





RSG REFERENCE: 3969-1\_bezandry-rickarlos Project update: Febroary 2025

# *Ex-situ* Conservation of three *Coffea* (Baracoffea group) species adapted to the semi-arid regions of northwestern Madagascar

Web site : https://www.baracoffea.org/



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This report was prepared following our October report. In that document, we outlined both the context and the objectives to be achieved. The present report focuses exclusively on the work completed between November 2024 and February 2025.

## 1- Growth and phenological monitoring

Monitoring of the growth and phenological stages of the 91 previously marked individuals has continued to date (February 2025) and will proceed over the coming months. Phenological stages, such as leafing out, defoliation, flowering and fruiting, were recorded at each observation (Photo 1). Additionally, growth parameters were also measured on the trunk and branches during each observation (Photo 2).



Photo 1 : Evolution of the phenological stage. A: Defoliation, B: Leafing, C, D, and E: Fruiting.



**Photo 2**: Overview of the growth observed in September (on the left) and in November (on the right), these are two different plants

During implementation, several difficulties were encountered. For example, the adhesive tape used for individual marking was damaged by unknown animals. Additionally, the identifiers inscribed on the tape sometimes faded, probably due to prolonged exposure to high heat and intense sunlight. To overcome these problems, the identification codes were painted directly onto the tree trunks.

In some of the trees studied, we also observed the death of the apical meristem after a mark had been applied to the last formed phytomer (Photo 3). This phenomenon highlights the fragility of the terminal bud. As a result, it was necessary to adapt our methodology by changing either the individual studied, or just the portion of branch monitored, depending on the situation.



Photo 3 : Non natural death of apical meristem

## 2- Recording environmental parameters

Since December 2024, with the arrival of the recording equipment, six environmental parameters have been recorded at each observation for each of the individuals studied. The aim is to analyse the relationship between these parameters and biological processes such as phenology and growth. The six parameters recorded were: soil fertility, soil pH, sunshine, soil moisture, soil temperature and ambient humidity.



Photo 4 : Device for measuring environmental parameters (left) and extract of recorded data

## 3- Setting up the nursery at the Botanical Garden of the University of Mahajanga

One of the fundamental aims of this project is to establish an *ex-situ* conservatory for species in the Baracoffea group, which are subject to multiple anthropogenic pressures, such as forest fires, illegal logging, charcoal burning and urbanisation. Within this framework, it is planned to integrate three specific species, namely *Coffea ambongensis*, *Coffea bissetiae* and *Coffea boinensis*, all of which are present in the study sites of the Ankarafantsika National Park and the Antsanitia forest. The project also aims to broaden its scope by including other species in the group in subsequent phases.

Work on setting up the nursery began in the third week of December 2024, with the harvesting of ripe *Coffea bissetiae* fruit first at Ankarafantsika, followed by Antsanitia. The fruit was collected in six different populations, four in Ankarafantsika and two in Antsanitia. After harvesting, the fruits were packaged in paper wrappers and store at room at room temperature. At that time, the fruits of *Coffea ambongensis* in Antsanitia and *Coffea boinensis* in Ankarafantsika were still maturing.

Photo 5: Coffea bissetiae fruit collection in Ankarafantsika National Park (20 December 2024)

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The second part of the collection took place towards the end of January and the beginning of February 2025, when the fruits of *Coffea ambongensis* (in Antsanitia) and *Coffea boinsensis* (in Ankarafantsika; Photo 6) had reached maturity. Following the same collection principle, we collected ripe fruits from 4 populations of *Coffea boinensis* in Ankarafantsika and from 2 populations of *Coffea ambongensis* in Antsanitia.

Photo 6 : Coffea boinensis fruit collection in Ankarafantsika National Park, 20 December 2024



In early January 2025, following the collection of *Coffea bissetiae* fruits, the installation of germination beds was initiated in the Jardin Botanique Éducatif Vololona Ranjamalala, located on the University Campus of the University of Mahajanga. The construction involved materials such as mangrove roundwood, bamboo, fir planks, nails, wire, nets and worms were used for the installation. To provide shade for the germination beds, a roof was constructed using weeds harvested from the garden, supported by nets and bamboo. A total of four germination beds were created, including one measuring 1 meter wide and 4 meters long, capable of holding 450 polyester pots (Photo 7).

