

Ecological Survey and Genetic Diversity of *Dalbergia* Species in the North-Eastern Region of Madagascar

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Abstract—Madagascar is well known by its eastern forest flora biodiversity richness which unfortunately receives heavy pressure due to natural disasters and anthropic actions such as slash and burn cultivation as well as selective logging of precious timber. This leads to an increasing degradation of habitats, to a depletion of the genepool of species and a reduction of environmental services offered former predestine habitats. To deal with this issue, applied research focused on in situ conservation by artificial regeneration for key species is needed. The objective is to conserve genetic resources of the heavily affected rosewood and “palissandre” species which are economically very valuable. However, in situ conservation needs knowledge on ecological traits of natural area of the targeted species and on their genetic diversity to insure safeguard in suitable habitat an optimal representative gene pool.

An, ecological study was made by following transects method in coastal area as main habitat of rosewood species and medium elevation forest for palissandre. Leaves samples were collected for genetic diversity analysis which was realized in EPFZ laboratory. Then, phylogenetic tree and Principal Coordinates Analysis were confronted with the ecological data set.

Results for 7 species (4 rose wood and 3 palissandre species) are presented for ecological survey and genetic diversity analysis. Also, relationship between ecological traits of habitat and genetic diversity was observed.

Keywords—rosewood, palissandre, *Dalbergia* species in Madagascar

I. INTRODUCTION

Madagascar is a very spacious country within various ecological characteristics presenting a great range of ecosystems or habitats. As consequence, an important diversity is known at interspecific and intraspecific levels for both fauna and flora. This diversity is supposed coming from environmental conditions and genetic aspects.

However, these parameters are influenced by different factors such natural disasters (e.g.: cyclones) and human activities like selective logging. The eastern forest of Madagascar is particularly affected by these phenomena, so forest degradation is very advanced and there is risk of biodiversity impoverishment.

The north-eastern rainforest of Madagascar is a region of frequent cyclones and forest overlogging and slash and burn practice although it is a great part of precious wood species natural range. These species become threatened and could be faced by risk of extinction. That is why, in 2013, *Dalbergia* species, including palissandre (p) and rosewood (rw) species, were registered into annex II of CITES. This is an international strategy to safeguard these species but it should be completed by national actions to really insure that a minimal population size should be maintained to keep genetic stability and future reproduction ability. This study, conducted in partnership with Zurich Zoo and ETHZ, aims to gather and analyzes current ecological data and genetic diversity of some species of *Dalbergia* in Makira-Masoala and Antongil's Bay region.

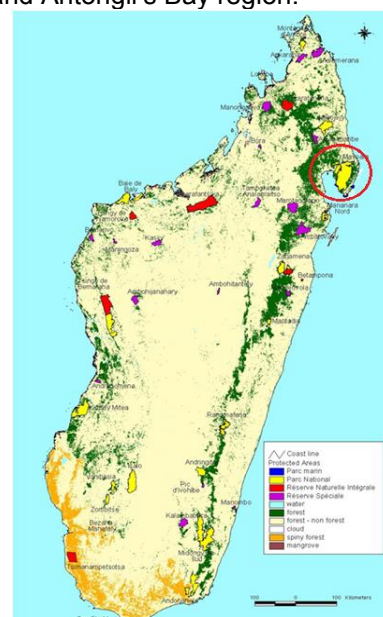


Figure 1: Map of Makira-Masoala and Baie d'Antongil region location

II. MATERIALS AND METHODS

A. Ecological assessment

Inventory was made by transects method, the same method as used by Missouri Botanical Gardens (MBG) and Madagascar National Parks (MNP) 's team in 2010 in Masoala's region. 3 transect per site and 3 sites per zone were established.

TABLE I. SITES OF TRANSECTS FOR ECOLOGICAL ASSESSMENT

| Zone | Site 1 | Site 2 | Site 3 |
|----------------|--------------|-----------|--------------|
| Makira | Mangabe | Anjiahely | Anjanaharibe |
| Masoala | Anjia | Sahabe | Ratsianarana |
| Antongil's Bay | Lohantrozona | Antalavia | Ambanizana |

Different data were recorded: species identity (vernacular name directly known by local guide or voucher for further identification), dendrometric traits (height and dbh), environmental factors (topography, soil, altitude, vegetation, state of ecosystem perturbation, hydric regime and anthropogenic activities).

B. Sampling and DNA extraction for genetic analysis

Leaves were collected from *Dalbergia* natural stand, and were dried in silicagel: 53 samples at Anjiahely (Makira) and 17 at Ambohitralanana (Masoala). Unfortunately, collecting samples from Antongil's Bay was not feasible.

