexane, dichloromethane, methanol and purified water.

A physicochemical part which includes, two tasks
(i) Analytical identification of compounds in the extracts (n-hexane extracts-shea butter, dichloromethane, methanol and water): dichloromethane and n-hexane extracts were analyzed by GC MS to determine the nature and relative percentage of each component and compare these samples. The analysis of the phenolic constituents of methanol and water fractions using high-performance liquid chromatography (HPLC) connected in series with two detection systems UV and Spectrometer of mass). The results obtained on hexane extracts shows important differences in the ratio of saturated and insaturated fatty acids esterifying the glycerol between Uganda and Mali shea butters.

(ii) Determination of relevant physical quality parameters of hexane extracts (rheology - DSC). The objective of the physical studies is to develop methodologies for hexane extracts of shea butter in order to discriminate shea butter from different geographical origins (Mali and Uganda). For a same extraction method, correlations are searched between physical responses and shea butters origins.

The physical methods realised are (i) Differential Scanning Calorimetry – DSC, chosen because melting behavior is one of the properties of fats that influences their functionality in many prepared food products (ii) rheological or texturometric analysis, retained because fat physical characteristics greatly influence the rheological properties and the stability of the products. Moreover, rheological properties of fats are important industrial impacts. The rheological behaviour of fats is mainly determined by the amount of fats crystals and the types of crystals present in the fat mixture. Texturometric analysis, more easy to realize than rheological measurements are also realised for a fast and coarse determination of the quality of shea butter.

The results of DSC show strong similarities between the different Mali butters while Uganda butter is slightly different. Finally, we note that, all the endotherms (melting peaks) of Uganda butter appear at slightly lower temperatures than these of Mali butter.

Rheological measurements realised with 3 different methodologies lead to identify relevant parameters for the screening of the shea butter. Uganda butter and Mali butters are easily differentiate by all the tests. For example, after modeling the flow curve of all shea butters, they shows a shear-thinning behaviour and the model parameters (structural parameter) could be correlated with the quantity of saturated or insaturated fatty acid on the glycerol. Therefore a correlation is found between physical parameters and components analysis.

A biological part which includes five tasks
(i) Study of cosmetic properties (i.e. absorption of UV radiation and moisturizing properties) : After the study of literature, the objectives were to identify the potential absorbance and calculate the Sun Protecting Factor of the 2 shea butters extracts obtained: Mali and Uganda Their photostability was evaluated. Then, they were compared with:
- a shea butter from Cognis (cetiol SB45)

- different fats (cited in reference 2) : sweet almond oil, coconut oil, paraffin
- a chemical sunscreen standard : P1

A standardized method issued from the cosmetic rules was used. In vitro SPF calculated from the spectral transmittance at UV wavelength from 290 to 400nm. The results show an absorbance of the Shea butter leading to SPF values of approximately 2.

The moisturizing properties of different shea butter extracts were measured by corneometry. This method allows evaluating the water content of the skin by measurement of the capacitance with an electrode. The results show that shea butter extracts present a good hydratation capacity comparing to mineral oil.

(ii) Study of skin care properties. The evaluation of the potential skin care properties of two shea butters in two in vivo rat models :

-Wound healing : healing bioactivity is evaluated after surgical calibrated induction and skin repair recording over time.

-Eschar healing : eschar production is induced by cycles of calibrated skin pressure producing ischemia followed by skin reperfusion.

Main results

- Rats treated with Uganda Shea butter have better healing than rats treated by Mali shea butter and rats from the control group in terms of speed.
- Eschar induction: after 15 days, all rats were healed but there was a higher rate of healing in the presence of shea butter. Both types of shea butter were identical.

(iii) Determination of anti-inflammatory activity in vitro of Shea Tree : Evaluation of the potential anti-inflammatory properties of two shea butters in an *in vitro* model: the murine cell line J774 stimulated by LPS/IFN  leading to the production of Nitric oxide. Uganda Shea butter dose-dependently reduced the production of nitric oxide by J774 activated cells.

(iv)Evaluation of the antioxidant capacity: Evaluation of the antioxidant capacity of shea phenolics compounds by measuring the radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl radical. Water extracts show a better efficiency to scavenge radicals than methanolic extracts and for each type of extract, mali’s extracts have a better activity than Uganda extract.

(v) Evaluation of antimicrobial properties. Evaluation of antimicrobial properties by disc diffusion tests carried out on Gram-positive (Staphylococcus aureus, Bacillus cereus and Listeria innocua), Gram-negative bacteria (Pseudomonas aeruginosa, Escherichia coli and Salmonella typhi) and fungi (Candida glabrata, Aspergillus ochraceus and Fusarium oxysporum). First assay shows a slight activity of methanolic extract on E. Coli and S. Typhimurium, this assay should be verified.

#### 1.3.7.3 WP7 - impact of the project on its industry or research sector

The results of the WP7 related to sources of raw materials for skin care actives, cosmetical actives, dietary supplements and pharmaceutical actives do not have for instant a direct impact on industry and research sector. They are just preliminary results that need to be confirmed by further experiments and research.

This work has allowed us to develop analytical methods for the determination of trace elements useful to differentiate the origin of the butter. In addition, we have demonstrated pharmacological effects of interest suitable to operate in cosmetics.

## 1.3.8- Work package 8 – *new post harvest processing techniques to improve quality of shea kernel and derived products*

Standardised methods and technologies, which are both socially and economically acceptable in rural locations, are the key to developing a production base that can keep both the buyer and supplier beneficially satisfied. In the shea industry, is expected that this can be achieved through improvements to the first few steps of post-harvest processing (accumulation, boiling and drying) by developing tools that save resources (time, labour, fuelwood and water) whilst increasing the consistency of "Quality @ Quantity". Although it has been proven through scientific research that there are major chemical effects on quality during these first few stages; cheap and appropriate technology is still lacking. Many of the methods that are currently favoured traditionally appear to have been developed to maximise extraction yields and efficiency of labour allocation, at the expense of quality, during a very demanding agricultural period. By linking modern researchers, materials and market demand it is anticipated that the adaptable nature of the African woman will be given the opportunity to adjust her traditions in a socially and economically beneficial manner. Additional and higher value products can be developed using premium quality shea butter and, together with research into traceability provided by links to production, it is hoped to offer additional methods for improving rural livelihoods.

General objective
To develop socially and economically acceptable 'post-harvest' tools for the production of traceable and consistent premium quality shea kernel.

Specific objectives
1 – To develop scaled-up designs appropriate for use by women's groups in the project area by testing methods for accumulation and boiling and testing different designs of dryers, de-huskers and boiling technology
3 – To identify potential markets and new products (that result from the results of this WP and other WPs).
4 – To assist with development of a 'rurally' appropriate traceability system (organic, fair-trade, quality or other certification).

### 1.3.8.1 WP8 - Methodologies and approaches employed

*Task 8.1: To conduct village-based research on the acceptability of new tools for post-harvest improvements*
Selected village-based women's groups will be interviewed to discuss and query current findings on the effects of traditional processing and potential options for making improvements to some of the key stages of post-harvest shea processing. The focus will be on identifying solutions that are both socially and economically acceptable, e.g. [current ideas only] donkey-carts for daily harvesting; metal cages for timed boiling of fresh nuts; tunnel-driers; smoke-less driers and 'geared-tumbling' barrels for fast sheanut de-husking, two sites from Central-North Ghana and Northern Uganda.

*Task 8.2: To conduct trials on optimal methods for accumulation and boiling of shea nuts*
Based on discussions from task 8.1 and from results obtained on previous research, field trials will be conducted to determine the effect of: 1) 'Time-from-harvest-to-boil', 2) 'Time-to-boil', on certain key chemicals in butter extracted from the dried shea kernels of these experiments: Free Fatty Acid (FFA), Peroxide Value (PV) and Unsaponifiables (Unsaps) in

order to determine the optimal recommended timing. Additional trials of small-scale models will be conducted at this stage to determine the most appropriate and acceptable technologies for these tasks. Develop database on results and trial designs for further testing.

*Task 8.3: To construct and trial different designs of dryers, de-huskers and boiling technology*
Designs and methods from the previous two tasks will be built into scaled-up working models for village based testing during the peak period of the sheanut season. Wherever possible local materials will be utilised to ensure cost-effective and sustainable designs for future use. To ensure cost effectiveness from a project point of view, all designs will be based on the successes and failures of similar projects. Using optimal accumulation/boiling methods determined from 'best chemical' results from task 8.2. a time and motion study will be carried out to determine the best ergonomic and economic designs for use by rural women shea processors, i.e. least time taken to produce highest quality shea kernel at lowest cost. The results from this task will be evaluated and findings used in connection with WP10.

