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Revisión | Review

Traditional uses, conservation status and biotechnological advances for a group of aromatic / medicinal native plants from America

[Usos tradicionales, estado de conservación y avances biotecnológicos para un grupo de plantas aromáticas / medicinales nativas de América]

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Abstract: Medicinal and aromatic plants are biologically and economically valuable species because of their intrinsic value as plants, ability to produce secondary metabolites, possible use in the pharmaceutical and food industries, germplasm availability and applications in traditional medicine. In addition, they hold social and economic importance due to the ancestral knowledge they represent and because they are part of the livelihood of many families. Most of them are collected from the wild and are in serious danger of extinction. Through biotechnological tools it is possible to develop their germplasm and obtain new and improved varieties from wild material, while advocating the alternative of production by cultivation instead of extracting it from nature. The objective of this review is to provide an updated perspective on the traditional uses, conservation status and biotechnological advances in a group of 30 plant species native to the American continent.

Keywords: Biotechniques; Aromatic-medicinal plants; Exploitation; Conservation

Resumen: Las plantas medicinales y aromáticas deben ser valoradas tanto por su valor intrínseco como tales, por su capacidad de producir metabolitos secundarios, su posible uso en las industrias farmacéutica y alimentaria y por sus aplicaciones en medicina tradicional. Además, tienen importancia social y económica debido al conocimiento ancestral que representan y porque son parte del sustento de muchas familias. La mayoría de estas especies son recolectadas de la naturaleza y están en grave peligro de extinción. A través de herramientas biotecnológicas es posible desarrollar su germoplasma y obtener variedades nuevas y mejoradas a partir de material silvestre; esta estrategia propicia la alternativa de producción por cultivo en lugar de extraerla de la naturaleza. El objetivo de esta revisión es proporcionar una perspectiva actualizada de los usos tradicionales, el estado de conservación y los avances biotecnológicos en un grupo de 30 especies de plantas nativas del continente americano.

Palabras clave: Biotecnicas; Plantas medicinales aromáticas; Explotación; Conservación

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INTRODUCTION

Biodiversity, defined as the variability of all of nature's living beings in a particular ecosystem or in the entire world (Pathak & Abido, 2014), exists in three different levels: genes, species and ecosystems, each with its own composition and structure. Biodiversity sustains every ecosystem, enabling its possible exploitation (Cardinale *et al*., 2012) and, as such, it is in the origins of agriculture and is the source of all the cultivars and domesticated livestock from the beginning of human civilization. A relevant part of biodiversity is constituted by medicinal and aromatic plants (MAPs), which are species that synthesize pharmacologically active secondary metabolites (active principles). Strictly speaking, aromatic plants are those whose active principles, responsible for their characteristic aroma, are volatile compounds (essential oils).

From the beginning of history, mankind has turned to the Plant Kingdom for food and medicine. In fact, the history of civilization began with the domestication of some plant species and the ensuing emergence of agriculture (which merits the question: who domesticated whom?). MAPs rapidly became part both of mankind´s kitchen and medicine cabinet. They made food more attractive by providing new aromas, tastes and colors. Furthermore, they also cured or alleviated illnesses and, in some species, expanded states of consciousness.

The importance of MAPs can be analyzed from different perspectives. First, they can be examined as living beings: their ecological niche, their intrinsic value as plants, their genes, and the nutrients and metabolites codified by those genes. This biological assessment of MAPs intermingles with their economic importance, since they have multiple industrial applications as pharmaceuticals, food or perfume (also determined by their genes). In addition, their social importance is manifold, because they not only embody ancestral cultural and religious knowledge, but also represent the livelihood of many families.

It is estimated that approximately 400,000 plant species exist and around 60,000 have medicinal properties (UN COMTRADE, 2013; Barata *et al*., 2016), but only a few hundred species are cultivated. Although these are estimates, the data presents an alarming view concerning the situation of the vast majority of MAPs used by man. Our native species are an important source of genetic variability and an invaluable gene pool, since most of them are in a wild state. However, they have not yet been properly and exhaustively explored.

