

Novel Therapeutic Uses of Legume Crops in Southern South America

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Abstract

The Argentine flora comprises more than 10,000 species, and many of them have been recorded as having medicinal, antimicrobial, and nutraceutical uses in humans as well as veterinary uses. In this chapter, native species/populations from the north of Argentina have been identified, selected, and characterized using morphological, chemical, and molecular techniques. *Bauhinia forficata* subsp. *pruinosa* was found to have anti-inflammatory, antidiabetic, diuretic, and analgesic activity and *Senna spectabilis* var. *spectabilis* has been found to have antibacterial, antifungal, and antioxidant properties. The characterization and conservation of the native germplasm will allow us to propose future protocols of adaptation and technological processes to improve the quality of life in the rural areas and sustainable growth. This process will be achieved through a future integral and rational use that contemplates the conservation of the wild populations and their habitat. Thus, new resources will be generated, and the native flora of the country will gain value, strengthening the regional and territorial development of the agricultural and agroindustrial system. In addition, the domestication practices oriented to an integral management of the crop without extraction of the biological resource from the natural habitat minimize the impact of ecosystem degradation by overexploitation associated with landscape fragmentation.

Keywords: native species, medicinal, germplasm selection, conservation, propagation

1. Introduction

Leguminosae (Fabaceae) with close to 770 genera and ca. 20,000 species is the third-largest angiosperm family after Asteraceae and Orchidaceae [1]. It has a global distribution and high ecological and economic importance. Along with Poaceae (the grass family), Leguminosae is the most important plant family in the production of food for humans and livestock, as well as in the production of industrial products. The total world exports of pulses (crops harvested for their dry seeds) have more than doubled between 1990 and 2012, expanding from 6.6 to 13.4 million tons, and in 2012, the value of pulse exports was estimated at US\$ 9.5 billion (Food and Agriculture Organization [FAO]: <http://www.fao.org/3/a-i5389e.pdf>). The Food and Agriculture Organization of the United Nations General Assembly

designated 2016 as the International Year of Pulses to promote awareness of their nutritional benefits, importance in food security, and sustainable agriculture, and mitigate biodiversity loss and climate change <http://www.fao.org/pulses-2016/en/>. Meanwhile, from now on, 10 February will mark World Pulses Day, keeping alive the positive momentum surrounding these healthy, nutritious, protein-rich, and nitrogen-fixing legumes after FAO's successful 2016 International Year of Pulses Campaign. Growing pulses contributes to sustainable crop production: <http://www.fao.org/news/story/en/item/1175295/icode/> and the legumes crops providing highly nutritious sources of protein and micronutrients that can greatly benefit health and livelihoods. This family is also uniquely important as fodder and green manure in both temperate and tropical regions and is used for their wood, tannins, oils, and resins, in the manufacture of varnishes, paints, dyes, and medicines, and in the horticultural trade. It has cosmopolitan in distribution, representing important ecological constituents in almost all biomes across the globe and occurs in even the most extreme habitats [2, 3]. The biomes represent significant elements in terms of species diversity and abundance, in lowland wet tropical forests in Africa, South America, and Asia [4], and they dominate dry forests and savannas throughout the tropics [5] and also occur in Mediterranean, desert, and temperate regions, up to high latitudes and at high elevations [6].

This high species richness is reflected in great morphological and chemical diversity, from which multiple uses are derived [7], that is, alkaloids, proanthocyanidins, and flavonoids can be present; pterocarpanes are found only in legumes and they have significant antimicrobial, anticancerous, antiinflammatory, and antimalarial report activities [8]. There are also many legumes containing toxic and indigestible substances, which may be removed through various processing methods.

In America, the cultivation of Leguminosae dates from prehistoric times [9] and it has a great ethnobotanical importance in medicinal uses [10, 11].

Medicinal evaluation has indicated the importance of the environment as an important factor in the selection of useful resources by human populations [12].

In South America, there are reports of the use of Leguminosae species with different therapeutic activities [13–17].