*Task 8.4: To identify potential markets and new products*
Samples produced from improved post-harvest processing methods developed in Task 8.3 and 8.4, and also using these methods on kernels from 'superior varieties' identified from other WP3 and WP4 will be selected for development into new products, i.e. shea butter with less odour, more consistent levels of FFA/PV, longer shelf-life, high levels of anti-oxidants, lower latex levels, etc. Key companies potentially able to develop, utilise and market this raw material into products in connection with WP7 will be sent samples from these trials and encouraged to partake in product development discussions.

*Task 8.5: To assist with development of a 'traceability system'*
Through links with WP6 (traceability), WP7 (new products) and WP9 (trade and market patterns) research will be conducted into how monitoring systems could utilise the improved processing methodology for increasing traceability and certification options. Key parameters to be tested will include evaluating the ability of rural processors to use of forms that maximises the use of pictures, symbols, maps, etc, but can still completed to demonstrate traceability from farm, improved processing and the use of fair-trade practices. Options will also be explored on whether shea butter field-testing kits can be developed to confirm quality at source and whether their results can be comparable to laboratory results. Discussions will be held with commercial certifiers to explain the opportunities these new post-harvest processing methods and traceability systems can offer, and identify whether there are any options for the addition of premiums to certified products (samples and evidence will be sent as required).

### 1.3.8.2 WP8 - Achievements of the project to the state-of-the-art.

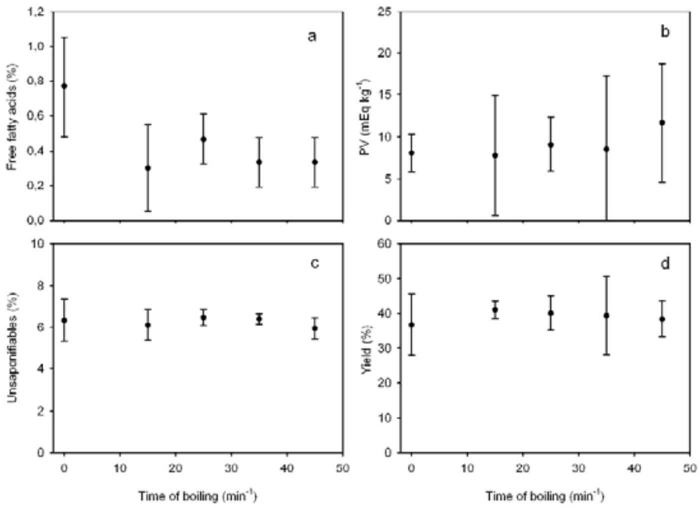
As a result of this project and activities under WP8, we now have a much clearer understanding of the impact of post-harvest processing of sheanuts on the quality of kernels and traditionally extracted shea butter.

- Increased time between fruit maturation and heat treatment is linked to lower quality, e.g. higher free fatty acid levels due to germination or kernel decomposition

- Equipment designs – that assist rural women to quickly immerse fresh sheanuts in boiling water and to remove them after a timed period – are possible to fabricate locally

Picture.1 & 2: Traditional boiling, versus boiling with research equipment

- Free fatty acid levels in traditionally extracted shea butter were recorded to decrease rapidly after immersing fresh sheanuts in boiling water for at least 15 minutes
- Increasing peroxide levels were recorded in traditionally extracted shea butter after boiling fresh sheanuts for 45 minutes or more
- The time taken to boil fresh sheanuts has little effect on unsaponifiable content
- Highest traditional extraction yields were recorded after boiling between 20-50 minutes




- Sheanut collecting communities have been demonstrated to quickly adopt innovations that improve their incomes, e.g. traceability (leading to premium payments with organic or fairly traded certification), optimising kernel quality (butter yield increased from 27% to 37% by weight, over a five-year period) and protection of local biodiversity (Hippo and bird levels in community sanctuary have remained constant or increased) led to increased local benefits
- Data was also collected for life cycle analysis of the shea butter's carbon footprint

### 1.3.8.3 WP8 - impact of the project on its industry or research sector

Results from INNOVKAR have been presented at many international conferences including Shea 2009 in Ouagadougou, Shea 2010 in Bamako, Shea 2011 in Accra and Shea 2012 in Cotonou. The *Global Shea Alliance* (formed in 2011 and now with ~350 members, represents all stakeholder groups in the shea sector, www.globalshea.com); has utilised the knowledge from the INNOVKAR project to develop recommendations for best practices on sheanut collection. In addition a working group has been formed (Shea 2013 in Abuja) to develop industry quality standards – to include premiums and penalties – that are being mainly based


on this shea kernel quality research.
Quality improvements, traceability and successful community conservation have also been proven in commercial projects which are now setting the bar in terms of how to do sustainable shea business with local African communities. The Savannah Fruits Company's *Hippo Shea* model was publically presented at The Union for Ethical Biotrade's annual conference (5th edition of its "Beauty of Sourcing with Respect", April 19th, Paris, http://www.ethicalbiotrade.org/) and was widely praised.
Projects assessing impact firewood-use during post-harvest processing, and analysis of shea's

carbon footprint, would not have started without initial data generated during INNOVKAR WP8 fieldwork.

## 1.3.9 -Work package 9 – Markets and Trade Patterns

Adding value to and diversifying food requires that the participants of the value chain get linked into appropriate marketing networks. A value chain perspective will be used to identify various ways by which the value of shea exports can be increased, focusing on strategies such as providing upgraded (semi-processed) shea to European importers, high quality products of well determined origin and certified products for which consumers can be expected to pay a price premium.

The activities conducted in the WP 9 will also contribute to the implementation of two crucial aspects
Traceability: Through the study of the value chain of shea, it will be understood in detail, how origins and qualities of traded shea are effectively traced, so far. Which traits serve as reference points to distinguish various qualities and origins Market research in Europe will provide a detailed picture of current practices to trace origins of shea nuts/butter and the preparedness to introduce alternative techniques to do so.

Certification: Certification is a market-driven approach to honour and promote endeavours to improve the management of natural resources and to make it sustainable. The success of certification systems is crucially determined by the willingness of consumers to honour efforts of sustainable forest management. The research will give a detailed assessment of this willingness to pay for shea products and the numerous benefits associated with its production

**General objective**
To frame a trade and policy environment which can assist poor producers and poor countries to participate more effectively in the global shea economy by analyse the value chain of shea products.

**Specific objectives**
1 – To increase the value of shea tree exports by analysing the shea value chain (map the shea value chain
product segments and critical success factors...)
2 – To improve the access to final markets by small producers
3 – To improve the fit of shea based enterprises into the international value chain

### 1.3.9.1 WP9 - Methodologies and approaches employed

In the following, the methodologies and approaches employed during work on WP 9 are described according to the WP tasks:

Task 9.1: To establish a point of entry for the shea value chain analysis
Value chains are complex and particularly in the middle tiers. Individual middlemen may feed into a variety of chains. The value chains were defined, delimited and entry points for its analysis determined. Setting the point of entry determined which links and activities in the chain were subject of special enquiry. In all cases individual producers/communities served as "entry points".

Task 9.2: To map the shea value chain
Starting from the selected entry points, the value chains were mapped forwards to local

processors, middlemen, regional wholesalers, international buyers and the European oil and fat industry. Along the various stages of the value chain, the physical and qualitative flow of shea nuts/butter, the flow of services and skills, gender ethnicity, destination of sales (wholesalers/retailers) by region (domestic/international) were determined.

Task 9.3: To determine product segments and critical success factors
A distinctive feature of the shea value chain is that it is "buyer-driven" or "market-pulled" as opposed to market demand. As competitive pressure among producers is high and increasing, shea markets are increasingly demanding. However, producers are often unaware of the exact requirements that determine high/premium quality of their product on the international markets. Through interviews with key respondents representing European buyers, the exact requirements and quality characteristics wanted on the international commodity markets were assessed. This helped to determine the Critical Success Factors (CSF). While low price were one of them other factors like quality, differentiation and origin played an important role.