One of the main problems facing wild plants is that, in recent years, the increased demand resulted in indiscriminate harvesting (Barata et al., 2016). For example, in the past decades in Argentina, the productive chain for MAPs was very simple and limited. It consisted of collectors from different regions that harvested the wild “yuyos” (‘weeds’) to obtain an additional income, selling them to tourists and to an incipient herbal industry.

This type of collection, carried out by expert hands, was performed in harmony with nature, favoring development while avoiding predation (Elechosa, 2009). This is part of the "good practices of collection" and it consists in respecting the plant´s cycles, harvesting only the aerial part and, if there are seeds, shaking the branches to distribute them, promoting their germination. These practices allow sustainable harvests because they exploit the part of the population that would otherwise die from natural causes. Therefore, if off-take does not exceed natural mortality rates, and simply replaces it, harvesting is sustainable (Tarciso *et al*., 2017). As can be observed, determining sustainable harvesting strategies requires basic ecological information and, although this detailed information is extremely useful, a considerable amount of local knowledge has not been documented or critically examined (Shanley & Luz, 2003).

Over the years, other protagonists with higher commercial influence, such as the liquor, herbal and food industries, came into play and, consequently, the demand increased significantly. Furthermore, the middleman, who sets prices by buying from the small producers/collectors and supplying the large-scale wholesalers, became increasingly important. The original saddlebag on a donkey became a cart, then a truck and, with time, dozens of trucks. The collection started to be performed in an unsustainable manner, using a metal roll that pulled out the material from its roots and left a razed mount. The future of MAPs worldwide was further endangered by the burning of lands that were ultimately assigned to cattle raising, tourism and real estate business. It is estimated that 15,000 of the global medicinal species are now endangered because of overharvesting and habitat loss (Schippmann *et al*., 2006; Barata *et al*., 2016). In Argentina, an outstanding example can be found in the province of Cordoba, where natural vegetation was lost in the past decades due, mainly, to unstainable collection practices, indiscriminate deforestation, urbanization, the advance of the agricultural frontier and periodic fires, among other factors (Menseguez *et al*., 2007; Arias Toledo *et al*., 2010). This situation demands that we become aware of the threats surrounding these genetic resources and address them in order to reverse the present state of affairs.

Modern biotechnology has several tools that are being increasingly applied to characterize the genetic diversity of plant germplasm. In this respect, it has an essential role in supporting plant conservation programs and the complementary plant breeding programs. The incorporation of biotechnology in conservation programs requires the interaction of multiple and diverse areas, and, in the near future, professionals from different disciplines and with diverse expertise should be included in this effort.

In modern biotechnology, three areas should be included as tools in conservation programs: i) molecular markers, ii) tissue culture, and iii) cryopreservation.

Considering that germplasm is a source of an indeterminate number of genes, molecular markers are an essential tool to recognize and measure the extant and available genetic variability, and to study the structure of different populations and the distribution of genes within the ecosystem. This would contribute towards the sustainability of *in situ* conservation programs and would also secure the progress of genomic studies in these species (Pathak & Abido, 2014).

Tissue culture is perhaps one of the most popular tools of modern biotechnology and, in this context, it is highly relevant for the *ex situ* multiplication of genetic resources, the sanitary status of materials and the recovery of plants in risk of extinction. It is also the groundwork for the application of biotechniques, such as transgenesis or polyploidization, in programs that aim to improve the production of interesting active principles. In addition, tissue culture is a fundamental tool to sustain germplasm banks through micropropagation techniques. These, in turn, enable establishing cultivars in the short to medium term, producing synthetic seeds by way of somatic embryogenesis and, due to their axenic nature, facilitating the transportation and exchange of germplasm between nations (Escandón et al., 2010).

As previously mentioned, tissue culture facilitates the preservation of phytogenetic resources through *in vitro* culture methods that, through adequate changes in the culture environment (Scocchi & Rey, 2010), can decelerate tissue growth and extend the time span between subcultures. The ultimate long-term technique for this strategy is cryopreservation, which consists in storage at liquid nitrogen temperature (-196º C) to suppress growth until a state of “suspended animation” is reached (Scocchi & Rey, 2010).