In the South of South America, Argentina conserves *ex situ* collections of germplasm banks in the National Institute of Agricultural Technology (INTA), provincial banks, national universities, and the National Research Council (CONICET). Most of the extra INTA collections hold native species to protect biodiversity from anthropogenic impact. Anthropogenic activity has accentuated the degradation of natural habitats, environmental changes, landscape fragmentation, pollution, the expansion of the agricultural frontier, and over-exploitation. The Argentine Flora Catalog (<http://www2.darwin.edu.ar/Proyectos/FloraArgentina/fa.htm>) has records of more than 10,000 species; many of them have been recorded as having medicinal, antimicrobial, and nutraceutical properties, as well as veterinary uses. The available information has been obtained mainly from ethnobotanical, chemical, and biochemical studies; however, up to now, the chemical compound to obtain phytopharmaceutical products has been recorded only in a few species, and the promising biotypes have not been selected or adapted as new crops; therefore, there is no material available to develop medicinal products that ensure therapeutic efficacy.

The project that initiated the bioprospecting of new medicinal bioactive compounds and agrochemical products in Argentina was conducted at the Institute of Biological Resources (IRB), Natural Resources Research Center (CIRN), INTA, between 1993 and 2003. The proposal was based on national and international legislation (Argentina Constitution: 1994, and Convention on Biological Diversity, 1993, ratified in 1994) and was implemented through the agreement between INTA and University of Arizona, USA (Bioactive Agents from Dryland Plants

of Latin America, INTA-Argentina/University of Arizona-USA, Grant UO1 TW00316 National Institutes of Health (NIH), National Science Foundation (NSF), U.S. Agency for International Development (USAID): International Cooperative Biodiversity Group (ICBG), 1993–2003). That evaluation allowed us to obtain new information on chemical and biochemical compounds of native species used in folk medicine [18, 19]. However, no species genotype or ecotype was introduced as a phytopharmaceutical product [19].

Within the frame of the project, and based on morphological, chemical, and molecular techniques (including the development of expression libraries), native species/populations were identified, selected, and characterized, via management in the natural habitat (*in situ*) and introduction in cultivation (*ex situ*). In addition, with the aim of conserving the identified biotypes, preservation was undertaken in the Germplasm Bank IRB, CIRN-INTA *ex-situ*), with subsequent introduction to cultivation in the different ecoregions where they grow. The characterization and conservation of the native germplasm will allow us to propose technological processes for the improvement of the quality of life in the rural territories; it will also promote sustainable growth through a future integral and rational use that contemplates the conservation of the wild populations and their habitat. In 2010 at the IRB, CIRN-INTA, we started the development of species with medicinal potential, each of them of regional importance in the central and northern regions of Argentina. Based on the results obtained, innovative lines (new-generation domestication) will be included.

- a. *Bauhinia forficata* Link. subsp. *pruinosa* (Vog.) Fortunato & Wunderlin “cow’s hoof, cow’s paw, Brazilian orchid tree, unha de boi or pata de vaca: Brazil”.
- b. *Senna spectabilis* (DC.) H.S. Irwin & Barneby var. *spectabilis* “spectacular cassia, mhomba, carnaval, calceolaria shower, cassia, yellow shower, and Pau-deovelha, São-jão, Parica: Brazil”.

2. Propagation

Bauhinia forficata subsp. *pruinosa* (=BFP): native to southern and eastern Brazil, Paraguay, Uruguay, and Argentina. In Argentina, it is distributed in the northeastern region, and through its introduction as ornamental, it has been naturalized also in the central and northwest regions. It is a deciduous to semi-evergreen tree up to 8 m tall, with twisted ascending branches that drop at the ends, an often-leaning trunk, and large, bilobed, dark green leaves; flowers are white, 8–13 cm in diameter, solitary, or arranged in axillary clusters; they bloom almost all summer. Fruits are dehiscent legumes with several flattened, oval, bright, and blackish seeds. It is used as ornamental and the leaves and young stems are consumed in infusions mainly as antidiabetic or hypoglycemic agent [20–25]. Anti-inflammatory, diuretic, and analgesic activities have also been reported in this species [26, 27]. In Southern South America, 10 species of *Bauhinia* are popularly used mainly to regulate glucose and lipids metabolism, but also for digestive, kidney, and urinary disturbances, for example, [28–31].

