



Biotechnology and bio-prospection of native species from Monte desert Patagonia, as strategies for the development of regional bio-economy

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Introduction

The dry-land surface (i.e. semi-arid and arid regions) covers approximately one-third of the world's land area and are inhabited by almost 400 million people. During the last 50 years, there has been growing interest in the cause of desertification due to its impact on the global environment, economy and society. These ecosystems provide a series of ecological services essential for the sustainability of human life and the development of productive activities. In Argentina, the arid and semi-arid environments represent approximately one-third of the country's surface. Particularly, the Patagonia region in Argentina has 80% of surface containing signs of deterioration. In Latin America and the Caribbean (LAC) interest for the bio economy has increased significantly. Argentina is at the forefront and the Province of Buenos Aires and Patagonia region has already approved a Bio economy Strategy. Argentina offers a wide range of options for the local development of bio economy. Our country has a vast territory, a remarkable climate variety and biodiversity, a significant natural and planted forested area, plus highly competitive agricultural and cattle breeding sectors. Besides, biotechnological advances were implemented at an early stage, and advanced scientific and technological skills are available. The conservation of biodiversity depends largely on knowledge generation about our own resources and enhances the native plants that are part of the cultural heritage of the region. The current boom of native species and the lack of studies forces improvement of conservation and propagation methods in addition to the quality of planting material and production in nurseries. At the Universidad of Rio Negro we carried out a Project named *Regional bioeconomy. Propagation and domestication of medicinal, ornamental, aromatic or forest species for the province of Rio Negro (Bioeconomía Regional. Propagación y domesticación de especies medicinales, ornamentales, aromáticas y/o forestales para la Provincia de Río Negro*” (PI-UNRN 40C352). The main purpose was to re-evaluate the properties of native species from argentine Monte (PATAGONIA) and their bio products as unconventional functional foods, nutraceuticals, and dietary supplements, cosmetics and phytotherapy and find propagation strategies through biotechnology to promote multiplication and reintroduction of species to the ecosystem and sustainably manage and conserve them.

The project objectives are:

General objective:

- Propagation and domestication of native species of potential economic value of the Flora Rionegrina, Patagonia.

Specific objectives:

- To apply and adjust techniques of sexual and vegetative propagation of native species.
- Develop and adjust commercial scale harvesting, processing and storage of seeds and in vitro plant production techniques.
- Promote in situ and ex situ conservation of native germplasm in order to maintain biological diversity.
- To form human resources in propagation and nursery of native plants.
- To disseminate the technical and/or theoretical knowledge obtained with the development of the research.
- Establish and strengthen relations in the field of extension and research with provincial government agencies on the issue of the propagation of native species, encouraging the exchange of knowledge and information achieved in the research.
- Integrate different disciplines and institutions with a common goal by training human resources and strengthening the pre-existing institutional infrastructure.

The aim of this communication is to contribute to the body of knowledge of native species from desert and semi-desert regions, in order to provide alternatives for conservation and sustainable use of these resources and to enable them to be included in ecological reforestation and restoration programs in degraded environments. The species studied were: *Prosopis alpataco*, *Prosopis caldenia*, *Geoffroea decorticans*, *Acantholippia seriphioides*, *Condalia microphylla*, *Senecio* sp, *Larrea divaricata* and *Bougainvillea spinosa* (Figure 1).