Photo 7 : Installation of four germination bed plots in the Jardin Botanique ÉducatifVololo-na Ranjamalala, University of Mahajanga, from January to February 2025Vololo-



The substrate was prepared using a specific composition of 1:1:2, i.e. 1 kg of potting soil, 1 kg of compost and 2 kg of sand. The sand, as the main component, was mixed with the potting soil and compost. After preparation, the substrate was placed in black nursery pots. A total of 1,800 pots were distributed over the four germination plots, requiring the use of 250 kg of compost. Each pot was identified by a unique code to facilitate monitoring.



Photo 8 : Preparing the substrate and germination pots

Once the four germination plots had been set up and the 1,800 pots had been placed (900 of them initially), the viable *Coffea bissetiae* fruits were sorted. Once the seeds had been depulped, they were dried at room temperature, away from direct sunlight. They were then measured and weighed individually, and placed in empty tea bags, each identified by a unique code corresponding to those on the jars. To test seed dormancy breaking, three types of pre-treatment were applied, with 30 seeds and fruits per population used as controls. The same methods were applied to the fruits and seeds of *Coffea boinensis* and *Coffea ambongensis* once they had reached maturity.

The first *Coffea bissetiae* seedling was sown on 15 January 2025. In all, 720 seeds and 180 fruits were germinated, distributed as follows:

- 30 seeds x 3 types of tests x 6 populations (4 at Ankarafantsika and 2 at Antsanitia);
- 30 control seeds x 6 populations;
- 30 control fruits x 6 populations.



**Photo 9 :** Preparation, measuring, coding, pre-treatment and sowing of *Coffea bissetiae* seeds and/or fruits

The second sowing was carried out on 12 February 2025 for *Coffea boinensis* and *Coffea ambongensis*. In all, 480 seeds and 120 fruits of *Coffea boinensis* and 200 seeds and 30 fruits of *Coffea ambongensis* were germinated, distributed as follows:

#### \*\*For Coffea boinensis (Ankarafantsika only)

- 30 seeds x 3 tests x 4 populations ;
- 30 control seeds x 4 populations;
- 30 control fruits x 4 populations.

#### \*\*For Coffea ambongensis (Antsanitia only)

• 30 seeds x 3 tests for population 1;

- 30 control seeds for population 1;
- 30 control fruits for population 2;
- 20 seeds x 3 tests for population 2;
- 20 control seeds for population 2 ;
- No control fruits for population 2.



**Photo 10 :** Preparation, measuring, coding, pre-treatment and sowing of *Coffea boinensis* and *Coffea ambongensis* seeds and/or fruit

The main challenges encountered during the project (installation of the nursery) included differences in fruit ripening among *Coffea species*. *Coffea bissetiae* ripened at least one month earlier than *Coffea boinensis* and *Coffea ambongensis*, requiring it to be sown ahead of the other species. In addition, the number of fruit trees was limited, particularly for *Coffea ambongensis*, which is located outside protected areas, resulting in insufficient fruit being collected for the nurse-ry. As a result, only 20 seeds per test and control were collected for *Coffea ambongensis*, instead of the planned 30, and no control fruit could be obtained. In addition, measuring and weighing individual seeds and fruits by species and population was a considerable task for the team. However, these measurements are considered crucial for establishing links between the morphology of seeds and fruits and their germination traits, as well as for analysing seedling vigour traits. They will also provide a better understanding of which populations produce the most efficient viable fruit.

It was initially planned to plant 200 seedlings per species as part of the project. However, given the preliminary results observed for the germination of Coffea ambongensis (very low germination rate) and Coffea bissetiae (no successful germination), this number was tripled where possible, depending on the number of populations and the availability of fruit.

### 4- Next steps

The next stages of the project will be to continue monitoring the growth and phenology of the seedlings over the coming months, carry out the necessary biological and anatomical measurements and analyses, ensure the transfer of skills in the management of *ex-situ* collections to FOFIFA technicians, depending on the availability of the trainers, maintain and service the nursery, then prepare a final report and finally publish the results.

## 5- Annexe

\*\*\*Administrative stage: The research authorization was renewed for a period of six months.

\*\*\***Purchase of equipment:** In early December 2024, field and laboratory equipment was purchased in France. This included an intelligent measuring device for analysing six environmental parameters, an optical microscope for studying stomata, a precision electronic balance for measuring individual fruits and seeds, and various other tools needed in the field.

\*\*\***Publication:** In December, we submitted a manuscript entitled « Architectural singularities in wild *Coffea* (Baracoffea) species: integrated morphological perspectives for climate-resilient coffee cultivation ». Pending the evaluation process, a preprint version of this article has been deposited on the bioRxiv platform (https://doi.org/10.1101.2024.12.18.628089).