BFP has potential for the treatment of Diabetes *mellitus*. Pharmacological studies performed on different plant extracts or purified flavonoids in normoglycemic and hyperglycemic models *in vivo* in general have confirmed the hypoglycemic activity, although some contradictory evidence has been found [26, 32].

It is important to note that the species is unclearly identified in most assays (it is published as *B. forficata* or *B. candicans*, present synonym of BFP); as a result, flavonoid profiles and some tested activities present differences that should be

analyzed in depth [33, 34]. In southern South America, the leaves and young stems are used to prepare a very popular tea due to their effective properties in reducing blood sugar levels. Wild specimens are collected, which violates the safety, quality, and efficacy requirements by WHO Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants (<http://apps.who.int/medicinedocs/es/d/Js5527s/>) and causes loss of biological resources and its habitat. Likewise, it is emphasized that herbal medicines have a variable concentration of active ingredients, which depends on population diversity, the phenological stage at the time of harvest, and the edaphoclimatic conditions in which they grow [35–37]. In our recent studies, one population analyzed from plant materials collected in the Botanical Garden, INTA, presented five glycoside derivatives of kaempferol and quercetin [38], whereas another population/specimens belonging to the same garden did not show the same bioactivity (unpublished data).

The strategy of our proposal to solve this problem is to introduce different types of propagation—asexual and sexual—to evaluate the bioactivity and select the biotypes of BFP that show the best expression of bioactive compounds.

Senna spectabilis var. *spectabilis* (=SSS): tree native to tropical America. In Argentina, it is distributed in the northwestern provinces of Salta and Jujuy, in the zones of the subtropical forest, between 400 and 700 m above sea level. It is also cultivated in the south and east of Africa as an ornamental tree (<http://www.buenosaires.gob.ar/noticias/senna-spectabilis>). It is a small, rounded deciduous tree, 7–10 m (max. 15) tall, and 30 cm in trunk diameter, with a spreading crown, compound leaves, yellow, fragrant flowers, and cylindrical or flattened pods ending in a short, narrow point, hard, not splitting open or slightly on one side, pendulous, more or less cylindrical or slightly compressed, blackish, divided internally by partitions in compartments, aromatic, with numerous compressed and brown seeds. It has various medicinal uses: the leaves are used to treat skin diseases and to prevent constipation and intestinal parasite infections, as well as for headaches and migraines [39], and the leaves, stem, and roots of the taxonomical variety *excelsa* from Brazil are used in depressant and anticonvulsant activities [14]. There are records of antimicrobial activities of leaf extract against *Candida albicans* [40] and *Fusarium graminearum* [41]. In addition, it was shown that extracts of leaves, flowers, stems, and fruits have significant activity against *Escherichia coli*, *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* [42, 43]. In phytochemicals, [44] isolated the alkaloid “(–) cassine” from the leaves and found antiinflammatory activity. On the other hand, [45] found that the anthraquinone compound emodin possessed antimicrobial activity and anti-inflammatory and laxative effects. There are also several reports [46] of populations of *S. spectabilis* from Brazil, whose fruits and flowers have pharmacological effects (analgesic and anti-inflammatory) due to the presence of more than 20 piperine and piperidine alkaloid compounds. It has been also presented important compounds report in leishmanicidal activity [47] and the strongly inhibit cell proliferation of hepatocellular carcinoma cells in alkaloids derived from flowers (in vitro antiproliferative and cytotoxic potentials of alkaloid mixture); if they represent promise antitumor compounds against liver cancer and should be considered for further anticancer in vivo studies [48].