Informatics and technology have also experienced significant advances, commensurable with Biotechnology. The progress in these fields has revolutionized the research paradigms of plant breeders and conservationists. Currently, it is possible to access DNA databases and available genes easily and rapidly, thus supplying a genomic map that allows a more efficient management of genetic resources. However, this is not available for the majority of the species mentioned in this review.

Secondary metabolites are organic molecules synthesized in some plant species and stored in relatively low concentrations. They don´t play a role in the growth or development of the plant, but they are significantly important in defense mechanisms and as pollinator attractants. These secondary metabolites bestow high commercial value to natural products due to their enormous potential as drugs and other uses for a wide array of industries. Among these active principles, we can mention pigments, essential oils, steroids, phenolic compounds (coumarins, flavonoids, tannins and lignin), saponins, cardiotonic and cyanogenic glucosides and alkaloids.

In the biotechnological productive paradigm, all these compounds could be produced *in vitro*, by way of cell, shoot or root cultures, avoiding exploitation of the wild resource. There are already several examples of substances that are being obtained *in vitro*, such as paclitaxel, ajmalicine, atropine, codeine, dopamine, digitoxin and morphine (Pathak & Abido, 2014). In the context of this paper, the Taxus and paclitaxel example is revealing: ten trees are needed to produce one dose of paclitaxel to treat only one patient, which turns the extraction of paclitaxel from the Taxus bark completely unsustainable. Consequently, biotechnology allows the implementation of strategies for economic exploitation without threatening the survival of the species (Tabata, 2006). Furthermore, it produces a positive social impact generating new sources of work and specialized workforce. This would be an alternative for regions rich in biodiversity whose population must exploit genetic resources to promote economic development, while preserving biodiversity for future generations.

The objective of this review is to provide an updated picture on the traditional uses, conservation status and biotechnological advances for a group of 30 MAPs native to the American continent. The species under study were chosen because most of them are employed by the pharmaceutical, cosmetic and food industries, but are exploited in an unsustainible manner, placing them in a vulnerable situation.

Regardless of the success achieved with those techniques, our aim was to show what has been accomplished and encourage the integration of biotechnology for the conservation and sustainable exploitation of MAP biodiversity.

MATERIALS AND METHODS

Criteria for the selection of plant species

We selected a group of medicinals and aromatic plants native to the American continent, used in traditional medicine and exploited by different industries. The botanical, taxonomical and geographic description of each plant can be found in the following literature: CONABIO (2009); Randall (2012); Instituto de Botánica Darwinion (2017); The International Plant Names Index (2017); Missouri Botanical Garden (2017); Flora do Brasil 2020 (2017); The Plant List (2017). The scientific names and current synonyms for each species were corroborated in The Plant List (2017).

Search strategy

We carried out an extensive review of the available literature to establish if the species involved were threatened with extinction and if any biotechnological tools had been applied in each case. We used the following databases: Google Scholar, Scopus and the MINCyT (Argentine National Ministry for Science and Technology) database. The keywords used were: "biotechnology", "tissue culture", "molecular markers", "cultivation" and "crops". We estimate that for every reference mentioned in this review, five references were examined. In many cases, only one or two references were found and all of them were used.

Criteria to evaluate the risk of extinction

To assess the risk of extinction, we checked the literature and further corroborated that information with the following bibliography: Martínez (2005); Elechosa (2009) and The IUCN Red List (2017).

RESULTS

1) ***Achyrocline satureioides* (Lam.) DC.** Asteraceae ("jate'i ka'a", "marcela", "marcela hembra", "vira-vira"). The aerial parts and the inflorescences have antispasmodic, hepatoprotective, antioxidant, colagogue, choleretic, smooth muscle relaxant, febrifuge and antitussive properties (Kadanian *et al*., 2002; Barboza *et al*., 2009; Retta, 2014). Cosmetic products containing its active principles (mainly quercetin derivates) are commercialized because of its antioxidant and antiinflammatory properties. It is also used in the manufacture of food products, such as tonic beverages. It was officially incorporated in the Brazilian National Pharmacopeia, 4th edition (Juliani *et al*., 2007; Retta *et al*., 2010).