TABLE II. SAMPLES COLLECT FOR GENETIC ANALYSIS

| Zone | Species | Number of samples |
|---------------------------|--|--|
| Anjiahely (Makira) | <i>Dalbergia occulta</i> (p) | AR001-AR003- AR004-AR005 - AR006 - AR007 - AR008 - AR010 - AR011 - AR012 - AR013 - AR014 - AR015 - AR016 - AR019 - AR020 - AR021 - AR022 - AR025 - AR027 - AR028 - AR034 - AR037 - AR050 |
| | <i>Dalbergia orientalis</i> (p) | AR002 - AR009 - AR023 - AR024 - AR026 - AR029 - AR031 - AR032 - AR033 - AR035 |
| | <i>Dajbergia chapelieri</i> (rw) | AR017 - AR018 - AR036 - AR038 - |
| | <i>Dalbergia madagascariensis</i> (p) | AR030 - AR053- AR054 - AR055 |
| | <i>Dalbergia maritima</i> (rw) | AR039 - AR040 - AR041 - AR042 - AR043 - AR044 - AR045 - AR046 - AR047 - AR048 - AR049 - AR051 - AR052 |
| Ambohitralanana (Masoala) | <i>Dalbergia madagascariensis</i> (rw) | AR057 - AR063 - AR064 - AR067 |
| | <i>Dalbergia normandii</i> (rw) | AR056 - AR058 - AR059 - AR060 - AR061 - AR062 - AR065 - AR066 - AR068 - AR069 - AR070 |

DNA was extracted using CTAB method with 10mg of dry leaves. CTAB solution was added to release

cellular membrane and B-Mercaptoethanol to stabilize DNA. Then, it was incubated at 65°C for 30mn. Phenol and protein were removed by addition of chloroform-isoamylalcohol to the samples in two times: Isopropanol was added to the second supernatant to precipitate the DNA into a pellet. This pellet is dissolved and stored in sterile water and DNA quantity for each sample was measured with Qubitfluorometer and quality with Nanodrop spectrophotometer (ETHZ, 2013).

C. Genetic analysis

Genetic analysis is based with DNA sequencing.

- Polymerase Chain Reaction (PCR):

The PCR protocol established by ETHZ in 2013 was used. PCR products are obtained by mixing of:

- DNA extracts to be amplified,
- Buffer to provide suitable chemical environment for optimum activity for DNA polymerase,
- Taq polymerase allowing the DNA replication,
- MgCl₂ in which Mg²⁺, cofactor of Taq polymerase to stabilize the DNA strands,
- dNTPs to build the copies of DNA fragment,
- two complementary primers (trnL and matK) for polymerase starting, but only data from trnL were treated in this article.

In a thermal cycler machine with heating and cooling alternative and repetitive steps, denaturation, annealing and elongation of DNA fragment are happening. Then, the quality of PCR products obtained are assessed by gel electrophoresis and cleaned up with ExoSap to remove the leftover of primers and of dNTPs.

- Sequencing with ABI Machine

- Big Dye v3.1, a phosphorescent reagent, and forward or reverse primers are added to the PCR products in order to create the DNA strand.

D. Data processing

Sequencing data from ABI Machine were imported firstly to Geneious 6.1 (© Biomatters 2005 - 2012). Forward and reverse sequences for each sample were selected and trimmed to remove low quality ends of sequences. The complement of reverse primer was created to get the same sequence as the forward one before assembling. The consensus sequences extracted from all samples are aligned to create the phylogenetic tree.

E. Crossed analysis between ecological distribution and genetic diversity

The analysis of intraspecific diversity, at samples level, was done by comparing ecological data for each species with phylogenetic trees; In case of existence of isolated samples, altitude factor which is related to soil and vegetation types was considered to explain the diversity. Ecological distribution (soil characteristics,

relief and vegetation type) were codified for analysis.

TABLE III. CODIFICATION OF ECOLOGICAL CHARACTERISTICS

| | Characteristics | Code |
|------------|--|------|
| Soil | Black claye soil | S1 |
| | Black claye soil with humus | S2 |
| | Black claye soil with humus and a thick litter | S3 |
| | Claye soil | S4 |
| | Sandy soil | S5 |
| | Yellow claye soil | S6 |
| | Yellow soil | S7 |
| Relief | Coastal lowland | R1 |
| | Lowland | R2 |
| | Slope | R3 |
| Vegetation | Degraded primary forest | V1 |
| | Secondary forest | V2 |

III. RESULTS

Rosewood species could occupy littoral forest (*D. normandii* and *D. madagascariensis rw*) or lowland and slope with black claye or yellow soil (*D. chapelieri*, *D. maritime* and *D. madagascariensis rw*). These species survive in degraded or secondary forests. Palissandre species (*D. occulta*, *D. orientalis*) are essentially met in slope within various soil types and in the two types of vegetation.

