RSG REFERENCE: 3969-1_bezandry-rickarlos

Project update: May 2026 (period from February 2025 to May 2026)

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

Web site of Baracoffea project : https://www.baracoffea.org/

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This report follows the one published in February 2025, in which we presented the progress made in growth and phenological monitoring, the recording of environmental parameters, and the establishment of the nursery at the Botanical Garden of the University of Mahajanga.

The present report focuses exclusively on the work carried out between February 2025 and May 2026.

1. Summary of progress

Overall, the project progressed steadily across the three objectives. Field monitoring and detailed measurements generated a consistent dataset on growth, phenology, and plant traits, improving understanding of variation in relation to environmental conditions. Germination trials provided initial evidence of delayed and staggered germination, with moderate success and sensitivity at early stages, while adaptive measures helped improve seedling survival. At the same time, the ex-situ system was reinforced through regular nursery management, expansion of plant collections, installation of a water supply system, and implementation of a capacity-building session, contributing to more stable conditions and continuity of activities.

2. Progress against objectives

2.1. Objective 1: Characterize growth strategy and phenology

Monthly monitoring of growth (length and diameter of the main and secondary axes) and phenological stages (leafing, flowering, fruiting, and defoliation) was carried out on the 91 previously marked individuals from populations located in Ankarafantsika National Park and the Antsanitia forest station. This monitoring was conducted continuously from September 2024 to July 2025.

In addition, detailed architectural and phenotypic measurements were performed on 40 individuals, focusing on both vegetative axes and leaf traits. These data enabled the initiation of analyses on phenotypic diversity and plasticity in relation to the ecological conditions of Ankarafantsika and Antsanitia. Environmental variables, including soil fertility, soil pH, sunlight exposure, soil moisture (%), soil temperature, and ambient humidity (%), were systematically recorded during each observation.

The phenotypic traits measured include trunk height and diameter; branch length, diameter, and insertion angle; leaf allometry; petiole length and diameter; number and diameter of internodes on both trunk and branches; number of growth units; number and length of internodes per growth unit; number of leaves per branch; leaf lifespan; and branching order.

Furthermore, stomatal studies were initiated to investigate physiological adaptation mechanisms, particularly in response to water stress conditions. A total of 120 stomatal impressions were collected in the field from 40 leaves belonging to 20 individuals in Ankarafantsika. For stomatal density analysis, 30 impressions per population were examined. For each impression, stomatal counts were recorded across up to 20 microscopic fields, allowing for robust estimation of stomatal variation.

Overall, these combined architectural, phenotypic and physiological datasets provide an informative framework for understanding the functional biology of species within the genus Baracoffea. They enable the analysis of phenotypic plasticity and growth dynamics in response to environmental constraints, highlighting how plant architecture and form are shaped by specific ecological conditions.

These results help to identify key adaptive traits associated with survival in semi-arid environments and improve our understanding of the relationships between plant structure, function and habitat. Ultimately, this work supports the promotion of Baracoffea species as a crucial genetic and functional resource for understanding the mechanisms by which coffee plants adapt to drought.

2.2. Objective 2: Study germination

It is important to recall that direct sowing in pots was conducted in January and February 2025, involving 1,800 seeds/fruits, with three types of seed pre-treatments (aimed at breaking dormancy) and two control conditions (seeds and fruits).

During the period covered by this report, the germination study reached a critical and highly informative phase, providing the first experimental insights into germination dynamics in Baracoffea species.

The first germination events were recorded in August 2025, approximately 24 weeks after sowing, marking the initial emergence phase. A major germination wave occurred in December 2025, followed by a second wave in January 2026 after the implementation of adaptive management measures.

Overall germination rates remained moderate across species, with comparable proportions observed among taxa, suggesting relatively consistent germination constraints at the genus level. Germination was clearly delayed compared to cultivated coffee species and occurred in a staggered manner over time, indicating complex dormancy mechanisms and strong environmental control of germination processes.

Preliminary observations indicate that germination success varied depending on the treatments. Control conditions using intact fruits tended to show better overall performance, while alternative treatments (such as seed extraction or simple soaking) showed more variable responses depending on the population.

The December 2025 germination peak was significantly affected by slug attacks (notably snails), resulting in substantial seedling losses and highlighting the ecological vulnerability of early developmental stages. Following the implementation of corrective measures (physical barrier using netting and application of Lymoxyl), the second germination wave (January 2026) showed improved survival, demonstrating the effectiveness of the adaptive response.

These findings provide key insights into germination ecology, including temporal dynamics, treatment effects, and early-stage sensitivity. They are particularly critical for conservation, as they improve understanding of recruitment constraints and support the development of effective propagation and restoration strategies for Baracoffea species.

