FELLOWSHIP SUMMARY REPORTS

Cover page

Name: Diego Muñoz
Research Theme: Managing Natural Capital for the Future (Theme 1)
Project title: Development of advanced in vitro protocols for plant biodiversity conservation
Host institution: Centre for Australian National Biodiversity Research – CSIRO, Australia
The name of your host collaborator: Dr. David Boshier
The dates of your fellowship: 28th November 2022 to 6th March 2023
I give my consent for this report to be posted on the Co-operative Research Programme's website, or alternatively, a short paragraph about my fellowship could be used anonymously.

1. What were the objectives of the research project? Why is the research project important?

Main objective: To develop advanced in vitro protocols for a range of threatened plant species.
Specific objectives:

a) Selection of targeted species and detailed design of experiments, accounting for specific traits of the species seeds such as recalcitrance, dormancy, size, and coat thickness.
b) Establish in vitro cultures and evaluate explant responses, with further short-term experiments assessing media and hormones on responsive plant material.
c) Disseminate and transfer the knowledge and methods developed, mainly by the production of a scientific manuscript for publication, and by presenting results to colleagues in Australia and to a wider academic community in the applicant's institution.

Research Project Importance: The protocols developed will allow ex situ conservation by tissue culture and the production of plants of endangered species for establishment of field populations, reintroduction or conservation at botanic gardens. These protocols are of high importance for the management of the natural capital that the targeted species represent. The techniques explored during the project, and the contact and joint work with Australian colleagues during the stay, are allowing the development of further insights and ideas in the field of natural capital and plant conservation, suitable to adapt and apply to Chilean situations. The tissue culture work started in the host institution during the research stay is ongoing and communication is maintained to coordinate culture evaluations.

2. Were the objectives of the fellowship achieved?

a) This objective was achieved. Species were selected, and experiments were planned according to species traits and after scientific literature review. The equipment and materials at the lab where checked, adapted for tissue culture when needed, and some new materials, and a pH meter, where purchased allowing to proceed with the techniques. Importantly, the work was performed in collaboration with staff, technicians and volunteers of the National Seed Bank.

b) This objective was achieved. In vitro cultures were established and several explant responses were evaluated. Shoots with constant growth were generated for 4 species, and a fifth species needs more evaluations in the future. Ferns spores were also cultivated using a specifically developed protocol in order to evaluate spore viability. Spore viability was tested from numerous herbarium specimens belonging to a range of fern species, although viability was not observed.

c) This objective was achieved. Several technicians at the host institution were separately introduced to some of the processes involved in plant tissue culture. Also, a training workshop was run for staff at the Australian National Botanic Gardens (ANBG) and CSIRO (28th Feb). A short talk and poster presentation on a previous research conducted by the visiting researcher and the host collaborator, were presented at a scientific conference (Ecological Society of Australia and the Society for Conservation Biology Oceania; Wollongong, 28th Nov – 2nd Dec, see poster N°63 at https://www.esascbo2022.org.au/wp-content/uploads/2022/11/Posters-at-ESA-SCBO-2022.pdf). A presentation was given for staff and researchers at CSIRO (National Research Collections Australia – NRCA, 9th Feb), and another one for the Plant Science Group of the Friends of the Australian National Botanic Gardens (13th Feb). Finally, a presentation was given at the applicant's institution (Universidad Católica del Maule, 18th March) to inform on the research project and activities.

3. What were the major achievements of the fellowship? (up to three)

a) The generation of protocols for the introduction and tissue culture for selected threatened plant species. This included successful protocols for in vitro shoot proliferation of 4 angiosperm species, specifying culture media, type and concentration of plant growth regulators, and in some cases the use of antioxidants. Additionally, a protocol was developed to assess fern spore germination in vitro, which is valuable to test the viability of spore accessions at the National Seed Bank (ANBG).

b) The introduction of plant material to in vitro culture. This comprised the establishment of shoots leading to stable growth of 4 species (*Eucalyptus recurva*, *Boronia imlayensis*, *Almeleea capitata* and *Leionema lamprophyllum*) that are presently kept in incubators at the National Seed Bank laboratory. This achievement will allow the regeneration of whole plants. In addition, a fifth species (*Eucalyptus imlayensis*) started to produce some buds at the end of the stay, which is considered a good sign for a recalcitrant species (most *Eucalyptus* are recalcitrant to tissue culture), and will need more time to confirm that growth for this species is sustained in time. Fern in vitro culture (gametophytes) was initiated and will be complementary to ex situ conservation of fern plants at the tropical glasshouse of the ANBG.

c) Dissemination of activities and the importance of tissue culture techniques applied to plant conservation were communicated through talks, seminars and a workshop: training workshop at National Seed Bank (28th Feb), presentation at NRCA (9th Feb), talk at the Friends of the ANBG (13th Feb), and seminar at Universidad Católica del Maule (18th March).