Task 9.4: To explore how small producers currently access final markets
Knowledge of the ways in which disparate producers are connected into different final markets is crucial to understand the nature and functioning of any particular value chain. The following steps helped to produce a concise picture of the access patterns of small producers and processing unit to the global shea market:

- identification of key buyers
- dynamics of the buying function
- charting the CSFs with each of these buyers
- acquiring strategic judgements of various buyers regarding specific sources of supply
- analysis of present supply-chain-management patterns and potentials for their upgrade

Task 9.5: To analyse Governance issues along the value chain of shea
The power relations governing the international shea chain and the institutions that mould and wield this power are distinctive and crucial features. The following governance issues were analysed:

- national and international regimes of rule-making and rule –keeping that are in place
- types of rules and codes (product and process rules, international codes, company standards)
- rule making processes affecting the shea value chain (past and imminent) and the reach of rulemakers (for which segments of the chain do the rules apply)
- positive and negative sanctions used to enforce these rules

Task 9.6: To identify opportunities to upgrade the shea value chain
To come to a proper benchmarking in the shea value chain both practices and performances of the value chain were documented. The potentials for improving the process efficiency and the improvement of existing products/introduction of new products were assessed. A number of blockers (inhibitive factors) and enablers (supportive factors) were in place; they were identified and described. These enablers/blockers were both inside the individual stage of the value chain (habits, traditions, beliefs, financial limitations, time constraints) and beyond of them (policies, trade practices, legislations).

Task 9.7: To clarify how shea based enterprises fit into the international value chain
The outcome of tasks 9.1 to 9.6 helped to address the question: how can small producers and smaller business units be integrated in the international shea value chain, thus contributing to the sustainable use of shea parklands of sub-Saharan Africa? To answer this question

especially results from the governance and value chain upgrading analysis were considered, i.e.:

- the role of negotiating power in the value-chains,
- degree of association and formalization of small scale producers and processors,
- possibilities of certification to improve small scale actors` market access and participation in international value chains,
- possibilities of new market opportunities to improve small scale actors` value chain participation and new income generation options (i.e. within the context of global warming, CO2 foot printing of industry products)

### 1.3.9.2 WP9 - Achievements of the project to the state-of-the-art.

The following table presents the deliverable and expected results of WP 9 and the state of achievements.
The first year's work on WP 9 was largely confined to the identification of the basic value chain structures, the stakeholders involved and the (formalised and non-formalised) relations between them.
In the second year of the project the research focus switched from the local / country level to the international perspective. The Shea value chain was analysed through an interview campaign aiming at international traders, processors and consumers of Shea raw material sand processed products.
The two major aims of this research approach were:

- to identify critical success factors for actors (producers, traders, processors and consumers) within the value chain; and
- to produce an inventory of international buyers and processors of Shea products, indicating their perceptions of the Shea value chain.

In the course of this campaign, more than 250 companies have been identified and directly contacted of whom about 50 gave answers to an online questionnaire and to personally led guideline interviews by phone.
The main products (as provided as deliverables for year two) are a database of international Shea buyers and processors and crucial information of success factors of actors within the Shea value chains.

The third period aimed at:

- Analysing governance issues along the value chain of Shea (Task 9.5). This was realised as case study in Ghana and will be continued with a second case study in the fourth period in Senegal or Mali.
- Identifying opportunities to upgrade the Shea value chain. (Task 9.6). The emphasis in the third period was put on analysing carbon finance opportunities for Shea management and marketing.

By the end of year 3, work on Task 9.5 included intensive desktop and literature research on governance in international commodity value chains. The fourth period continued work on governance research along the international Shea value chains and work on identifying upgrading opportunities for the respective value chains. Hence, in 2010:

- Governance research on the Ghana Shea value chain was completed.
- Governance research on the Mali Shea value chain was completed.
- Identification of upgrading opportunities continued and was completed.

In the final project year a synthesis of WP deliverables was prepared. The synthesis comprises a comprehensive report and forms report of the final project report. The report will be completed by mid January 2012.
In the following the summaries of the two case studies conducted in 2010 are presented. Both studies have been submitted in full length to the project coordinator.

**Summary of WP 9 Research Activities**

**<u>Summary of Ghana case study results</u>**

The case study of governance and upgrading the Shea value chain in Ghana tries to identify patterns of incorporation of African actors into a global network of production. Methods of analysis include qualitative participant observations and semi-structured expert interviews with actors or groups of actors at the upstream end of the Shea value chain.
Shea often plays a significant role in both diet and subsistence income generation of poor actors in Ghana and Africa because it grows semi-wild and is readily available to most rural women to harvest. In contrast to alternative tree cash crops, Shea collection, production and trade have relatively low barriers to entry. Because of different trade regimes, variation and unpredictability of species and output quantity, performance in domestic and export production and trade varies greatly between the countries of the Sub-Saharan Shea belt.
The governance of Shea trade in Ghana and other African producing countries was heavily influenced by the state until structural adjustment took place in many Sub-Sahara African countries. Today, state involvement is either still or once again an important factor of governance in the industry. However, most markets have undergone liberalization and have experienced concentration at downstream nodes of the value chain. Oligopolistic transnational oil processors and global manufacturers of food and cosmetic products have captured increasing shares of value. After economic liberalization, new forms of governance evolved in producing countries due to increased demand from global chocolate manufacturers, growing transaction costs as well as risk of supply failure. First, especially in networks of nut supply, vertical integration of trade in Africa became important to secure steady supply. Second, along the value chain, Shea transactions are increasingly done through social networks and along family or ethnic ties of often globally connected entrepreneurs. Third, proliferation of standards and certification of products are greatly shaping the cosmetic value chain. In this case transactions are governed from outside the value chain by nongovernmental organizations, and these organizations are becoming players of growing importance.
For actors at upstream nodes of the Shea value chain, different upgrading opportunities appear. Industrial processing is largely done outside Africa in European, South American or Asian countries. Therefore, production of value-added products for chocolate manufacturing, but also cosmetic markets would be of particular benefit to African actors. However, a prerequisite is that governments provide better financial and transport infrastructure. In order to enhance involvement of producer groups´ and shorten supply chains as well as to prevent purchases on speculations in the stock market, trade in West Africa should be regulated.
At the traditional manual collection and processing levels, organization and capacity building should be facilitated to enhance and upgrade livelihoods of rural women. In contrast to other commodities from Africa, Shea is unique in that it grows with a variety of species and concentrations in various areas. If quality and quantity constraints are overcome, different markets can be tackled. In particular, connections to niche markets of certified or specialized products for the exportation and the new invention of products for domestic markets are promising fields for upgrading.
In the further course of the research project, carbon finance opportunities will be assessed as

well as the options to increasingly involve in the markets for organic products in Europe and North America.

**Summary of Mali case study results**

Thinking about future threats and chances it should be kept in mind that, in the long term, markets for Shea products will probably be changing. The so far, very high importance of Shea butter on the National market is threatened by a possible decline as substitutes become more available and traditions (which also imply the utilization of Shea for traditional uses) might lose significance. Thus, in order to hold up this probable decline, it should be worked on diversifying and promoting the uses of Shea butter in the National market. One option could be the promotion and the development of the Cosmetic use of Shea which already lost its traditional importance in favor of “modern” western products. Therefore, improving products and searching access to customers within Mali as well as a joint marketing campaign from all Malian Cooperatives could be possible approaches.

Improving the image of Mali as a Shea producing country and therefore working on the quality of Malian nuts are necessary to enhance options for Malian actors and widen their opportunities to enter international value chains. Thus, information regarding the quality standards has to be spread to all actors within Mali. Furthermore, it seems crucial that Malian actors get a clear benefit for enhanced quality, for example through higher prices. Without incentives, demands for higher quality will not change customs and traditions.

Nevertheless, the international aim of increasing the Malian export potential by changing local conservation and production methods should also be questioned. Generally speaking, the boom of 2007/2008 was a beneficial time for most actors in the Shea sector selling into the export value chain. However, the National market had collapsed as a consequence of the high international demand for nuts. Consequently all the actors who are limited to the National Market Value Chain (especially Shea butter merchants and some of their middlemen) were knocked out by this boom as they were taken by surprise and had limited adjustment capacities. It seems very likely that a similar boom in the near future would again dry out the National Market Value Chain as all the Shea nuts will again be channeled into the Nut Export Value Chain. In case this situation prevails longer than in 2008, it remains unsure whether the National market will easily recover afterwards. More affordable substitutes might have replaced Shea butter in case of long lasting high prices for Shea. It thus has to be considered that any increased potential for export also carries a risk for the National market which provides employment and income to many Malians as well.

Furthermore, Shea butter is the cheapest available cooking oil for a big part of the poorest Malians. A price increase or non availability of Shea butter forces them to switch to more expensive substitutes.

In the past and current projects, the focus is very often laid on securing a sustainable and increased income for the very upstream end of the value chain, namely the producing women. However, when talking about small scale and vulnerable actors of the Shea value chain, the merchants and their middlemen need to be considered as well. Often the small scale merchants are specialized on a single product and appear to be extremely vulnerable to market dynamics as has been seen in 2007/2008. A further vertical integration of the value chain, linking exporters and/or other end consumers directly to the women by bypassing the intermediary step of the merchant/middleman, induces the loss of business for a whole range of small scale actors on the Malian market.

