Progress Report: June 2007 – November 2007

Introduction

The present study partially funded by the Rufford Small Grant for Nature Conservation, is a component of a baobab (*Adansonia digitata* L.) conservation and domestication research program being undertaken in Benin. The project is planned to be executed for a period of 18 months (December 2006 – June 2008) and aims at combining molecular analyses (AFLP) and ethnobotanical surveys to develop optimal strategies for conservation and sustainable utilization of baobab genetic resources in Benin.

A first report (December 2006 – May 2007) focused on the ethnobotanical studies and allow to point out local perception, preferences and folk classifications of baobab products in Benin. A publication titled *"Folk classification, perception and preferences of baobab products in West Africa: consequences for species conservation and improvement "* has been submitted and already accepted and should be published in the next issue of *Economy Botany*, a peer-review international scientific journal.

The second step of the study consisted in baobab leaves sampling for DNA extraction and fingerprinting using AFLP markers. The objectives of the work were to assess (i) pattern of genetic variation in Benin of baobab populations and, (ii) combine modern molecular tools and previous ethnobotanical surveys to assess the relevance of folk classification of baobab by indigenous people in Benin. To this aim, the genetic diversity and differentiation within and between baobab populations and locally recognised morphotypes (group of baobabs sharing some linked traits identified by local people) were studied.

Based on the found results, *in situ* and *ex situ* conservation strategies of the baobab genetic resources have been proposed.

Main results

Six baobab populations with 30 individuals per population (180 individuals in total) have been sampled from the three climatic zone of Benin, specifically in the areas where local ethnic groups have an outstanding and important knowledge on the species.When bands from all 180 sampled individuals were considered, levels of polymorphism within populations varied between 89.4 % and 98.2 %, reflecting a high level of polymorphism and variation within populations.

The highest estimate of the likelihood of the data, conditional on a given number of clusters, was obtained when clustering all genotypes into six gene pools. Results indicated that the genetic structuring of the sampled individuals was correlated with their geographic origin. Nei's gene diversity (expected heterozygosity) within populations ranged between 0.26 and 0.37. A three level AMOVA partitioned 14.70% among the three regions of Benin and 5% of genetic variation among populations within regions. Analysis of population structure with allele-frequency based *F*-statistics revealed a global F_{ST} of 0.127 ± 0.072 (P = 0.001). The total gene diversity (Ht) was estimated to be 0.355 ± 0.02 while the mean gene diversity within populations (Hw) and the average gene diversity among populations (Hb) were estimated at

0.309 and 0.045 ± 0.072, respectively. Pairwise genetic distances between populations (F_{ST}), calculated using AFLPsurv 1.0, were statistically significant ($P < 0.001$). Within the same climatic region, the genetic distance is generally lower than 0.05, whilst genetic distance between populations located in the different climatic zones were larger than 0.05. Mantel tests comparing genetic differentiation and geographic distance per population showed a significant correlation of 0.758 ($P < 0.001$), indicating isolation by distance.

The current high levels of genetic variation present within populations of Benin implies that large numbers of samples from a few populations will capture a sufficient amount of the species' genetic variability for conservation programmes. Also, seeds can be sampled based on the found intra-specific genetic variation and conserved *ex situ* in seed banks.

In rural area, the most commonly used criteria to differentiate among baobab individuals were: leaf taste, pulp taste, the slimness of fruit pulp and the fertility of baobab trees. Based on locally recognized variants, the survey revealed that local people are able to recognize 8 local morphotypes, a morphotype being defined as a group of baobabs sharing some linked traits identified by local people. Nei's gene diversity within morphotype ranged between 0.29 and 0.37 indicating a substantial amount of variation within locally recognised morphotype. Analysis of population structure with allele-frequency based *F*-statistics revealed that morphotypes are not significantly differentiated from each other at genetic level. A non-significant F_{ST} value was observed within the country. Pairwise F_{ST}-values confirmed that none of the analysed morphotypes are genetically differentiated. As such genetic fingerprinting with AFLPs did not correlate with the traditional morphological classification of the identified morphotypes of baobab in rural areas of Benin. Since no genetic differentiation is found between the locally recognized morphotypes, a morphotype-based approach in the collection of genetic variation for conservation programs is not advisable. However, farmers are able to guide breeders in collecting germplasm from trees since they have knowledge to distinguish types of baobab. This can allow selecting the *plus tree* for propagation, and planning domestication programme, combining indigenous knowledge and genetic findings. As desirable traits of baobab vary according to ethnic groups, conservation strategies should be specific for each locality/ethnic groups. *In situ* conservation of living trees should mainly target desired baobabs in the traditional agroforestry systems while *ex situ* programmes should target the non-desired baobabs in rural areas.

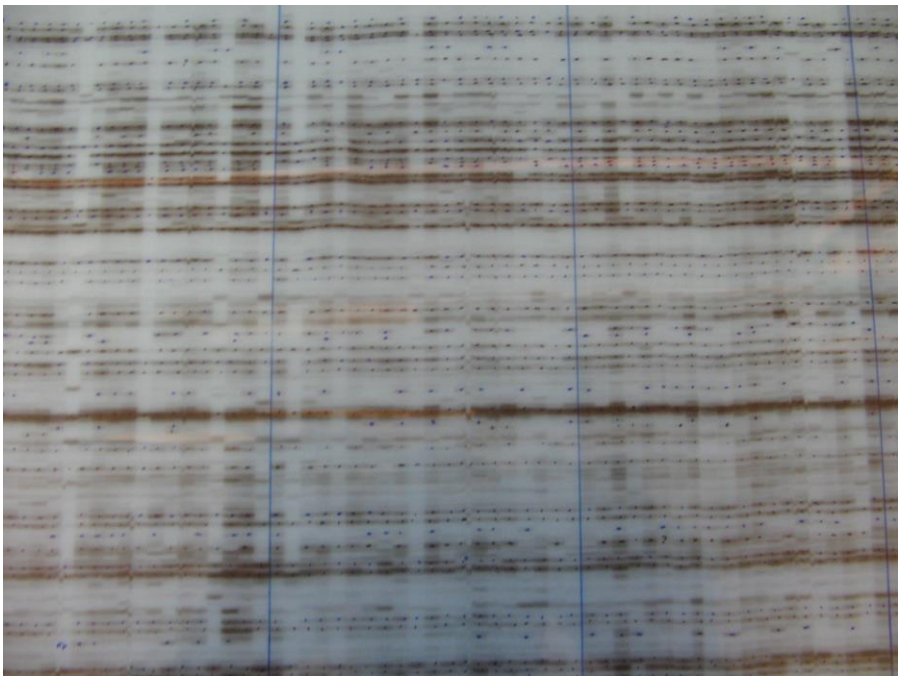
Planning for the next 6 months (December 2007 – June 2008)

<u>*Submission of scientif article:*</u> A publication related to the combination of folk classification and molecular analyses in locally recognized morphotypes of baobab will be submitted to *Agroforestry Systems*, a peer-review international scientific journal.

<u>*Propagation tests for domestication*</u>: seed longevity and germination tests will be carried out to test for easy propagation of the species. Natural populations of baobab will be surveyed based on ethnobotanical studies and genetic diversity within the species and germplasm will be consequently sampled for propagation tests.

<u>*Dissemination plan:*</u> a workshop with local communities, NGOs and other stakeholders will be organized to discuss conservation and domestication strategies related to the species.

Also, a final report related to the project will be submitted and uploaded on the website of RSG.



AFLP Markers.

Lab work for DNA fingerprinting.