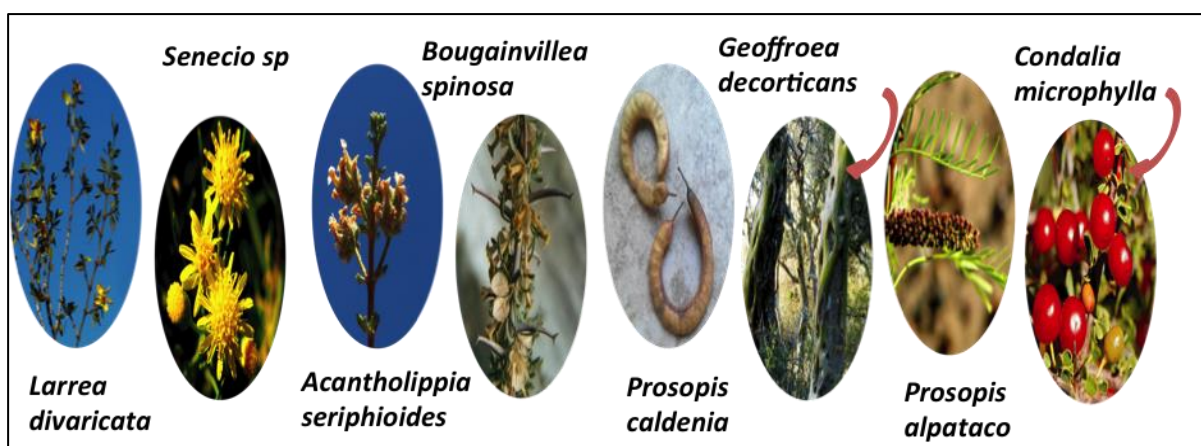
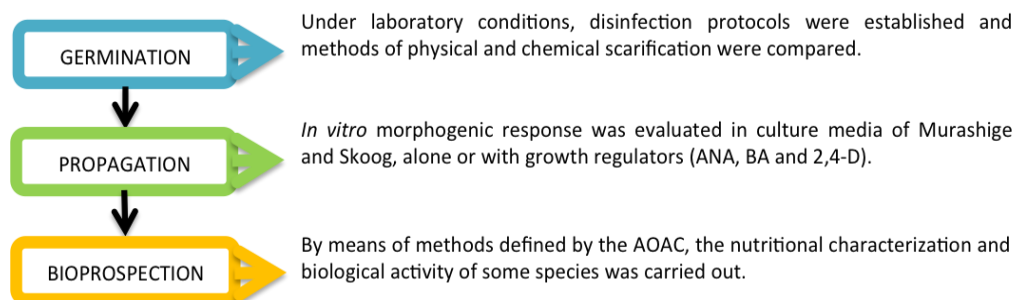


Figure 1. Species studied.

Material and Methods

The mother plants were collected from the field in the Monte Plant Formation, in Rio Negro Province, Argentina. We assayed germination, propagation and bio prospection in different species:



For *in vitro* studies we used as explants the seeds, cotyledons, embryos, and shoot and nodal section from vitroplants. The selected species from arid areas of the Monte desert, generally share the presence of hard and impermeable integuments. Therefore, for each case, we used different types of scarification treatments to optimize the germination process (see Boeri et al., 2018). The embryogenic callus induction of *P. alpataco* from cotyledonary explants was developed as described in Boeri and Sharry (2018). All the culture steps were incubated in a plant growth chamber under controlled conditions of temperature (25 ± 2 °C), under a 16 hr light: 8 hr *dark photoperiod* (provided by fluorescent tubes) and average photosynthetic photon flux of $50 \mu\text{mol m}^{-2}\text{s}^{-1}$. Basic medium composition was Murashige-Skoog (1962). The pH was adjusted to 5.8 with 1N KOH or 0.5 N HCl prior to autoclaving at 121°C for 18 min. The nutritional value was determined according to the Association of Official Analytical Chemists (AOAC) methodology, and phenolic compounds were quantified by diode-array detection (HPLC-DAD). Antioxidant activity *in vitro* and *in vivo* was analysed through the use of the radical species 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and zebra fish model, respectively (Boeri et al, 2017a).

Main Results

Micropropagation was achieved through direct and indirect organogenesis of *P. alpataco* (Fig. 2 a), *G. decorticans* (Fig. 2b), *B. spinosa* and *A. seriphioides* (Fig. 2c) and Somatic embryogenesis of *P. alpataco* (Fig. 2d) (Boeri and Sharry, 2018).