Finally, the different ways in which Shea butter and Shea side products are used in the rural areas as well as within the whole National market in Mali preserve a precious cultural heritage. When trying to increase the quantity of Shea exports, these features and the vulnerability of the National market should definitively be kept in mind.

### 1.3.9.3 WP9 - impact of the project on its industry or research sector

Results of WP 9 contribute to knowledge about and upgrading of Shea value chains as follows:

**Impact on small scale producers and processors**

- The research results provide knowledge on international buyers of Shea products, there regional distributions and their quality requirements to small producers and processors in Subsaharan Africa.
- A list of buyers with address and product portfolio is available and can be used by small scale producers and multipliers (such as NGOs).
- The governance and upgrading studies clearly indicate that organization, association and quality management positively influence market access and value chain participation of mall producers. This is documented through best practices and can be used by multipliers to promote such activities.

**Impact on the value chains as such**

The case studies have shown that upgrading of value chains only works if producers and buyers cooperate and generate win-win situations. Most promising approaches to achieve these aims are provided through certification schemes (organic, social, climate and ecological). Successful experiences are described in the studies and can be used by NGOs, associations and international buyers for replication, modification and transfer. The studies were disseminated to multipliers in the case study countries.

# 2. Dissemination and use

The Publishable results of the Final plan for using and disseminating the knowledge are listed for each work package in the tables hereafter.

# INNOVKAR

# LIST OF THE MAIN PUBLISHABLE RESULTS

## *Work package 1 – Shea tree parkland dynamics and production: characterisation of natural regeneration, prediction of recovery using modelling and quantification of fruit production*

| Publishable result title and reference (authors journal) | Type of publication (article, student report, booklet, | Deliverables concerned | Date of publication | Project Partners involved | Corresponding scientist |
|---|---|---|---|---|---|
| KABORE S.A., 2010 - Etude de la dynamique de régénération du karité (*Vitellaria paradoxa* C.F. GAERTN.) dans le terroir de Sobaka (zone sud soudanienne du Burkina Faso) en champs et en jachère. Mémoire de DEA option Biologie et Ecologie Végétales. Université de Ouagadougou, Burkina Faso, 74 p. | Student Thesis | D1.2: data base on growth, mortality in each site (12, 24, 36) | 2010 | CNRST | Brigitte Bastide |
| S.A. Kaboré, B. Bastide, S. Traore, and J.I. Boussim (2011). "Dynamique du karité dans les systemes agraires du Burkina Faso" (Submitted to: *Bois et Forêts*) | Paper in International Scientific Journal | D1.2: data base on growth, mortality in each site<br><br>D1.6 : manuscript of scientific papers | 2011 | CNRST | Brigitte Bastide |
| S.A. Kaboré, B. Bastide, and J.I. Boussim. (2011) Dynamique de régénération du karité (*Vitellaria paradoxa* C.F. GAERTN.) dans la zone sud soudanienne du Burkina Faso | Poster presented at the INNOVKAR Conference | D1.2: data base on growth, mortality in each site | 2011 | CNRST | Brigitte Bastide |
| S.A. Kaboré, B. Bastide, M. Poudyal, B.A. Kelly, and J.I. Boussim. "Dynamic of regeneration of shea tree (*Vitellaria paradoxa* C.F. Gaertn.) on fields and fallows in Sobaka (Burkina Faso, West Africa)" (**Manuscript First Draft Ready**) | Paper for International Scientific Journal | D1.2: data base on growth, mortality in each site<br><br>D1.6 : manuscript of scientific papers | 2012 | CNRST, IER, UoY | Brigitte Bastide |
| Nyarko, G., Mahunu, G.K., Chimsah, F.A., Yidana, J.A., Abubakari, A.-H., Abagale, F.K., Poudyal, M., and Quainoo, A. "Leaf and fruit characteristics of Shea (*Vitellaria paradoxa*) in Northern Ghana" (Draft Manuscript for Ghana Journal of Horticulture) | Article in Scientific Journal | D1.3: data base on phenology studies<br><br>D1.6 : | 2011 | UDS, UoY | George Nyarko |

| | | | | | |
|---|---|---|---|---|---|
| | | manuscript of scientific papers | | | |
| Chimsah, F.A., Nyarko, G., Yidana, J.A., Abubakari, A.-H., Abagale, F.K., Mahunu, G.K., and Quainoo, A. "Diversity of tree species in cultivated and fallow fields within Shea parklands of Ghana | Poster presented at the Final INNOVKAR Conference | | 2011 | UDS | Francis Chimsah |
| B.A. Kelly, M. Poudyal, J.-M. Bouvet. "Variation of fruits production of Shea trees (*Vitellaria paradoxa*) along the north - south gradient in Mali (West Africa)" (**Manuscript under preparation for African Journal of Plant Science**) | Paper in International Scientific Journal | D1.3: data base on phenology studies<br><br>D1.6 : manuscript of scientific papers | 2011 | IER, UoY, CIRAD | Bokary Kelly |
| B.A. Kelly, M. Poudyal, J.-M. Bouvet. "Variation of *Vitellaria paradoxa* phenophases along the north - south gradient in Mali (West Africa)" (**First Draft of Manuscript Ready for Agroforestry Systems journal**) | Paper in International Scientific Journal | D1.3: data base on phenology studies<br><br>D1.6 : manuscript of scientific papers | 2011 | IER, UoY, CIRAD | Bokary Kelly |
| Patrick Byakagaba, Gerald Eilu, John Bosco L. Okullo, Susan B. Tumwebaze, and Edward N. Mwavu (2011) "Population structure and regeneration status of *Vitellaria paradoxa* (C.F.Gaertn.) under different land management regimes in Uganda", *Agricultural Journal* 6(1): 14-22. | Paper in International Scientific Journal | D1.2: data base on growth, mortality in each site<br><br>D1.6 : manuscript of scientific papers | 2011 | UNIMAK | Patrick Byakagaba |
| Patrick Byakagaba, Gerald Eilu, John Bosco L. Okullo, Susan B. Tumwebaze, and Edward N. Mwavu. "Shea butter tree (*Vitellaria paradoxa*, Gaertn.) fruit yield under different environmental conditions in Uganda" (**Manuscript ready for African Journal of Ecology**) | Paper in International Scientific Journal | D1.3: data base on phenology studies<br><br>D1.6 : manuscript of scientific papers | 2011 | UNIMAK | Patrick Byakagaba |

## *Work package 2 – adaptation and resilience of shea tree facing climate change and drought using ecophysiological and modelling approaches*

| **Publishable result title and reference (authors journal)** | **Type of publication (article, student report, booklet,** | **Deliverables concerned** | **Date of publication** | **Project Partners involved** | **Corresponding scientist** |
|---|---|---|---|---|---|
| BAZIE P., JOURDAN C., BASTIDE B., BAYALA J., ZOMBRE G. and ROUPSARD O. - Linking leaf and root phenologies of *Vitellaria paradoxa* in an agroforestry parkland system in Sobaka, Burkina Faso. **Lead author: JOURDAN C**..**Targeted Journal: Annals of Botany–(first quarter of 2012)** | Paper in International Scientific Journal | | 2012 | DRFP/CNRST Cirad | Bastide B |
| ABUBAKARI A.H., NYARKO G., YIDANA J.A., MAHUNU G.K., ABAGALE F.K., QUAINOO A., CHIMSAH F. and AVORNYO V. Comparative studies of soil characteristics in three shea parklands of Ghana. First Draft ready. | Paper in International Scientific Journal | | 2012 | DRFP/CNRST Cirad | G Nyarko |
| BAYALA J. *et al.* – Morphological traits photosynthesis of seedlings of *Vitellaria paradoxa* under water stress in nursery in Burkina Faso. “Science for Better Shea” INNOVKAR conference, Ouagadougou, 24-26/10 2011. | Conference | | 2011 | DRFP/CNRST | J Bayala |
| BAZIE P., BAYALA J., JOURDAN C., ROUPSARD O., ZOMBRE G., BASTIDE B. - Linking leaf and root phenologies of *Vitellaria paradoxa* in an agroforestry parkland system in Sobaka, Burkina Faso. “Science for Better Shea” INNOVKAR conference, Ouagadougou, 24-26/10 2011. | Conference | | 2011 | DRFP/CNRST | B Bastide |
| PLATTS P.J., POUDYAL M. and Mc CLEAN C.J. – Shea distribution under climate change. “Science for Better Shea” INNOVKAR conference, Ouagadougou, 24-26/10 2011. | Conference | | 2011 | DRFP/CNRST | M Poudyal |
| SANON Z., 2009 – Fonctionnement physiologique du karité (Vitellaria paradoxa Gaernt. F. Hepper, Sapotaceae) sous différents régimes d’eau. Diplôme d’Ingénieur du Développement Rural. Option : Eaux et Forêts. Université Polytechnique de Bobo-Dioulasso, Burkina Faso. 62 p. | Student master report | | 2009 | DRFP/CNRST | B Bastide J bayala |
| BAZIE P., 2009 – La physiologie du karité (Vitellaria paradoxa Gaertn. F.) en fonction des modes de gestion de l’espace dans les écosystèmes à Sobaka. Mémoire de DEA Sciences Biologiques Appliquées. Option : Ecophysiologie. Université de Ouagadougou, Burkina Faso. 47 p. | Student master report | | 2009 | DRFP/CNRST | J Bayala |