TABLE IV. MATRIX OF ECOLOGICAL DISTRIBUTION FOR SPECIES

| | Relief | | | Vegetation |
|------|---|--------------------------------|---|------------|
| | R1 | R2 | R3 | |
| Soil | | | | |
| S1 | | <i>D. madagascariensis (p)</i> | <i>D. chapelieri</i> - <i>D. maritime</i> - <i>D. occulta</i> | V1 |
| S2 | | | <i>D.occulta</i> - <i>D. orientalis</i> | V2 |
| S3 | | | <i>D.occulta</i> - <i>D. orientalis</i> | V2 |
| S4 | | <i>D. madagascariensis(p)</i> | <i>D. orientalis</i> | V1 |
| S5 | <i>D. madagascariensis (rw)</i> - <i>D. normandii</i> | | | V2 |
| S6 | | | <i>D. chapelieri</i> | V1 |
| S7 | | | <i>D. occulta</i> | V2 |

In terms of intraspecific genetic diversity, 2 species have differences among their samples: *D. normandii(rw)* and *D. orientalis(p)*.

The phylogenetic tree (Fig.2) show also that 3 rosewood species (*D. normandii*, *D. madagascariensis* and *D. maritime*) are genetically closed, except *D. chapelieri* which is far isolated. This species is closer to palissandre group and particularly to *D. occulta*.

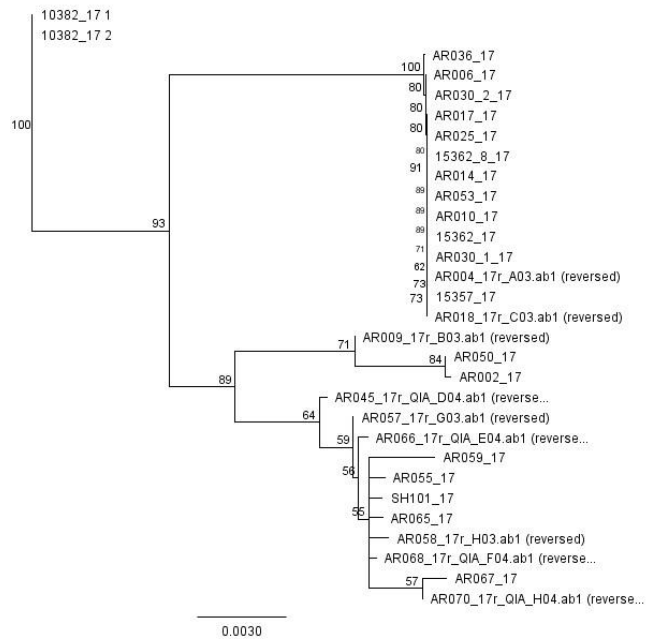


Figure 2 : Phylogenetic tree with trnL

At interspecific genetic diversity level, PCoA results (Fig.3) show four major groups:

- Group 1 : one species *Dalbergia occulta* with only one sample (AR050)
- Group 2 : 3 different species composed by 3 samples : *Dalbergia madagascariensis* palissandre(AR030), *Dalbergia baroni* and *Dalbergia normandii*
- Group 3: 2 species that are *D. normandii*(AR059 and AR065) and *D. madagascariensis* rosewood (AR067)
- Group 4: 2 species, *D. occulta*(AR010, AR014, AR025) and *D. chapelieri*(AR017 and AR036)

The main results from crossed analysis between genetic diversity and ecological distribution are:

- *Dalbergia normandii*, a rose wood species, within intraspecific genetic diversity, is occupying secondary littoral forest;
- *Dalbergia orientalis*, a palissandre, within intraspecific genetic diversity, grow on slopped land with claye soil where forest is degraded or converted into secondary vegetation.;
- Rose wood species (*D. normandii*, *D. maritime* and *D. madagascariensis*) are genetically related even if ecological traits of their sites are various, particularly for *D. maritime* which could be met in slopped land
- *Dalbergia chapelieri*(which is also presumed to be a rosewood species)could occupy the same ecological site as *D. maritime* but is genetically close to palissandre species.
- Palissandre species (*D. occulta*, *D. orientalis* and *D. madagascariensis*) form a genetic

cluster and have similar ecological distribution (clay soil, slope and degraded or secondary forest).

Existence of single sample for some targeted species which has isolated genetic traits allows formulating new hypothesis:

- "ecological factors are not influencing individual's genetic adaptation"

- or "individuals occurring the same site could be not related".

For *D. normandii*, sample n° AR070 was collected from a regeneration in a very low altitude (9m).