2.3. Objective 3: Establish an ex-situ collection

The ex-situ conservation system has been significantly strengthened during this reporting period.

The nursery has been regularly maintained, including continuous monitoring of seeds and fruits under germination, maintenance of substrates and environmental conditions, and progressive improvement of infrastructure and protection systems. These efforts have contributed to enhancing seedling survival and ensuring more stable growth conditions.

It is important to recall that four germination beds were established between January and February 2025. In addition, a shaded structure was set up to accommodate seedlings directly transplanted from Antsanitia. To date, 25 young plants of *Coffea*

ambongensis and one individual of *Coffea bissetiae* have been successfully established in the Botanical Garden of the University of Mahajanga.

Seed-derived seedlings currently maintained in the nursery include 140 individuals of *Coffea bissetiae*, 82 of *Coffea boinensis*, and 32 of *Coffea ambongensis*, reflecting encouraging establishment despite germination constraints.

Recent efforts have further expanded the living collection through the integration of additional plant material, including naturally sourced seedlings, rooted cuttings, and grafted individuals (*C. bissetiae* grafted onto *C. ambongensis*). These activities were carried out both at Antsanitia and within the Botanical Garden.

In addition, a water supply system was installed to ensure reliable irrigation of the nursery and ex-situ collection. This system connects an external water source to two plots within the Botanical Garden, addressing a major limitation in water availability and ensuring continuous watering during both dry and rainy seasons.

A major milestone was achieved through a capacity-building and skills transfer session organized in March 2026. This activity was planned within the project framework and supported by the Rufford Foundation, with the objective of strengthening local capacity at the University of Mahajanga. The training was coordinated by the project lead (Dr BEZANDRY Rickarlos) and delivered by the FOFIFA coffee program team based in Kianjavato (Fianarantsoa), who are responsible for the long-term management of living coffee collections (Mascarocoffea) since the 1960s. It covered key aspects of ex-situ conservation and nursery management, including substrate preparation, seed handling, germination techniques, vegetative propagation (cuttings and grafting), and nursery maintenance.

This initiative significantly strengthened local capacity, involving technicians, nursery staff, and gardeners from the Botanical Garden of the University of Mahajanga, as well as a representative of the local community-based management association (VOI) of Antsanitia, alongside academic staff and students.

Overall, these combined efforts substantially enhance the effectiveness, resilience, and long-term sustainability of the ex-situ conservation strategy for Baracoffea species.

3. Key activities carried out

3.1. Continued field and biological monitoring

- Continued field and biological monitoring
- Regular monitoring of growth and phenology
- Tracking of developmental stages
- Strengthening of long-term datasets

Photo 1 : Field and biological monitoring activities

3.2. Architectural and phenotypic analyses

- Measurement of plant architecture
- Measurement of phenotypic traits
- Initiation of phenotypic diversity analyses