## *Work Package 3: Potential of shea tree origins based on multitrait approach*

| **Publishable result title and reference (authors journal)** | **Type of publication (article, student report, booklet,** | **Deliverables concerned** | **Date of publication** | **Project Partners involved** | **Corresponding scientist** |
|---|---|---|---|---|---|
| Zénor Ablah Logossa, François Allal, Létizia Camus-Kulandaivelu, Alexandre Vaillant, Haby Sanou, Kouami Kokou, Jean-Marc Bouvet Molecular data reveal isolation by distance and past population expansion for the shea tree (Vitellaria paradoxa C.F. Gaertn) in West Africa. Molecular Ecology accepted | Article in international scientific journal | D3.4 : data base on molecular variation (month 30)<br>D3.6 : manuscript of scientific papers on genetic analysis (month 46) | 2011 | Cirad, IER | Jean-Marc Bouvet |
| Allal F., Sanou H., Millet L., Vaillant A., Camus-Kulandaivelu L., Logossa Z.A., Lefevre F. and Bouvet J.-M. 2011 Past climate changes explain the phylogeography of Vitellaria paradoxa over Africa. Heredity, 1-13. | Article in international scientific journal | D3.4 : data base on molecular variation (month 30)<br>D3.6 : manuscript of scientific papers on genetic analysis (month 46) | 2011 | Cirad, IER | Jean-Marc Bouvet |
| Allal F., Vaillant A., Sanou H., Kelly B.A., Bouvet J.M. 2008. Isolation and characterization of new microsatellite markers in shea tree (Vitellaria paradoxa C. F. Gaertn). Molecular ecology resources, 8 (4) : 822-824. | Article in international scientific journal | D3.4 : data base on molecular variation (month 30)<br>D3.6 : manuscript of scientific papers on genetic analysis (month 46) | 2008 | Cirad, IER | Jean-Marc Bouvet |
| Phylogéographie de Vitellaria paradoxa (CF Gaertn): mise au point et analyse comparative de différents types de marqueurs moléculaires.<br>Laurent Millet | Master of Science dissertation Master 2 Biodétection, Biotraçabilité, Biodiversité | D3.4 : data base on molecular variation (month 30) | 2008 | Cirad | Jean-Marc Bouvet |
| Variabilité des tocophérols et du gène VTE 2-2 de la voie de biosynthèse chez Vitellaria paradoxa. | Master 2 : Biologie, Géosciences, Agroressources et Environnement<br>Spécialité : Bio - Ingénieries | D3.4 : data base on molecular variation (month 30) | 2009 | Cirad | Jean-Marc Bouvet |

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| Structure de la diversité génétique et test d'association.<br>Anna Antolik | Parcours : Biotraçabilité, Biodétection, Biodiversité<br>Année Universitaire 2008-2009 | | | | |
| "Fatty acid and tocopherol patterns of variation within the natural range of the<br>shea tree (Vitellaria paradoxa)", submitted to Agroforestry Systems<br>François ALLAL, Georges PIOMBO, Bokary A. KELLY, John B. L. OKULLO, Massamba THIAM, Ousmane B. DIALLO, George NYARKO, Fabrice DAVRIEUX, Peter N. LOVETT and Jean-Marc BOUVET<br>Accepted Journal of agoforestry system | Article in international scientific journal | D3.4 : data base on molecular variation (month 30)<br>D3.6 : manuscript of scientific papers on genetic analysis (month 46) | 2012 | Cirad | Jean-Marc Bouvet |
| Davrieux F., Piombo G., Allal F., Bastianelli D., Bouvet J.M. 2011. Quality characterization of commercial Shea nuts using NIRS. In : *15th International Conference on Near-Infrared Spectroscopy (NIR 2011), 13-20 May 2011, Cape Town, South Africa*. s.l. : s.n., 1 p. International Conference on Near Infrared Spectroscopy. 15, 2011-05-13/2011-05-20, Cape Town, Afrique du Sud. | Article in international scientific journal | D3.6 : manuscript of scientific papers on genetic analysis (month 46) | | | |
| Bouvet J.M., Kelly B.A., Sanou H., Allal F. 2008. Comparison of marker- and pedigree-based methods for estimating heritability in an agroforestry population of *Vitellaria paradoxa* C.F. Gaertn. (shea tree). *Genetic resources and crop evolution*, 55 : 1291-1301. http://dx.doi.org/10.1007/s10722-008-9328-8 | Article in international scientific journal | D3.6 : manuscript of scientific papers on genetic analysis (month 46) | | | |
| F. Allal, L. Millet, V. Vaillant, F. Davrieux, G. Piombo and J.-M.Bouvet<br>Genetic patterns of Vitellaria paradoxa: SNP *vs* Chemical traits in relationship with environment characteristics<br>Poster IUFRO Conference 2009 2009 IUFRO Tree Biotechnology Conference, Whistler, BC, Canada. | Poster in International conference | | | | |
| Past climate change explain the phylogeography of Vitellaria paradoxa over Africa. F. Allal1, L. Millet1, A. Vaillant1, L. Camus-Kulandaivelu1, Z. Ablah Logossa2, H. Sanou3, F. Lefèvre4, J.-M. Bouvet1<br>SMBE 2010 - Annual Meeting of the Society for Molecular Biology and Evolution, Lyon, France. | Poster in International conference | | | | |

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| Some insights into shea tree (*Vitellaria paradoxa* C.F. Gaertn) history in West Africa<br>Zénor Ablah LOGOSSA[1], Haby Sanou[2], François Allal[3] Létizia Camus-Kulandaivelu, Alexandre Vaillant, Kouami Kokou, Jean-Marc Bouvet<br>SMBE 2010 - Annual Meeting of the Society for Molecular Biology and Evolution, Lyon, France. | Poster in International conference | | | | |
| Ph.D. thesis in population genetics – University of Montpellier II (Montpellier, France)<br>« Patterns of variability in *Vitellaria paradoxa* (Shea tree): phylogeographic study and combined analysis of the variation in fatty acids, tocopherols and candidate genes » | PhD thesis | | | | |

## *Work package 4 – domestication and Pre-breeding of shea tree : characterisation and mobilisation of local varieties by a multitrait and participative approach*

| **Publishable result title and reference (authors journal)** | **Type of publication (article, student report, booklet,** | **Deliverables concerned** | **Date of publication** | **Project Partners involved** | **Corresponding scientist** |
|---|---|---|---|---|---|
| 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 | Article in international scientific journal | D4.1. Results from the interview to identify varieties (month 14) D4.8: manuscript of scientific papers on local varieties identified by farmers (month 46) | 2011 | Makerere University | Sam Gwali |
| S Gwali, JBL Okullo, G Eilu, G Nakabonge, P Nyeko & P Vuzi (2011). Traditional management and conservation of shea trees (Vitellaria paradoxa subspecies nilotica) in Uganda. *Environment, Development and Sustainability*. DOI 10.1007/s10668-011-9329-1 | Article in international scientific journal | New deliverable not planned in the original document | 2011 | Makerere University | Sam Gwali |
| S Gwali, G Nakabonge, JBL Okullo, G Eilu, P Nyeko and P Vuzi. Morphological variation among shea tree (*Vitellaria paradoxa* ssp. *nilotica*) 'ethno-varieties' in Uganda. Submitted to *Genetic Resources and Crop Evolution*. | Article in international scientific journal | D4.2 : data base on morphological traits of each variety (month 24). D4.8: manuscript of scientific papers on local varieties identified by farmers (month 46) | Expected for 2012 | Makerere University | Sam Gwali |
| S Gwali, G Nakabonge, JBL Okullo, G Eilu, N Forestier-Chiron, G Piombo, F Davrieux. Fat content and fatty acid profiles of shea tree (Vitellaria paradoxa subspecies nilotica) ethno-varieties in Uganda | Article in international scientific journal | D4.3 :data base on chemical characteristics of each variety (month 30). D4.8: manuscript of scientific papers on local varieties identified by farmers (month 46) | Expected 2012 | Makerere University | Sam Gwali |
| Quainoo, A., Piombo, F., Davrieux G.: Determination of biochemical properties of Shea varieties from Ghana based | Article in international scientific journal | D4.3 :data base on chemical characteristics of | Expected 2012 | | Quainoo Albert |