In Argentina, antifungal activities were detected in studies of methanolic extracts from leaves of SSS against *Fusarium graminearum*. In addition, our studies determined antifungal activity against *F. verticillioides*, the causal agent of maize ear rot in fruits and flowers, adding a new record of bioactivity to this plant species [49]. The chromatographic reaction observed suggests that biological activities could be related to the anthraquinone emodin compound, as reported by [50] in *Senna*. *Fusarium* species are mycotoxin producers infecting crops during their

development in the field, as well as their products during storage. The losses in the crops associated with fungi are due not only to yield reduction and the alteration of the commercial and nutritional quality, but also to the production of mycotoxins, which pose a risk for human and animal health [51].

2.1 Long-term Ex situ conservation

The germplasm of the populations that showed promising biotypes is preserved in the germplasm bank (Genetic Resources Conservation at the National Institute of Agricultural Technology (INTA): gene bank/germplasm bank).

According to the records, the seeds of *Bauhinia* and *Senna* are orthodox and can be conserved in long-term banks for the future access of selected germplasm [52]. All seed samples were dried in a controlled environment at between 5 and 20°C and with a relative humidity of 10–25%. After drying, they were placed in an airtight container suitable for long-term storage. In the cases of humidity, 25% of the total, they were stored in nonhermetic containers (medium-term: active collections). The long-term samples (base collections) were stored at $-18^{\circ} \pm 3^{\circ}\text{C}$ and a relative humidity of $15 \pm 3\%$. About 25% of the samples under medium-term conditions (active collections) were preserved at 5 and 10°C and with relative humidity of $15 \pm 3\%$.

2.2 Plants multiplication

BFP and SSS are commonly known species that are cultivated as ornamental tropical plants; however, no selection of the promising biotype with medicinal properties has been attempted. As a consequence, there is a lack of improved cultivars, and there are many difficulties in processing and marketing new crops.

Mature fruits were collected from specimens cultivated in the Botanical Garden, INTA (BFP) in March 2017 and from plant populations growing at different altitudes of the dry land forest in Salta province, northwestern Argentina, in March–April 2017 and from specimens cultivated in the Botanical Garden, INTA (SSS) in April 2017. The fruits of both species were packed in paper bags and transported to the laboratory, where they were thoroughly cleaned and stored in paper bags until the start of the experiments. The seeds were stored in cloth bags under room conditions (20–23°C and 50–75% relative humidity). Most experiments were conducted when the seeds were less than 1 year old (**Figure 1**). Only fully developed, undamaged seeds previously conditioned and disinfected were used for germination experiments: approximately 1000 seeds were mechanically scarified using fine sandpaper (**Figure 2**). The germination test was carried out in May with seeds previously hydrated for 24/48 hours [53]. The experiments were conducted at a constant temperature of 20°C in the greenhouse. Seeds were planted in soil in large pots (6 cm diameter $\times$ 10 cm height) and multi-celled plastic plug-trays: cell length 40 mm, width 41 mm, and depth 50 mm. The substrate mixture used was 70% sifted soil, 15% sand, and 15% perlite (**Figure 3**). The seeds were covered with 5 mm of substrate (**Figure 4**).

2.3 Preliminary results of adaptation

Seeds with 2-mm long radicles were considered germinated. Seedling emergence and height were recorded after 45 days.

In BFP: mechanical scarification increased germination (from 0.4 plants/day in non-scarified seeds to 1.2 plants/day in treated seeds); after 28 days, the percentage of germination was 15% for nonscarified seeds to 86% for scarified seeds. Therefore, scarification favored seedling uniformity (**Figure 5**).



Figure 1.
Mature seeds.