The current demand is met by collecting material in the wild. This not only affects its ecology, but it is also frequently found mixed with similar species that partially share the distribution area, like *Achyrocline flaccida* (Weinm.) DC. and *Pseudognaphalium gaudichaudianum* (DC.) Anderb. synonymous *Gnaphalium gaudichaudianum* DC. (Retta *et al.*, 2010). *A. satureioides* is one of the few native plants that has been subjected to domestication and cultivation processes, mainly in Uruguay and Brazil (Davies, 2011). However, the traditional propagation method of this species is by seeds or by cuttings. With the first method, there is no uniformity in the population obtained; and with the second one it is possible to obtain few individuals for commercial plantations (Severin *et al.*, 2008). In this sense, the use of biotechnology through *in vitro* culture techniques has been proposed in order to obtain a high number of plants with controlled quality.

In the last two decades there have been important advances concerning the *in vitro* introduction and multiplication of "marcela" (Gattuso *et al*., 2007; Severin *et al*., 2008; Kotik *et al*., 2014; Rosso *et al*., 2015). All these authors have been successful regenerating shoots and producing plantlets from nodal segments, cotyledons, hypocotyls and leaf cultures. Among these reports, Kotik *et al*. (2014) informed the most promising results, with interesting multiplication rates and *ex vitro* plants transferred and tested in the field.

The National Institute of Agricultural Research (INIA) in Uruguay has amassed an extensive and outstanding experience in the management of this and other related species, both in traditional cultivation and breeding (Davies, 2011), and in different aspects of tissue culture. Recently, a new variety was developed by the State University of Campinas (Brasil), which was obtained through classic breeding. This variety presents less variability in different phenotypic aspects (Garcia, 2017). However, no improved varieties were yet obtained through biotechniques.

2) ***Actaea racemosa* L.**, synonymous: *Cimicifuga racemosa* (L.) Nutt. Ranunculaceae ("cimicifuga", "hierba de cascabel", "hierba de San Cristóbal", "hierba rica", "raíz de cascabel", "raíces de cohosh negro", "raíz de culebra negra", "raíz de serpiente de cascabel"). This plant has been employed in the treatment of menopause symptoms (nocturnal hot flashes and vaginal dryness, among others) as well as in irregular menstrual cycles, premenstrual syndrome and vaginal discharge. In fact, the "cimicifuga" rhizome is used mainly as a dietary supplement aimed at treating pre-menstrual syndrome, menopause and related gynecological problems (Laboratorios Bagó, 2012). In addition, it is used to treat rheumatism, arthritis, dizziness, sleep disorders, pain and muscular spasm. Its use as antiinflammatory, tonic, diuretic, aperitif, antitussive and antidiarrheal (Vademécum Colombiano de Plantas Medicinales, 2008) has also been reported. The plant is extensively used as an ornamental.

Notwithstanding the important commercialization of this species, cultivated plants represent a minimum part of the total harvest. It was included in the list of rare species (Robbins, 1999) and it may become an endangered species since wild populations have declined because of collection pressure (Lata *et al*., 2002; Hyung *et al*., 2005).

Tissue culture techniques have been reported for conservation and propagation of this endangered medicinal plant, but only one report on *in vitro* culture for plantlet regeneration through leaf-derived calli was found (Lata *et al*., 2002), and its purpose was plant conservation to increase the number of propagules for cultivation or to replace of natural populations. However, this report did not indicate if the process included the transfer of *ex vitro* plants to soil. Also, there is only one communication on hydroponic culture (Hyung *et al*., 2005) and the purpose of this study was to determine the potential for "black cohosh" production in perlite. Although closely related species could be mistakenly included in or substituted for "black cohosh" products on the market, we found only one citation pertaining to the use of the "fingerprint" technique, Amplified fragment length polymorphism (AFLP), to identify the species among other related sympatric species to ensure quality control (Zerega *et al*., 2002).