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| on NIR. 1 draft manuscript. Target journal: Current topics in Biotechnology. To by submitted Dec 2012. | | each variety (month 30) D4.8: manuscript of scientific papers on local varieties identified by farmers (month 46) | | | |
| Sanou, Korbo. Farmers perception on shea: Selection criteria and folk classification (draft under preparation). Target journal: Agroforestry Systems. To be submitted on March 2012 | Article in international scientific journal | D4.1. Results from the interview to identify varieties (month 14) D4.8: manuscript of scientific papers on local varieties identified by farmers (month 46) | Expected 2012 | IER | Sanou Haby |
| Yossi, Sanou. Domestication of shea tree: Impact of fertilisation and weeding on the survival and growth in the nursery and after plantation. Target journal: Journal of Horticulture. To be submitted on April 2012 | Article in international scientific journal | D4.10 : Manuscript on guidelines for propagation of Karité (month 46) | Expected 2012 | IER | Sanou Haby |
| | | | | | |
| Sanou, Bouvet, Thiam, Gwali , Quainoo, Diallo, Davrieux, Bouvet, Kjær. The shea trees of Africa: unique trees identified by farmers analysed by objective quality parameter and genetic relationship. Suggested: Journal of biogeography | Article in international scientific journal | D4.8: manuscript of scientific papers on local varieties identified by farmers (month 46) | Expected 2012 | IER, CIRAD, ISRA, UDS, UNIMAK, FLD | Sanou Haby |
| Lompo, Kambou, Ouédraogo, Sinna, Kjær: Potential gains in growth rate, fruit set age, fruit production and fruit quality from using from selected and tested clones in Shea. Draft manuscript to be submitted to Agroforesty systems. Expected: jan 2012.systems. | Potential gains in growth rate, fruit set age, fruit production and fruit quality from using from selected and tested clones in Shea. | D4.7 manuscript of scientific papers on genetic and economical feasibility of breeding for different traits (month 46) | Expected 2012 | UCP | Erik D. Kjær |
| S Gwali (2008). Famine foods for uganda – domesticating the shea tree for food security. NaFORRI Newsletter Issue 1 July 2008. | National Newsletter | D4.9: Manuscript on guidelines for domestication and breeding of Karité (month 46) | 2008 | Makerere University | Sam Gwali |
| S Gwali (2011). Revealed: Folk classification and nomenclature of shea trees in Uganda? NaFORRI newsletter Vol.1 (1). June 2011 | National Newsletter | D4.8: manuscript of scientific papers on local varieties identified by farmers (month 46) | 2011 | Makerere University | Sam Gwali |

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| Tahiru Fatawu, Yidana**:** Management practices of grafted shea in Northern Ghana. | BsC report | D4.10 : Manuscript on guidelines for propagation of Karité (month 46) | 2010 | UDS | J.A.Yidana |
| Salifu Fuseini, Yidana: Effects naphthalene acetic acid on air layering of shea | BsC report | D4.10 : Manuscript on guidelines for propagation of Karité (month 46) | 2008 | UDS | J.A Yidana |
| Wuni David Dawuda, Yidana: Dissertation some management practices of grafted shea (*vitellaria paradoxa*) at zoolanyili in the tolon-kumbungu district | BsC report | D4.10 : Manuscript on guidelines for propagation of Karité (month 46) | 2010 | UDS | J.A.Yidana |
| Yahaya Aminu, Gustav Mahunu: Response of epicormic and regular shoots to rooting of shea by air-layering | BsC report | D4.10 : Manuscript on guidelines for propagation of Karité (month 46) | 2010 | UDS | Gustav Mahunu |
| Yahaya Dana Saka: Studies on natural and hand pollination of shea trees (Vitellaria paradoxa | BsC report | Not planned in the project | 2010 | UDS | J.AYidana |
| Anders S. Larsen and Erik Kjaer. Analysis of clonal trial | Protocol for analysis of clonal trial at Sapone | D4.7 manuscript of scientific papers on genetic and economical feasibility of breeding for different traits (month 46) | 2009 | UCP | Anders Søndergaard Larsen and Erik Dahl Kjær |
| Anders Søndergaard Larsen, Boukary Diallo, and Erik Dahl Kjær. Analysis of seed dispersal by parental analysis and simulation: the case of the West African Parkland species *Vitellaria paradoxa*. Manuscript submitted in Tree gene and Genomes 2011 | Article in International scientific journal | In cooperation with WP1 | 2011 | INERA, UCP | Anders Søndergaard Larsen, Boukary Diallo, and Erik Dahl Kjær |
| Lompo, Kambou, Oudraogo, Sinna, Kjær Estimation of growth rate, fruit set, heritabilities and age of first flowering from selected and tested clonal trial. Draft manuscript to be submitted to Agroforesty in January 2012 | Article in international scientific journal | D4.7 manuscript of scientific papers on genetic and economical feasibility of breeding for different traits (month 46) | 2011 | CNSF, UCP | Erik Kjaer |
| Farmers recognition of superior trees. Submitted to 'Africa focus' that reports observations from interviews with farmers in Northern Ghana | Article in international scientific journal | D4.7 manuscript of scientific papers on genetic and economical feasibility of breeding for different traits | 2011 | UPC | Jesper Vind Erik.D.Kjær |

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| Guidelines for domestication: Based on the input from the present meeting and - drawing on the results from all WPs - we will develop a technical guideline: *Domestication of Shea*. The target group will be relevant organisations potentially involved in domestication. | International Booklet (ICRAF) | D4.9: Manuscript on guidelines for domestication and breeding of Karité (month 46) | 2012 | UPC | Erik Kjaer |
| Ousmane Seck: Comparative studies of germination, survival, root development and growth of shea 25 progenies from three sites in Senegal.. To be transformed into publication with Thiam as co-author, Target journal: Agroforestry systems during August 2012. | BSc study (Forestry) in progress | D4.10 : Manuscript on guidelines for propagation of Karité (month 46 | 2012 | ISRA | Ousmane Seck, Massamba Thiam |
| Yidana. A guide to shea grafting (poster) | Poster | D4.10 : Manuscript on guidelines for propagation of Karité (month 46 | 2011 | UDS | Yidana |

## *Work package WP5: Chemical analysis using conventional and near infrared spectrometry (NIRS)*

| Publishable result title and reference (authors journal) | Type of publication (article, student report, booklet, | Deliverables concerned | Date of publication | Project Partners involved | Corresponding scientist |
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## *methods*

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| Near Infrared Spectroscopy for High-Throughput Characterization of Shea Tree (Vitellaria paradoxa) Nut Fat Profiles<br>Fabrice Davrieux,*,† Francois Allal,‡ Georges Piombo,§ Bokary Kelly,) John B. Okulo,^Massamba Thiam,# Ousmane B. Diallo,R And Jean-Marc Bouvet<br>J. Agric. Food Chem | Article | D5.6: data base Nirs and wet chemistry 2008 | 2010 | IER, MAKERERE INERA, ISRA | FABRICE DAVRIEUX |
| *Conference Euro fed lipid*<br>Shea nuts oil characterization using near infrared spectroscopy<br>Allal F.[1], Piombo G.[1], Villeneuve P.[1], Fores C.[1], Forestier N.[1], Kelly B.[2], Okulo J.B.[3], Bouvet J.M.[1], Davrieux F.[1] | Conference Euro fed lipid Muchen | D5.3: data base Nirs and wet chemistry of Fat contents, H2O,fatty acids profils 2007 | 2010 | IER, MAKERERE INERA, ISRA UDS | F.ALLAL |
| *Global Shea 2010*<br>Quality characterization of commercial Shea nuts using NIRS | Conference Bamako | D5.9: data base FFA 2010 Nirs and wet chemistry | 2010 | IER, MAKERERE INERA, ISRA UDS | FABRICE DAVRIEUX |
| Beurre de karité<br>Alchimie du bien être ? | Conference<br>Le vigan-France | D5.6: data base Nirs and wet chemistry 2008 | 2010 | IER, MAKERERE INERA, ISRA UDS | G.PIOMBO |
| Rapport Licence Professionnelle Département Chimie-Université Clermont Ferrand | student report | D5.6: data base Nirs and wet chemistry 2008 | 2008 | IER, MAKERERE INERA, ISRA USD | FABRICE DAVRIEUX |
| Rapport IUT(Institut Universitaire de Technologie Montpellier-UM2) 2009 | student report | D5.7: data base Determination of tocopherols 2007-2008 | 2009 | IER, MAKERERE INERA, ISRA USD | G.PIOMBO |
| Rapport IUT(Institut Universitaire de Technologie Montpellier-UM2) 2010 | student report | D5.10: data base FFA 2010 Nirs and wet chemistry | 2010 | IER, MAKERERE INERA, ISRA USD | G.PIOMBO |
| Rapport IUT(Institut Universitaire de Technologiede Saint Nazaire Université de Nantes) 2011 | student report | D5.11: data base FFA 2011 Nirs and wet chemistry | 2011 | IER, MAKERERE INERA, ISRA USD | G.PIOMBO |