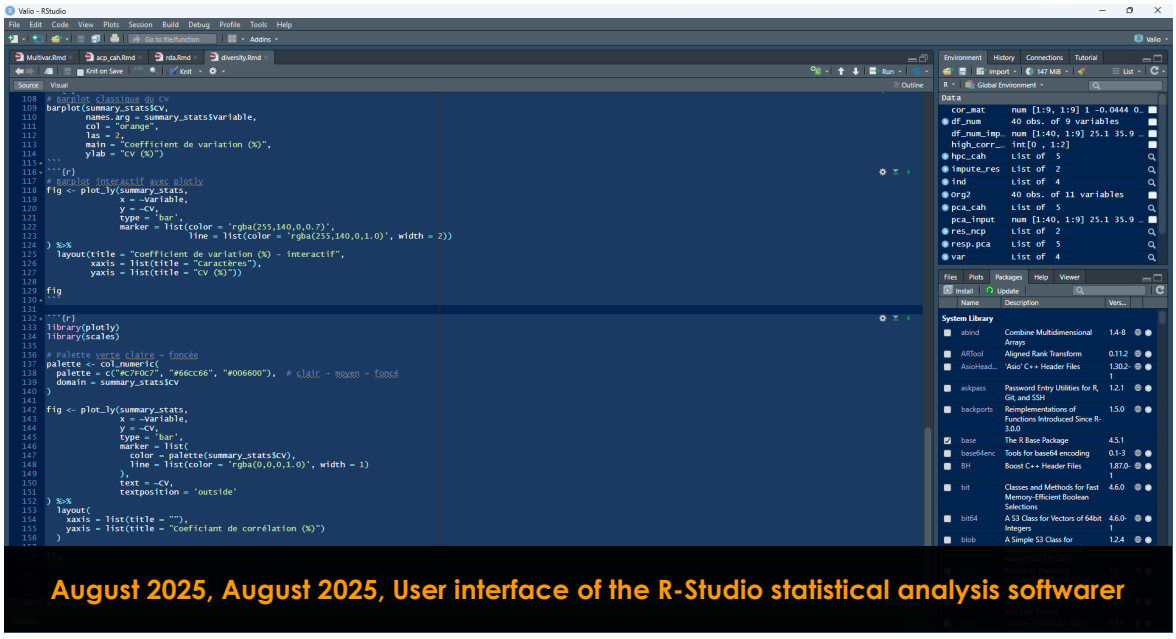

Photo 2 : Field measurements of plant architecture and phenotypic traits in Baracoffea, contributing to the analysis of phenotypic diversity

3.3. Physiological investigations

- Collection and processing of stomatal imprints
- Microscopic analysis and quantification of stomatal density
- Data analysis to support links between physiological traits and environmental conditions

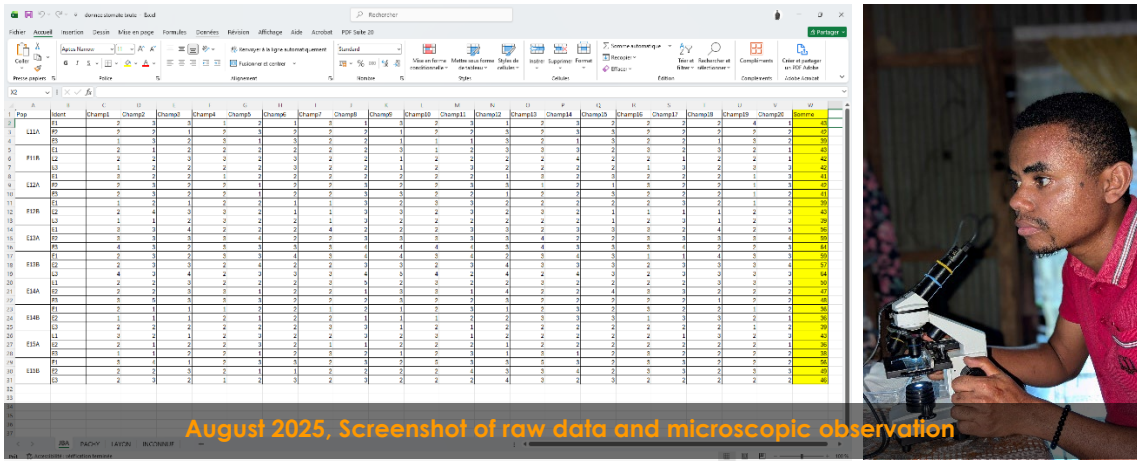

Photo 3: Stomatal sampling and analysis to assess physiological traits in relation to environmental conditions

3.4. Nursery management, germination monitoring, and ex-situ development

- Continuous maintenance of the nursery (substrates, watering, environmental conditions, infrastructure)
- Monitoring of seddlings, seeds and fruits under germination
- Increased monitoring frequency during key germination periods
- Initial attempt at vegetative propagation (cuttings and grafting) to ensure the long-term sustainability of the collection

Photo 4: Germination dynamics between November and December 2025, accompanied by continuous nursery maintenance

3.5. Pest management and nursery protection

- Installation of protective netting (Preventive measure)
- Identification of slug attacks as a major threat to seedling survival
- Application of pest control solution (Lymoxyl)

Photo 5 : Key nursery management actions, including protective netting installation, detection of slug-related damage, and Lymoxyl application for pest control

3.6 Infrastructure development and water supply

A water supply system was installed to connect an external source to the Botanical Garden plots, significantly improving irrigation capacity for both the nursery and the ex situ collection. This intervention addressed one of the major constraints of the garden, namely the lack of a permanent water source, and ensured continuous watering throughout both the dry and rainy seasons.

Both spatially separated plots benefited from this system, each being equipped with a dedicated tap.