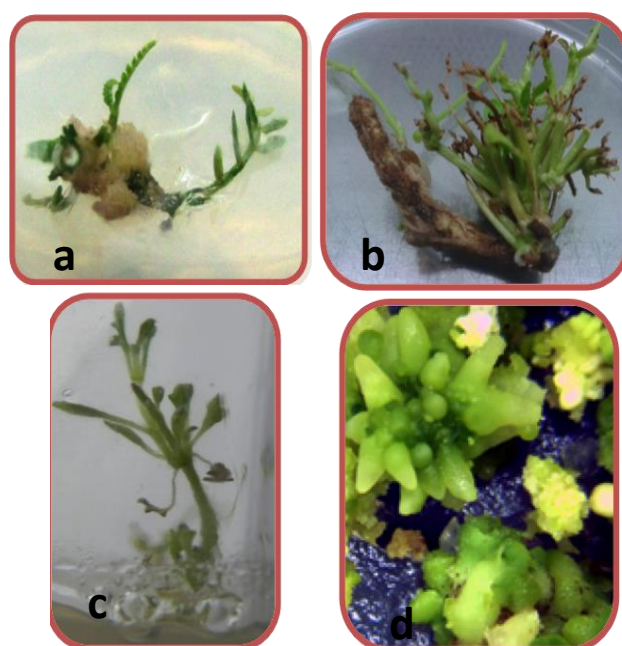


Figure 2. Indirect organogenesis of *P. alpataco* (a), Direct Organogenesis of *G. decorticans* (b) Organogenesis *A. seriphioides* (c) and Somatic embryogenesis of *P. alpataco* (d)

The genetic diversity of regional resources provides the ability to obtain a wide selection of products by bioprospecting, in order to find new foods with high nutritional value for human consumption. The pods of *Prosopis alpataco* constitute a potential resource for both forage and human food. Whole flour was obtained from the full pod. To assess the nutritional value of integral flour (317.15 Kcal / 100 g) the percentage composition was determined. Whole pod flour has a high content of protein and carbohydrates (10 and 62% of dry weight, respectively) and thus pods of this species are a potential food resource. The fat content determination showed that the flour of *P. alpataco* has a low lipid content (3.23%) and the ratio of unsaturated / saturated fat was 4: 1. The presence of antinutritional factors such as polyphenols and phytoagglutinins were evaluated. The flour contained 33.8 mg GAE / 100 g and 0.35 weight HA / mg ml total protein, respectively. In conclusion, the results suggest that integral flour could be used as a dietary supplement for the food industry, either in the preparation of food for human or animal consumption (Boeri et al, 2017b). The integral flour of *P. alpataco* and the pulp of *C. microphylla* indicate could be an important source of carbohydrates. *P. alpataco*, in addition, had a high content of fibers and proteins. *C. microphylla* possesses antioxidant and hemagglutinating activity (Boeri et al, 2017a) (Figure 3).

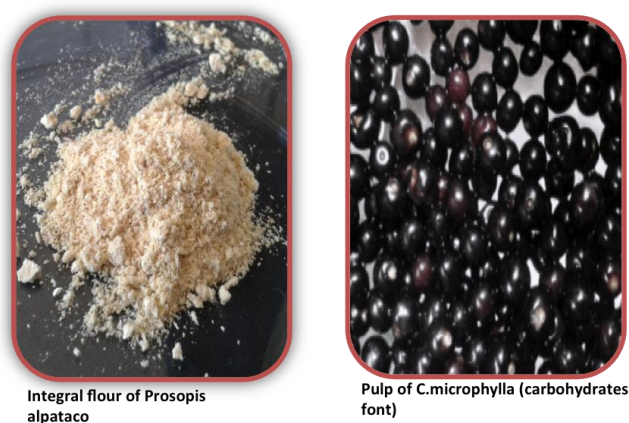


Figure 3. *Prosopis alpataco* flour and *Condalia microphylla* fruits

Condalia microphylla has hemagglutination activity (Boeri et al, 2017a). The results of the biological characterization indicated that the drupes of *C. microphylla* possess a significant antioxidant activity *in vitro* and *in vivo*. Therefore, piquillin fruit is a promising source of antioxidant molecules that could exert a preventive effect against oxidative stress-associated disorders and might be used as a natural additive for food. In addition, the information generated in this work could lengthen the list of bioactive compounds for current scientific evaluation. Further studies are needed to investigate the bioavailability and mechanism of action of the compounds included in piquillin fruits.