For *D. chapelieri*, the sample AR018 was from a cut down adult tree but the others samples were from natural regeneration.

For *D. madagascariensis* (p), AR055 extracted from a juvenile individual belongs apparently to another family.

For *D. occulta*, only AR050 was collected from an adult tree.

IV. DISCUSSION

In terms of methodology discussion, firstly, samples collecting were done without pre-defined protocol because of lack of data about species distribution. Also, forest fragmentation and selective exploitation did not allow having the necessary data, so results might be biased because limited data was available for a random sample planning. The consequence was that number of samples for species is varying.

In addition, quality of DNA extracted and PCR products was not sufficient for sequencing has affected the results.

About relationship between ecological characteristics and species distribution, of the survey conducted by DBEV (2013) in the south eastern region of Madagascar, from Kianjavato to Manombo shows that all the species were found in moderately degraded or intact rainforest, contrary to the ecological survey in MaMaBaie where most of the species can be found in a secondary forest. For *D. normandii*, it has been met only in Masoala's littoral forest, at coastal lowland, while it can be found in top of slope in the south eastern region, but always at low altitude. The difference among species repartition from the northern and the southern region can be explained mainly by the variation of latitude and the degree of fragmentation of the forest which can cause a loss of individuals of a species from its natural habitat.

Low genetic diversity was generally observed for the sampled *Dalbergia* species from MaMaBaie, except for *Dalbergia orientalis* and *Dalbergia normandii*. This lowness can be explained by the high degree of fragmentation of Anjiahely and Ambohitralanana's forest. The forest fragmentation

led to a genetic and ecologic drift (Vellend&Orrock, year). As *Dalbergia* genus was subjected to a highly selective logging; the consequence may be dysgenic selection, loss and or depletion of locally adapted populations and reduced seed set and / or increase in selfing or autocross associated with the degree of isolation between remnant trees (FAO & al, 2004), that can explain the low genetic diversity. However, this aspect was not observed by Andrianoelina (2009) with genetic diversity analysis for *Dalbergia monticola*. The possible cause may be the recent fragmentation as the species is less exploited than the studied ones. Further research should be done about *Dalbergia monticola* which can be found in MaMaBaie's forest. The study about *Justicia adhatoda* (Gilani&al, 2011), a medicinal plant from Indonesia and South East Asia, confirms also that recent fragmentation does not affect genetic diversity.

Species from Ambohitralanana's littoral forest are more under pressure than species from Anjiahely. Adding to the fact that Ambohitralanana's rosewood species were subjected to an overexploitation since decennia, this region is oftenstricken by cyclones. However, cyclones may play two "paradoxal" roles in genetic diversity. First, they cause a degradation of forest and consequently a loss of genetic diversity. But in another hand, rosewood species pollination and sexual reproduction can appear mainly after a cyclone happening. This could explain the important genetic diversity observed with *Dalbergia normandii* species.

About genetic diversity among species, rosewood species which are *Dalbergia madagascariensis*, *Dalbergia normandii* and *Dalbergia maritima* seem to be close each other, even if *D. maritime* is geographically separated. Concerning *Dalbergia chapelieri*, which is described to be likely palissandre than rosewood is closer to the palissandre species group. Advanced determination of this species should be done. For the palissandre species, which are all from Anjiahely, they also constitute a group genetically close. In fact, this situation confirms Sukhjiwan& al. (2014) in the framework of assessment of genetic diversity in Faba bean: geographical origin is a major origin of genetic pattern.

V. CONCLUSION

This study conducted on *Dalbergia* precious wood species (rosewood and palissandre) in the North-eastern zone of Madagascar includes two areas: ecological distribution survey and genetic diversity analysis. The collaborative research between SNGF, EPFZ and Zurich Zoo was agreed face to necessity of getting scientific data for future genetic resources conservation to contribute to safeguard precious wood species which are threatened because of overexploitation. The finality of the research is to prepare in situ conservation of genetic resources for targeted species. Inventory by transects protocol and DNA testing process were the methods adopted. The main results are:

- rosewood species could be met in littoral

- forest, in lowland or in slope and grow in different types of soil; palissandre species are mainly on sloped land; the two groups survive in degraded primary or in secondary forest;
- an intraspecific genetic diversity for *Dalbergia normandii* and *Dalbergia orientalis*;
- genetic neighboring for the group of rosewood species in one hand and for palissandre species in other hand;
- *Dalbergia chapelieri* which was botanically identified as a rosewood species is genetically close to palissandre.

Crossed analysis between ecological repartition and genetic diversity present a low level of relationship. So, genetic diversity within species seems to be more influenced by mating system and species traits than ecological factors.

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