Photo 6 : Water supply system installation enhancing irrigation capacity and ensuring continuous water availability across both plots

3.7 Capacity building and skills transfer

- Organization of a capacity-building session (16-17-18 and 19 March 2026), planned within the project and supported by the Rufford Foundation
- Training delivered by the FOFIFA coffee program team (Kianjavato, Fianarantsoa)
- Practical training on ex-situ conservation and nursery management (seed handling, germination, vegetative propagation, nursery maintenance)
- Participation of technicians, nursery staff, gardeners, a representative of the local community (VOI Antsanitia), as well as academic staff and students

Day 1, Field visit by participants and trainers to the natural habitat of Baracoffea in Antsanitia, including plant material collection and observation of the substrate and surrounding environment

Day 2, Practical activities at the Botanical Garden: substrate preparation, transplanting, cuttings, grafting, and small greenhouse installation using plant material collected in Antsanitia

Photo 7 : Training activities combining field visits, practical work, and theoretical sessions with participants and trainers

3.8 Student supervision and academic contribution

Three Master's students are supported by the project and are supervised by myself, the project leader, during fieldwork. Their research is directly aligned with the project's research themes, notably growth dynamics and phenological dynamics, architectural and physiological analyses, as well as studies on germination.

One of the students successfully defended his Master's thesis on 2 March 2026, as part of the 'Biodiversity and Conservation' programme at the University of Mahajanga. The other two students are currently finalising their thesis reports.

Photo 8 : Master's thesis defense on the phenotypic diversity of three Baracoffea species (Coffea boinensis, C. bissetiae, C. ambongensis), Ankarafantsika National Park and Antsanitia forest station, 2 March 2026

4. Challenges and adaptive solutions

4.1. Challenges

- Severe slug attacks affecting young seedlings
- Delayed and irregular germination patterns
- High mortality during early seedling stages
- Administrative constraints: six-month renewal of research permits, causing delays
- Fieldwork disruptions due to the political situation in Madagascar (October 2025)
- Intrinsically long germination duration and annual phenological cycles, extending the project timeline

4.2. Adaptive solution

- Adaptive solutions
- Installation of protective nets
- Application of Lymoxyl for pest control
- Reinforcement of nursery monitoring
- Increased frequency of observations during critical periods
- Adaptive adjustment of project timeline and activities

5. Scientific output

5.1. Publication

Bezandry, R., Ranarijaona, H. L. T., Vavitsara, M. E., Sabatier, S., Guyot, R., & Anest, A. (2026). Architectural singularities in wild *Coffea* species: integrated morphological perspectives for climate-resilient coffee cultivation. ***Plant Ecology & Diversity***, 1–13. https://doi.org/10.1080/17550874.2026.2637118

5.2. Communication

Bezandry R. '' Architectural singularity and phenotypic plasticity of wild *Coffea* species (Baracoffea group): Responses to environmental constraints in Madagascar'', ***IRN LiStat***, 3 February 2026 [Guest webinar]. Available at https://www.youtube.com/watch?v=qW6pG8uxdcM

Bezandry R. *et al.* '' Toward Sustainable Conservation of Baracoffea: Endemic and Drought-Tolerant Species in Madagascar'', ***30th Coference ASIC 2025***, 27-31 October 2025 [Presented by Romain Guyot, as a guest speaker]. DOI: 10.13140/RG.2.2.31002.09920

Bezandry R. '' Architecture and evolution of three representative species of the Coffea genus (Baracoffea group), illustrating remarkable adaptation to the hot and dry climates of western Madagascar », ***AUF-Ma Thèse en 180 seconde édition 2025***, Mai 2025 [National final]. Available at https://www.youtube.com/watch?v=A5plkbOfhc0&t=3s

5.3. Student supervision

- Successful defense of one MSc thesis (March 2, 2026)
- Ongoing supervision of two MSc students (thesis in progress)

6. Next steps

- Expand architectural, phenotypic, and physiological analyses
- Maintain and optimize nursery management and protection systems
- Strengthen data analysis and integration across datasets
- Support completion of ongoing MSc theses and associated outputs
- Disseminate results through publications and scientific communications
- Transplant nursery-derived seedlings during the next rainy season, as recommended by technical experts
- Prepare and submit the final project report

7. Annexe

7.1. Administrative stage :

- Initial research permit: No. 270/24/MEDD/SG/DGGE/DAPRNE/SCBE.Re, issued on 19 August 2024
- First renewal: No. 013/25/MEDD/SG/DGGE/DAPRNE/SCBE.Re, issued on 22 January 2025
- Second renewal: No. 452/25/MEDD/SG/DGGE/DAPRNE/SCBE.Re, issued on 19 November 2025

7.2. Website update :

Regular updates of the dedicated project website **https://www.baracoffea.org/** to support communication, visibility, and dissemination of project activities and results.