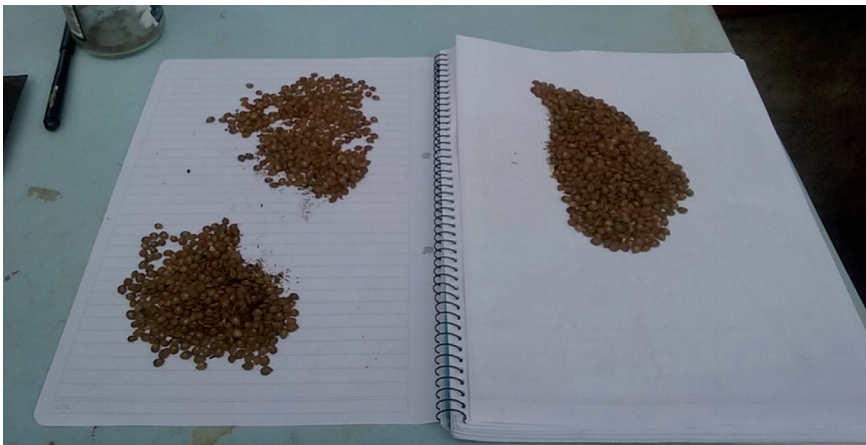


Figure 2.
Seeds scarified and hydrated.

SSS: plants emerged after 3–9 days, and 65% of plants germinated; the minimum and maximum temperatures were controlled. No frost damage was recorded, with the absolute minimum being 13°C. Planting was performed at the end of autumn, in a greenhouse with an automatic convection heating system. The seedlings (60 days



Figure 3.
Substrate mixture.



Figure 4.
Multi-celled plastic plugs.



Figure 5.
Seedling.



Figure 6.
Senna spectabilis transplanted in individual pots.



Figure 7.
Bauhinia forficata subsp. pruinosa: specimens planted.



Figure 8.
Planting scheme.



Figure 9.
Bauhinia forficata subsp. pruinosa: annual growth post-transplant.

old) were transplanted into individual pots: **Figure 6** (8 cm diameter × 19 cm height). They were ready for planting out 4–5 months later.

BFP: to test the propagation and management of plants that were already initially adapted, in September 2018, 3-year-old specimens were collected from Los Robles Park, Moreno, Buenos Aires (34°40'12.2"S 58°51'21.8"W). Those specimens were planted in spring 2015 with seeds from the established mother plants (**Figure 7**).

At the time of transplanting, the plants did not have leaves, since in the province of Buenos Aires, the species behaves as semi-tropical, due to the low winter temperatures (average minimum temperature 8°C). The specimens were approximately 1.52 m tall and 0.65 cm in diameter. Planting scheme was 8 plants/plot, 2 m between plants and 2 m between lines (**Figure 9**), in a randomized complete block design using R (www.r-project.org).

At present, survival, annual shoot growth, and different phenological stages post-transplanting are being monitored (**Figures 8 and 9**).

3. Conclusion

In the adapted specimens, harvesting of the organs of interest will be carried out in the four seasons, coinciding with different crop phenological stages (vegetative, flowering, and fruiting), to measure fresh and dry weight with the aim of determining biomass yield (kg/ha). In the next growing season, the harvested specimens will be evaluated according to regrowth capacity to determine the new available biomass.

Likewise, BFP might be reproduced asexually by the vegetative propagation technique, with plant cutting management. Agamic multiplication will be carried out in ligneous and semi-woody nursery cuttings, using different concentrations of rooters with synthetic auxins and different types of substrate [54].

4. Final remarks

Next-generation domestication: in the age of big data, gene editing, and next generation sequencing (NGS), we have the opportunity to document the transition from wild plant to crop. NGS provides a powerful tool for discovery of domestication genes in crop plants and their wild relatives [55]. The accelerated domestication of new plant species as crops may be facilitated by this knowledge. Re-sequencing of domesticated genotypes can identify regions of low diversity associated with domestication. Novel allelic variation in close or distant relatives can be characterized by NGS; the results give support to the selection and adaptation as new crops and ensure that biomass with therapeutic efficacy will be obtained. The characterization and evaluation of these model species have a phase of construction and validation of genomic cDNA expression libraries with the objective to accelerate the domestication of forest trees in a changing world. This strategy has allowed us to propose plans and technological processes to improve the quality of life in rural territories and to support sustainable growth of wild plant populations and their ecosystem.

Acknowledgements

This work was supported by the National Institute of Agricultural Technology (INTA), the National Research Council (CONICET) and Faculty of Agronomy and Agri-Food Sciences, University of Moron. This evaluation was included in the Project PNHFA-1106094.

The author also thanks María Monsalvo for her adviser during the figure editions.

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