The disinfection and germination of the seeds of "thyme", "Senecio", "Chañar" and "Caldén" were adjusted for their subsequent introduction in *in vitro* conditions (Figure 4).

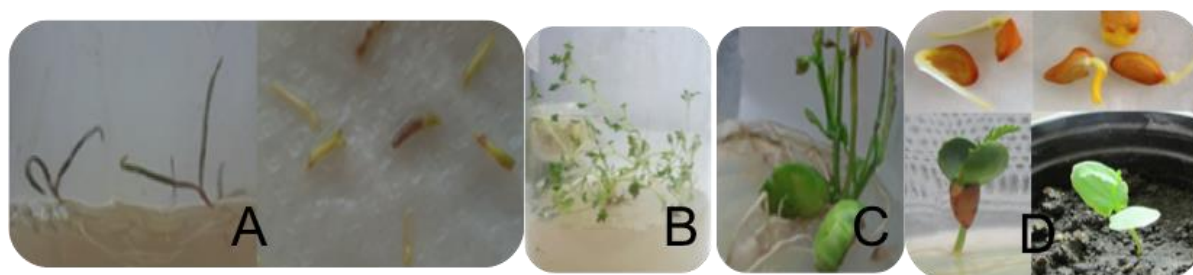


Figure 4. *In vitro* germination of seeds. A. *A. serifoides*, B. *Senecio* sp. C. *Geoffrea decorticans* D. *Prosopis caldenia*.

Transfer-Social Impact

The results of the project, mainly the plants obtained were transferred through agreement and support to the Ministry of agriculture, livestock and fisheries of the province - project PFIP-09 (Cofecyt): "Multiplication of native species of Rio Negro xerophyte monte, from a basic laboratory of plant biotechnology" (Figure 5) (Boeri et al, 2018b).



Figure 5. Plantlets of “alpataco” after rustication and the field of Ministry of agriculture, Rio Negro, Argentina.

Conclusions

The species under study have been systematically used as sources of energy, food or even medicinal use. This has exposed them to extractive type conditions, compromising their future availability, such as *A. seriphioides*, a species that is currently in danger of extinction. This project has allowed to generate knowledge about the propagation of the native flora of the arid and semiarid region, indispensable for the implementation of restoration strategies of degraded ecosystems and the *ex situ* conservation of these species. The conservation of biodiversity depends to a large extent on generating knowledge of our own resources and re-evaluating native plants, which are part of the cultural heritage of the region. The lack of studies of this native species obliges to optimize strategies of propagation for its use-based bioenergy and to generate methodologies of bio prospection that allow knowing its nutritional and medicinal value. The development of the bio-resources area has become a strategic axis for the country, with a strong impact on the regional socio-economic and environmental areas. The evaluations of the medicinal and nutritional properties of the selected species, allowed the identification of products with current or potential uses, and are fundamental for the use and rational protection of biodiversity. On the other hand, this project has allowed to generate knowledge about the propagation of the native flora of the arid and semiarid region, indispensable for the implementation of restoration strategies of degraded ecosystems and the *ex situ* conservation of these species. The great physiological and morphological variability of these species allows their inclusion in programs of reforestation of arid lands in Argentina. The optimization of different propagation strategies and the increased knowledge of chemical prospecting of these resources, adapted to the environmental stress factors that characterize the region, allow us to initiate plans for the restoration of degraded ecosystems in the Monte desert of Patagonia Argentina. This work allowed us to expand the information base of Patagonian biodiversity, recover threatened species and collaborate with the maintenance of regional biodiversity. Biodiversity is the basis of the bio-economy and the sustainable management of regional resources is a fundamental principle of the new circular economy paradigm. In this context, the development of the bio-resources area has become a strategic axis for the country, with a strong regional socio-economic impact.

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