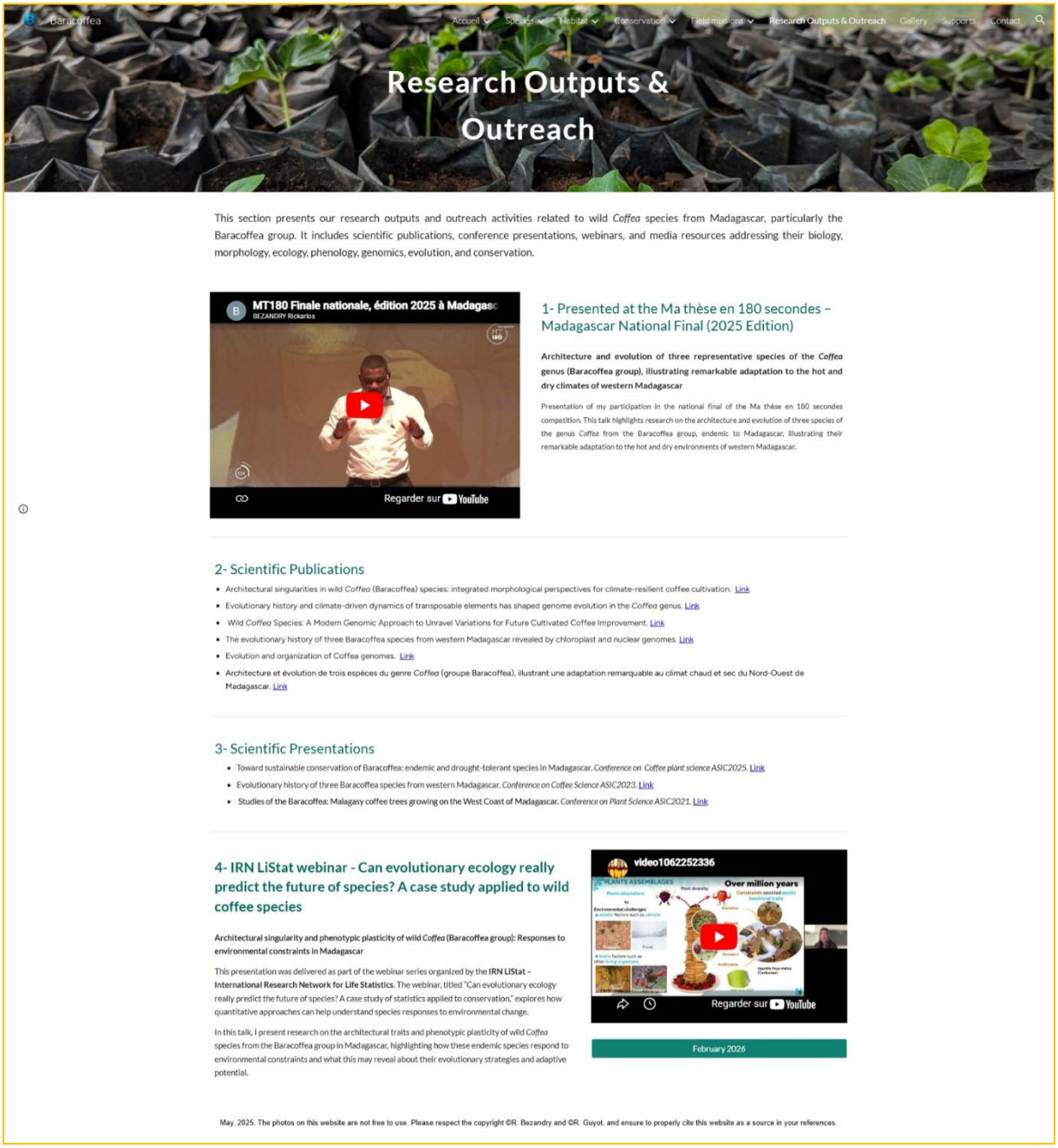

Photo 9 : Screenshot of the Baracoffea project website highlighting regular updates and the dissemination of research activities and outcomes

https://www.baracoffea.org/research-outputs-outreach

7.3. Social media post

- Regular dissemination of project activities and results through Facebook and LinkedIn
- Posts covering fieldwork, nursery development, capacity building, and preliminary findings
- Contribution to increasing project visibility and outreach to both scientific and broader audiences

Some examples of the publication poster

Ex Situ Conservation of Three Coffea (Baracoffea) Species Adapted to the Semi-Arid Regions of Northwestern Madagascar

https://www.baracofea.org

7.4. Nursery labeling update and vegetative propagation activities (May 2026)

This annex presents selected photographs illustrating recent improvements in nursery management, including the renewal of pot labeling for better traceability, as well as the progression of vegetative propagation experiments (cuttings and grafting) conducted during practical training sessions on ex-situ conservation and nursery management.

Photo 9 : New labeling system for improved plant identification and traceability

Photo 10 : Vegetative propagation (cuttings and grafting) initiated on 17 March 2026, with high initial success observed by early May 2026