## Work package 6: *Development of new scientific tools to assure the determination of foodstuff origin: promoting the traceability of Shea*

| Publishable result title and reference (authors journal) | Type of publication (article, student report, booklet) | Deliverables concerned | Date of publication | Project Partners involved | Corresponding scientist |
|---|---|---|---|---|---|
| El Sheikha A. F. (2011). Détermination de l'Origine Géographique des Fruits: Exemples du Karité et du Physalis par l'Utilisation d'Empreintes Génétiques sur la Communauté Microbienne par PCR/DGGE. Edited by: Éditions Universitaire Européennes. GmbH & Co. KG, Sarrebruck, Germany isbn:978-613-1-59473-1 | Book with international publisher | D6.6: manuscript of scientific papers on origin analysis and traceability (month 46) | 2011 | CIRAD | Aly Farag El Sheikha |
| El Sheikha A. F., Bouvet J-M., Montet D. (2011). Novel molecular fingerprinting for the geographical origin of fruits. Mansoura Journal of Biology, 37 (2): 35-43. | Article in national scientific journal | D6.6: manuscript of scientific papers on origin analysis and traceability (month 46) | 2011 | CIRAD | Aly Farag El Sheikha |
| El Sheikha A. F. (2011). Nouvel outil de détermination de l'origine géographique des aliments et des points critiques microbiens pour les usines agro-alimentaires. Industries Alimentaires et Agricoles, 128: 16. | Article in international scientific journal | D6.6: manuscript of scientific papers on origin analysis and traceability (month 46) | 2011 | CIRAD | Aly Farag El Sheikha |
| El Sheikha A. F., Bouvet J-M;, Montet D. (2011). Biological bar-code for the determination of geographical origin of fruits by using 28S rDNA fingerprinting of fungal communities by PCR-DGGE: An application to Shea tree fruits. Quality Assurance and Safety of Crops & Foods, 3 (1): 40-47. | Article in international scientific journal | D6.6: manuscript of scientific papers on origin analysis and traceability (month 46) | 2011 | CIRAD | Aly Farag El Sheikha |
| El Sheikha AF, Bouvet J-M, Montet D. (2010). Diversity of fruit origin by using 26S rDNA fingerprinting of yeast communities by PCR-DGGE: An application to Shea tree fruits. In: 17th World Congress of the International Commission of Agriculture Engineering (CIGR). Symposium on Nanotechnologies Applied to Biosystems | Proceeding Paper in International Conference, Québec , Canada | D6.6: manuscript of scientific papers on origin analysis and traceability (month 46) | 2010 | CIRAD | Aly Farag El Sheikha |

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| Engineering and the Environment, Section VI: Postharvest Technology and Process Engineering. Hosted by the Canadian Society for Bioengineering (CSBE/SCGAB), 13-17 June 2010, p. 317, Québec City, Canada. | | | | | |
| Determination of geographical origin of Shea tree and Physalis fruits by using the genetic fingerprints of the microbial community by PCR/DGGE. Analysis of biological properties of some fruits extract.<br>Aly F. El Sheikha | PhD Degree in "Biotechnology-Microbiology" from Montpellier University II | D6.3 : Data base on microbial analysis (month 30) | 2010 | CIRAD | Aly Farag El Sheikha |
| El Sheikha A. F. (2010). Determination of the geographical origin of fruits by using 26S rDNA fingerprinting of yeast communities by PCR-DGGE: An application to Shea tree fruits. Journal of Life Sciences, 4 (6): 9-15. | Article in international scientific journal | D6.6: manuscript of scientific papers on origin analysis and traceability (month 46) | 2010 | CIRAD | Aly Farag El Sheikha |
| Montet D., El Sheikha A. F. (2010). Innovative traceability molecular technique PCR-DGGE to determine the geographical origin of fruits by using fingerprinting of yeast and fungi. International Conference on: "Food Security during Challenging Times", Universiti Putra Malaysia "UPM", Malaysia, 5-7 July 2010. | Poster was presented in International conference, Malaysia | D6.3 : Data base on microbial analysis (month 30) | 2010 | CIRAD | Aly Farag El Sheikha |
| El Sheikha A. F., Montet D. (2010). Universal biological bar-code for determining the geographical origin of fruits by using PCR-DGGE. One of the top 6 Posters in the Posters Competition In: 2nd MoniQA International Conference "Emerging and persisting food hazards: Analytical challenges and socio-economic impact", Krakow, Poland, 8-10 June 2010. | One of the top 6 Posters in the Posters Competition in International Conference, Krakow, Poland | D6.3 : Data base on microbial analysis (month 30) | 2010 | CIRAD | Aly Farag El Sheikha |
| Montet D., El Sheikha A. F., Bouvet J-M. (2009). Universal innovative molecular fingerprinting for geographical origin: applications to fruits. 1st Prize of Posters Competition was granted to El Sheikha Aly In: TRACE Final Conference entitled: "How to trace the origin of food?". Autoworld, Brussels, Belgium, 2-3 December 2009. | 1st Prize of Posters Competition was granted to El Sheikha A. F. in: International Conference, Autoworld, Brussels, Belgium | D6.3 : Data base on microbial analysis (month 30) | 2009 | CIRAD | Aly Farag El Sheikha |

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| Montet D., El Sheikha A. F., Bouvet J-M. (2009). New Strategies of Traceability for Determining the Geographical Origin of Foodstuffs: Creation biological Bar-Code by PCR-DGGE. The Second Bi-Regional EU-SEA S&T Stakeholders Conference on “Climate change adaptation and mitigation – Strengthening SEA-EU S&T cooperation to find common solutions”, Bogor, Indonesia, 11–12 November 2009. | Poster was presented in International conference, Bogor, Indonesia | D6.3 : Data base on microbial analysis (month 30) | 2009 | CIRAD | Aly Farag El Sheikha |
| El Sheikha A. F., Le Nguyen D. D., Métayer I., Montet D. (2009). Application of PCR-DGGE in determining geographical origin of fruits: Cases studies of Physalis and Shea tree fruits. 1st Prize of Posters Competition In: Trace 5th Annual Meeting and International Conference entitled: "TRACE in practice (New methods and systems for confirming the origin of food)", Freising near Munich, Germany, 1st - 3rd April 2009. | 1st Prize of Posters Competition in International Conference, Freising near Munich, Germany | D6.3 : Data base on microbial analysis (month 30) | 2009 | CIRAD | Aly Farag El Sheikha |
| Montet D., Le Nguyen D. D., El Sheikha A. F., Condur A., Loiseau G. (2008). Application of PCR - DGGE in Determining Food Origin: Case Studies of Pangasius fish from Viet Nam and Shea tree from Sub Saharian Africa. International conference entitled: "Increasing Trust in Rapid Analysis for Food Quality and Safety", Rome, Italy, 8-10 October 2008. | Poster was presented in International conference, Rome, Italy | D6.3 : Data base on microbial analysis (month 30) | 2008 | CIRAD | Aly Farag El Sheikha |
| Development the method of PCR/DGGE concerning the bacteria ecology on Shea tree seeds. Christophe Leroy | Master 2 (Master of Science and Technology of Health): University Montpellier II | D6.3 : Data base on microbial analysis (month 30) | 2007 | CIRAD | Didier Montet & Aly Farag El Sheikha |