3) ***Adesmia emarginata* Clos.** Fabaceae, ("paramela"). The plant is used in traditional medicine, mainly because of its analgesic and diuretic properties (Fischer *et al*., 2011). Also, infusions or decoctions of the dry leaves are used as aphrodisiacs (Montes & Wilkomirsky, 1985).

Wild harvesting and the increase in the use of land for crop and forest cultivation (mainly in Chile) have resulted in the decrease of plant population density. However, only a few attempts have been made to domesticate and cultivate this species to prevent its extinction. Fischer *et al.* (2011) reported a preliminary study to characterize the environment and to rescue, propagate and characterize plants both *in situ* and *ex situ*. We found no literature regarding the use of biotechniques.

4) ***Aloysia citriodora* Palau.**, synonyms: *Aloysia triphylla* (L'Hér.) Britton, *Lippia citriodora* (Palau) Kunth, *Lippia triphylla* (L'Hér.) Kuntze, *Verbena citriodora* (Palau) Cav. Verbenaceae ("cedrón", "cidrinha", "hierba luisa", "salvia limão", "verbena de Indias"). This plant is characterized by its lemon-like aroma.

In traditional medicine, the leaves are used in infusions and decoctions as stomachic, digestive, tonic, carminative, eupeptic, in the treatment of dyspepsias, indigestion, nauseas and vomits (Shanley & Luz, 2003), as well as in the treatment of coughs and colds (Barboza *et al*., 2009).

As a result of its digestive properties, "cedrón" is in high demand worldwide by the herbal industry, and is used as an infusion, either alone or mixed with other herbs (Juliani *et al*., 2007).

It is assumed that its essential oil and phenolic compounds (flavonoids, and mainly verbascoside) are responsible for the digestive properties (Pascual et al., 2001). Concerning the chemical composition of the essential oil, Elechosa (2009) reported a large variety of aromas in the same population, and determined the presence of citral, citronellal, carvone-dihydrocarvone, thujones and cineol-carvone. The citral type is the most abundant and the most accepted in the market. In Argentina, up to ten chemotypes have been reported in studies of populations in different provinces, being citral-neral the preferred one. However, several chemotypes are also very interesting due to their composition and possible application in the aromatic industry, such as the ones rich with limonene-citronellal, β-thujone, linalool, carvone, and citronellal (Di Leo Lira *et al*., 2008; Juárez *et al*., 2012). In turn, in samples collected in Chile, the main compounds were sabinene and 1,8-cineol (Di Leo Lira *et al*., 2008).

Progress in several aspects of the propagation of "cedrón" has been made. The specifications for its culture are described in Alonso & Desmarchelier (2015).

The plant has been subjected to different *in vitro* biotechniques, such as direct propagation (Severin *et al*., 2005; Severin *et al*., 2006; Moradi *et al*., 2014) and, very recently, propagation through a previous callus stage (El-Hawary *et al*., 2012; Boustani *et al*., 2016). All these reports evaluated the production of active principles in the cultures, highlighting the differences found between *in vitro*-derived materials and wild-types. Both Severin *et al*. (2006) and Moradi *et al*. (2014) reported high essential oil content in micropropagated plants; whereas El-Hawary *et al*. (2012) suggested that further studies are still required to increase the flavonoid content in the callus obtained. Besides, a protocol for the obtention of hairy roots was described (Figueroa *et al*., 2013), but no references of the effects on the production of metabolites were found.

Although the taxonomical identification was confirmed as *Aloysia citriodora* (The Plant List, 2017), there are reports of synonymia for this species, so it can be found with the names *Lippia citriodora*, *L. triphylla*, *A. triphylla* (Boustani *et al*., 2016). However, very little is known about genetic diversity levels and genetic structure within and between populations, not only in this species but between genera (Suárez González *et al*., 2007). We found only a pilot study concerning chloroplast and nuclear DNA polymorphisms cpDNA, Internal transcribed spacer (ITS) and Inter Simple Sequence Repeats (ISSR) with the aim to document the level of genetic structure with contrasting distributions (natural environments versus home gardens) and contrasting modes of propagation (natural versus human-induced clonal propagation) (Suárez González *et al*., 2007).