4. Will there be any follow-up work?

- Yes, rooting and acclimation will have to be tested in order to complete the propagation process to regenerate whole plants in all selected species. Technicians that worked with the applicant, as well as those who attended the training workshop will be able to undertake this activities.
- Is a publication envisaged? Will this be in a journal or a publication? When will it appear? Yes it is, although more data is needed before starting a manuscript preparation. In the coming months, shoot growth and rooting are expected to be evaluated in order to progress. If enough data is gathered allowing the preparation of a publication, a manuscript may be produced within a year.
- Is your fellowship likely to be the start of collaboration between your home institution and your host? Yes, it is a start. Contacts are underway with two researchers at the Herbarium. In one case, the formulation and application of a research grant is expected. In the second case, a collaboration involving plant specimen collections from Chile is expected for sending material to the Australian National Herbarium.
- Is your research likely to result in protected intellectual property, novel products or processes? No protected intellectual property is expected. The knowledge and techniques developed during this research are expected to be freely communicated to the scientific community.

5. How might the results of your research project be important for helping develop regional, national or international agro-food, fisheries or forestry policies and, or practices, or be beneficial for society?

Please express this in terms of environmental/food security/food safety/economic/health (human and livestock and plant) benefits, etc.

The research conducted helped to set up new procedures and practices at an institution in charge of the conservation of plant biodiversity, especially threatened species. This represent an important aspect for the conservation of natural capital in the long term, and so is beneficial for society. The in vitro protocols developed can be considered as part of practices or tools, while the plant material established in culture is a real product (living plant material). Both can be viewed as actions that help the conservation of plant germplasm and biodiversity. This is especially important when threatened species are involved, because of the current trend in biodiversity loss, which along climate change are the two main environmental negative processes humanity is facing today. During the research stay, the applicant also participated in field trips aimed to collect seeds for rare plant species, contributing with photographic records (see page 5 of the journal 'Fronds' at https://friendsanbg.org.au/sites/default/files/pdf/Fronds103April2023.pdf). Additionally, the applicant collaborated with information generated from the research done at the National Seed Bank, for the production of species fact sheets as part of the project Survive and Thrive, run by the National Seed Bank and the ANBG Nursery.

6. How was this research relevant to:

- The objectives of the CRP?

The research performed strengthened scientific knowledge regarding tissue culture of threatened plants that haven't been previously investigated. This provides relevant scientific information on techniques and tools that can be used to support conservation, and thus inform future policy decisions for a sustainable management of natural resources involving plant biodiversity. The talks and presentations delivered were aimed to inform the public, contributing to the debate on available alternatives for the conservation of biodiversity. The research done also contributes to the application of plant tissue culture techniques for the conservation and propagation of threatened species, by finding and developing specific protocols that will allow micropropagation of selected species. The activities undertaken during the research stay promoted international scientific understanding among Australia and Chile, hopefully opening new opportunities for future collaborations.

- The CRP research theme?

Theme 1: Managing natural capital for the future. The research was relevant mainly for biodiversity issues, but also for land, forests and integrated agricultural production systems. The work done on tissue culture techniques and the culture of material from several plant species with conservation concern, will help for the ex situ conservation of biodiversity, affected by a range of processes such as agriculture, forestry, urbanisation or mining. Importantly, it will also help to propagate those species, providing plants for in situ conservation, restoration or offsetting projects. Thus, scientific information developed by the research will help management practices for a sustainable use of biological natural resources and agricultural systems.

7. Satisfaction

- Did your fellowship conform to your expectations?

  Yes it did, and it was actually over my expectations in the sense that the work developed helped to set up new procedures and techniques that the National Seed Bank will continue to use in order to pursue its goals of ex situ germplasm conservation. The team (researchers, technicians, staff) was all very helpful and extremely kind during the stay, which made the work enjoyable.

- Will the OECD Co-operative Research Programme fellowship increase directly or indirectly your career opportunities? Please specify.

  I strongly consider that it will. The work done will hopefully conduct to a scientific publication, which is a good outcome that contributes to the scientific career. The links strengthened and created with researchers at CSIRO and ANBG will help to collaborate on new proposals (research grants, scientific activities), supporting my scientific career.
- Did you encounter any practical problems?

  The period of the original proposal (17 weeks) was shortened and I had some concern regarding the times to get plant responses (which in tissue culture may take several months). Indeed, rooting was not observed and consequently acclimation was not able to be tested. Hopefully, staff at the National Seed Bank will continue observations and we may have more data in order to build a manuscript.

  A second observation, more personal, was my family life. It was a big financial burden to bring my family with me, even for part of the stay. I am aware that this was my own decision and responsibility, but having a family clearly limits the time I could spend out of the country. At some point (before the departure) I even considered the option of rejecting the fellowship for family reasons. With more funds I may have been able to stay longer and get more research results, having an even richer research work and to strength research links. However, I again stress that I am very grateful to OECD for the fellowship and the research experience was great.
- Please suggest any improvements in the Fellowship Programme.

  Applicants may be more aware that selected proposals may be shortened, which may influence the research itself. Financial family support may be considered.

8. Advertising the Co-operative Research Programme

- How did you learn about the Co-operative Research Programme?

  Through a message that was circulated within the University where I work.
- What would you suggest to make it more “visible”?

  I think word of mouth is very important. You may explicitly ask/suggest (old) applicants to share a message when applications are open.
- Are there any issues you would like to record?

  Getting the wire transaction details took some time and messages, but it worked.

  I am sincerely grateful to CRP OECD for the fellowship, it was a truly nice opportunity and amazing research experience in Canberra. I enjoyed very much the work with people at CSIRO and ANBG teams, to whom I am very grateful too.