***tree products***

## Work package 7 – Evaluating the suitability of different shea butters as raw material sources for cosmetic and pharmaceutical applications

| **Publishable result title and reference (authors journal)** | **Type of publication (article, student report, booklet,** | **Deliverables concerned** | **Date of publication** | **Project Partners involved** | **Corresponding scientist** |
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| Laetitia NOWACKI: Extraction, purification et analyses chimiques et biologiques des métabolites d'intérêt nutritionnel et thérapeutique du karité | Master 2 Biologie-Santé Université Montpellier I 2011 | D7.2, D7.3 | 2011 | Faculté de Pharmacie, Université Montpellier 1 | MUNIER Sylvie, LARROQUE Michel |
| Anne-Sophie ARNAUD: Etudes rhéologique et de protection solaire du beurre de karité | Master 2 Biologie-Santé Université Montpellier I 2011 | D7.2, D7.3 | 2011 | Faculté de Pharmacie, Université Montpellier 1 | DELALONDE Michele, NIELLOUD Françoise, PELISSIER Yves |
| Jessica OBRUN: Etudes et valorisation des propriétés cicatrisantes du beurre de karité sur un modèle de plaie chirurgicale | Master of Science dissertation Master 1: Valorisation des agroressources | D7.3 | 2011 | Faculté de Pharmacie, Université Montpellier 1 | Alain MICHEL, Patrick POUCHERET |
| Agnès HOF: Etude des effets cicatrisants du beurre de karité sur un modèle de plaie ischémique induite chez le rat, dans le cadre du projet européen INNOVKAR | Master of Science dissertation Master 1: Valorisation des agroressources | D7.3 | 2011 | Faculté de Pharmacie, Université Montpellier 1 | Alain MICHEL, Patrick POUCHERET |
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## *Work package 8: – new post-harvest processing techniques to improve quality of shea kernel and derived products*

| Publishable result title and reference (authors journal) | Type of publication (article, student report, booklet, | Deliverables concerned | Date of publication | Project Partners involved | Corresponding scientist |
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| Lovett PN (2010, 2011, 2012, 2013) Quality @ Quantity @ Price (revised and updated annually) | Presentations given at international annual shea conferences | D8.3, D8.4 | 2010, 2011, 2012, 2013 | UDS, SFC, CIRAD and others (USAID-WATH and GSA) | Peter Lovett |
| Lovett PN (2011) The importance of good nuts – sheanut harvesting, boiling and drying | Presentation given at Science for Better Shea INNOVKAR conference, Ouagadougou | D8.3, D8.4 | 2011 | UDS, SFC, CIRAD | Peter Lovett |
| Vind J & Lovett PN (2011) Boiling freshly harvested shea nuts and its effect on shea butter quality | Presentation given at Science for Better Shea INNOVKAR conference, Ouagadougou | D8.1, D8.3, D8.4 | 2011 | UDS, SFC, CIRAD and Forest and Landscape, Faculty of Life Sciences, University of Copenhagen | Peter Lovett |
| Lovett PN (2011) Linking quality, conservation and revenue – Hippo Shea | Presentation given at Science for Better Shea INNOVKAR conference, Ouagadougou (also invited for presentation at UEBT's annual international conference *Sourcing with Respect* Paris, April, 2013 | D8.3, D8.4 | 2011 | UDS, SFC, CIRAD plus others (NCRC, WCHS and Calgary Zoo) | Peter Lovett |
| Vind J, Lovett PN (in prep.) Effects of boiling freshly-harvested sheanuts (*Vitellaria paradoxa* CF Gaertn) on butter quality: Case study from north Ghana – being reviewed for publication after comments from editor of Forests, Trees and Livelihoods. | Paper for International Scientific Journal | D8.1, D8.3, D8.4 | 2013 TBC | UDS, SFC, CIRAD and Forest and Landscape, Faculty of Life Sciences, University of Copenhagen | Peter Lovett |
| Ojeda O & Lovett PN (in prep.) Calculating the carbon footprint of traditional West African shea production. Final draft prepared and choice of | Paper for International Scientific Journal | D8.1, D8.3, D8.4 | 2013 TBC | UDS, SFC, CIRAD plus others (University of | Peter Lovett |

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| journal under consideration | | | | Aberdeen) | |
| Glew D & Lovett PN (in prep.) Assessing the Carbon Footprint of Shea Butter using Life Cycle Assessment. Final draft prepared and choice of journal under consideration | Paper for International Scientific Journal | D8.3, D8.4 | 2013 TBC | UDS, SFC, CIRAD plus others (ICCO and UoY) | Peter Lovett |
| Lovett et al (TBC) Chemical effects of processing methods on traditional sheanut and butter quality – A Review. First draft in preparation | Paper for International Scientific Journal | D8.1, D8.3, D8.4 | unknown | UDS, SFC, CIRAD plus others TBC | Peter Lovett |
| Quality Sheanuts: best practices for processing (Fr and En versions) | Poster developed for training village women – during 2012 poster and trainings disseminated to 6,590 women in Benin, Cote d'Ivoire, Ghana and Nigeria in collaboration with GSA and members | D8.3, D8.5, D8.6 | 2012 | UDS, SFC, CIRAD plus others (USAID-WATH, GSA, etc) | Peter Lovett |
| Proposed Sheanut Norms for the Global Shea Alliance's Quality Grades | GSA draft Technical report developed for discussion by working group | D8.3, D8.5, D8.6 | 2013 | UDS, SFC, CIRAD plus others (USAID-WATH and GSA) | Peter Lovett |
| Lovett PN. 2010. Sourcing shea butter in 2010: a sustainability check. In: Global ingredients & formulations guide 2010. The Green Book of Cosmetics. H. Ziolkowsky GmbH, Augsburg, Germany: Verlag fur chemische Industrie. p. 62–68. | Paper for International Industry Publication | D.8.4 | 2010 | UDS, SFC, CIRAD plus others (USAID-WATH) | Peter Lovett |
| Lovett PN and Haq N (2013) Progress in developing in vitro systems for shea tree (*Vitellaria paradoxa* C.F. Gaertn.) propagation. Forests, Trees and Livelihoods, http://dx.doi.org/10.1080/14728028.2013.765092 | Paper for International Scientific Journal | N-A | 2013 | Outside INNOVKAR | Peter Lovett |
| Various MOUs with NRC, WCHS and women's processing groups detailing partnerships, quality control methods and provision of increased benefits above local market prices | Memorandum of Understanding signed by partners | D8.5, D8.6 | 2008 (ongoing revisions and signing of additional MOUs continues) | SFC, CIRAD plus others | Peter Lovett |
| Ecocert organic certification for WCHS sheanuts | Commercially accepted certificate | D8.6 | 2009 (annual renewal inspections continue) | SFC, CIRAD plus others | Peter Lovett |

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| IMO Fair for Life certification for Tamale area processing groups | Commercially accepted certificate | D8.6 | 2013 (audit commenced) | SFC, CIRAD plus others | Peter Lovett |
| Union for Ethical Bio Trade membership | Membership to international body through adherence to audited guidelines | D8.6 | 2012 (provisional membership granted and management plan under development) | SFC, CIRAD plus others | Peter Lovett |
| Sheppard, D.J., A. Moehrenschlager, J.M. McPherson and J.J. Mason (2010) Ten years of adaptive community-governed conservation: valuating biodiversity protection and poverty alleviation in a West African hippopotamus reserve. Environmental Conservation. 37(3): 270–282. | Paper for International Scientific Journal | D.8.4 | 2010 | Independently published by SFC partners and acknowledges SFC's 2008 involvement | Axel Moehrenschlager e-mail: axelm@ calgaryzoo.ab.ca |
| Reinecke W and Lovett P (2011) Developing a Conservation Certification System for NTFPs in West Africa: *'Hippo Friendly Shea Butter'* Strategy Report. | Technical report prepared for NCRC with funding from Sustainable & Thriving Environments for West African Regional Development (STEWARD) based on experiences developed through INNOVKAR | D8.5, D8.6 | 2011 | SFC, CIRAD plus others | Peter Lovett |
| Lovett PN et al (TBC) Development of "conservation" certification systems for African Parkland products. First draft in preparation | Paper for International Scientific Journal | D.8.4, D8.5, D8.6 | TBC | SFC, CIRAD plus others | Peter Lovett |

## *Work package 9 – Markets and trade patterns*

| **Publishable result title and reference (authors journal)** | **Type of publication (article, student report, booklet,** | **Deliverables concerned** | **Date of publication** | **Project Partners involved** | **Corresponding scientist** |
|---|---|---|---|---|---|
| Odongo, et al: Marketing and Trade Patterns for Shea Nut Productions in Uganda | MSc thesis Makarere University, Uganda | D 9.1. and D 9.3 | 2009 | UNIMAK, UNIQUE | Okullo, Odongo Statz |
| Scholz, Kai: GOVERNANCE AND UPGRADING IN HIGH-VALUE CHAINS OF NON-TIMBER FOREST PRODUCTS: THE CASE OF SHEA IN GHANA | MSc thesis University of Frankfurt, Germany | D 95. and D 9.6 | 2010 | UNIQUE | Scholz, Held |