5) ***Aloysia polystachya* (Griseb.) Moldenke**, synonymous: *Lippia polystachya* Griseb., Verbenaceae ("burrito"). The infusion of leaves and flowers is used to treat stomach aches, hepatic disorders, slow digestions, indigestions ("empachos"), acid reflux, nausea and vomiting (Menseguez *et al*., 2007; Barboza *et al*., 2009). It is demanded by the herbal ("yerbatera") industries for the formulation of compounded herbs (Juliani *et al*., 2007). Also, the plant has a promising potential for the pharmaceutical industry as eupeptic, carminative and sedative (Mora *et al*., 2005; Hellión-Ibarrola *et al*., 2008).

In Argentina, only two chemotypes were described (Elechosa, 2009): carvone-limonene, with digestive and carminative properties attributed to the presence of carvone (Burdyn *et al*., 2006), and α-thujone-β-thujone, with possible toxic effects since α-thujone is mentioned as an animal neurotoxin (Millet, 1981).

There are references regarding experimental cultivation of "burrito" in Argentina and Paraguay. This species is usually cultivated in home gardens (Alonso & Desmarchelier, 2015), and recent studies have suggested that these may be important reservoirs of unique genetic diversity (Watson & Eyzaguirre, 2002). Despite these reports, there are references of harvesting from the wild (Martínez, 2005) but no reliable ones concerning the cultivation of "burrito".

In vitro propagation is mentioned only in one paper (Burdyn *et al*., 2006). In one report, the authors described the use of these "burrito" micropropagated plantlets for the manufacture of tablets by direct compression of the extract (Aguado *et al*., 2006). The *in vitro* cultivation of plantlets provided uniform materials for the formulation of this product. Also, the authors highlight the importance of starting from uniform plant material (in this case micropropagated plants) when a pharmaceutical product is developed.

6) ***Aniba rosaeodora* Ducke.** Lauraceae ("palo de rosa"). It is a species with great economic importance in the international market due to its linalol-rich essential oil, used as a fixative in the perfumery industry (Sampaio, 1999). The oil is extracted almost entirely from the wood, and the extraction method results in the destruction of the tree (May & Barata, 2004). The high demand for this product has resulted in the overexploitation of the species through the indiscriminate extraction of complete adult

individuals. Besides, there are factors related to the physiology and ecology of the species, such as the intensive predation of the seeds by birds and insects, and the extreme recalcitrance and low germination rate of the seeds (Yepes *et al*., 2010).

This has led to the decline of many natural populations and the loss of important variability, to the point that it has been included as a species threatened with risk of extinction in countries such as Colombia, Brazil, and Surinam (IBAMA, 1992; Calderón, 1997; Oldfield *et al*., 1998; May & Barata, 2004; The IUCN Red List, 2017). Due to this situation, the export of essential oil has drastically fallen in the last 30 years.

To reverse this situation, multiplication procedures for this species have been developed (Viera, 1970; Sampaio, 1987; dos Santos Britto & Sampaio, 2003; Yepes *et al*., 2010). However, the results have not been satisfactory in terms of increasing survival through the production of seedlings, due to the slow growth of the species (Valencia *et al*., 2010).

Concerning biotechnology, *in vitro* culture techniques to promote the propagation of the species have been established. Only two reports dealing with micropropagation were found (Handa *et al*., 2005; Jardim *et al*., 2010). These works analyzed aspects of the *in vitro* culture, such as the use of embryos´seeds and bud explants (Handa *et al*., 2005) or the effects of different growth regulators (Jardim *et al*., 2010). Although these reports showed successful results, there were no references concerning further uses of the developed materials in conservation programs.

Molecular markers were developed for this species to estimate genetic diversity in the populations which remained in their natural habitat. To contribute to effective strategies for the conservation and management of this threatened Amazonian forest resource, Angrizani *et al*. (2013) evaluated SSR to estimate gene flow and characterize population genetic diversity, structure and mating system of the species. Santos *et al*. (2008) used Random Amplified Polymorphic DNA (RAPD) and they informed that populations retain high diversity, mainly in a population that had been protected for more than forty years in a Reserve. They suggest the creation of a new reserve as a strategy for the protection of the variability of this threatened species.

7) ***Argemone mexicana* L.** Papaveraceae (“cardo amarillo”, “cardo santo”, “chicalote”). It is considered a weed in various parts of the world (Randall, 2012). Different parts of this plant are used in chronic skin diseases, and also as emetic, expectorant, demulcent and diuretic; the seeds and seed oil are employed as a remedy for dysentery, ulcers, asthma and other intestinal affections (Brahmachari *et al*., 2013). This plant produces alkaloids (protopine, berberine, sanguinarine, inter alia) in all its organs and it is reputed to have sedative, sleep-inducing, antiinflammatory and antibacterial effects (Harborne & Williams, 1983; Brahmachari *et al*., 2013).

An interesting work reports the use of leaf extract of *A. mexicana* for the synthesis of nanoparticles in the search of value-added products for biomedical and nanotechnology based industries (Singh *et al*., 2010).

The plant that is used for medicinal purposes is harvested in the wild. However, some blog spots indicate that in the United States and Europe it is cultivated as an ornamental.

In vitro cell cultures of this species have been established, and they produce and accumulate sanguinarine at levels comparable to those in plant tissues (Ziegler & Facchini, 2008; Trujillo-Villanueva, 2010). However, we did not find further references concerning the use of these cell cultures as a source of metabolite production at industrial scales. In addition, there is a protocol that describes transient transformation via *A. tumefaciens* (Godoy-Hernández *et al*., 2008), but there are no recent reports concerning transgenic *A. mexicana*.

8) ***Baccharis articulata* (Lam.) Pers.** and 9) ***B. trimera* (Less.) DC.** Asteraceae, (“carquejas”). Several species of the genus *Baccharis* are known as “carquejas” because they are very similar morphologically and cannot be easily distinguished (Simões-Pires *et al*., 2005). Also, their uses in traditional medicine are similar. The aerial non-woody parts of both species are mainly used in the manufacture of non-alcoholic bitter beverages because of their hepatoprotective, digestive and antiinflammatory properties. Furthermore, infusions and decoctions are traditionally used as antidiabetics due totheir antihyperglycemic properties (Juliani *et al*., 2007; Barboza *et al*., 2009). Hepatoprotective and antihyperglycemic properties in both species are mainly attributed to the presence of luteolin, quercetin and 4'-O-beta-D-glucopyranosyl-3',5'-dimethoxybenzyl caffeate (BaII) (Soiche & Leng

Peschlow, 1987; Oliveira *et al*., 2005; Kappel *et al*., 2012). In addition, *B. articulata* has been ascribed aphrodisiac and sexual stimulant properties (Barboza *et al*., 2009). With respect to its important volatile fraction, whereas the main principles of the essential oil of *B. articulata* are β-pinene, spathulenol, (E)-nerolidol, β-caryophyllene and bicyclogermacrene (Zunino *et al*., 2004), in the case of *B. trimera*, carquejyl acetate is the most important constituent, not only for its antispasmodic activity (Torres *et al*., 2000) but also because it could be a chemical marker for this species (Simões-Pires *et al*., 2005; Minteguiaga *et al*., 2018).

The exploitation of these species is carried out by wild harvesting and, due to its high demand (Martínez, 2005), the wild populations in Argentina are subjected to immense pressure. In the case of *B. trimera*, many of its populations are exposed to the risk of extinction (Céspedes de Zárate *et al*., 2009). However, we could not find data regarding its cultivation, except for staking assays (De Bona *et al*., 2005) and small-scale gardening.

Regarding biotechniques, in the reviewed literature only in *B. trimera* there are reports on studies of inter-population genetic variability through isoenzymes (Cúneo, 2012) and molecular markers of the alu ISSRs type in studies of genetic diversity between species (Vaco, 2011); no references were found concerning *B. articulata*. The propagation under *in vitro* conditions is not documented, neither for *B. articulata* nor *B. trimera*.

10) ***Bauhinia forficata* Link.** subsp. *pruinosa* (Vogel) Fortunato & Wunderlin. Fabaceae (“bauhinia”, “pata de chivo”, “pata de vaca”, “pezuña de vaca”). This species has been claimed to possess antidiabetic activity and strong hypoglycemic effects, making it a therapeutic alternative for the treatment of diabetes mellitus (Marques *et al*., 2013). The active principle responsible for this pharmacological effect is trigonelline, which is extracted from the leaves (Toloza-Zambrano *et al*., 2015). Currently, in Uruguay, a product (drops) developed from *B. forficata* is commercialized for the treatment of diabetes mellitus Type 2 (Diabenat, 2011).

Concerning the development of biotechnological tools, micropropagation of the genus *Bauhinia* has been extensively reviewed by Teixeira da Silva (2013). According to this author, the studies on the *in vitro* culture of *Bauhinia* species are still at an early stage. As their medicinal and ornamental value increase, greater attention will be paid to tissue culture. Currently, reliable protocols exist for the micropropagation of several *Bauhinia* species. In this context, it is important to highlight that there are a few reports on the use of cell cultures (Mello *et al*., 2000; Appezzato-da-Glória & Machado, 2004; Lima, 2009). Mello *et al*. (2000) proposed the establishment of a protocol for *in vitro* plant regeneration from cells of *B. forficata* that could be used in plant propagation and transformation assays. However, no further reports concerning transformation were found. Lima (2009) established a cell culture protocol to produce phenolic compounds and proteins with hypoglycemic activities, but we could find no current advances for this work.

Despite its medicinal importance, commercial cultivation is not feasible since its propagation is negatively affected by the long period (10-12 months) between flowering and seed production (Lorenzi, 1992; Mello *et al*., 2000). However, in Brazil it is included in the National Policy of Medicinal Plants and Phytotherapics and the National Program of Medicinal and Phytotherapeutic Plants within the Unified Health System (SUS), with common objectives aimed at guaranteeing safe access and rational use of medicinal plants and phytotherapics (Brasil, 2006).

11) ***Carapichea ipecacuanha* (Brot.) L.** Andersson, synonyms: *Callicocca ipecacuanha* Brot., *Psychotria ipecacuanha* (Brot.) Standl., *Cephaelis ipecacuanha* (Brot.) A. Rich. Rubiaceae, (“ipecacuana”). It is well known for its expectorant and emetic properties, and it is also effective in the treatment of amoebiasis. Its main active principles are the isoquinolic alkaloids cephaeline, emetine and dehydroemetine (Teshima *et al*., 1988).

For approximately 300 years, “ipecacuana” has been subjected to over-extraction practices in Tropical America, mainly in Brazil. At that time, harvesting was uncontrolled and there was no replanting after the uprooting of native populations (Oliveira & Martins, 1998). Around the mid-20th century, plantations were initiated under the arboreal cover of the tropical rainforest, first in Nicaragua and later in Costa Rica. Hence, the cultivation of high quality roots, aimed exclusively at satisfying the international market demand, was established. In contrast, the root´s supply in Brazil is procured through wild harvesting of the populations in the Mato Grosso state. As a result, the roots present higher variability in their secondary metabolite content and the plant is at high risk of extinction (Ocampo, 2007). A similar situation has been described in Colombia (Yoshimatsu *et al*., 2003; Lucía Aterhotua, personal comunication).

Biotechnological tools for this species have been extensively developed, beginning with reports on micropropagation (Jha & Jha, 1989; Yoshimatsu & Shimomura, 1994), indirect regeneration (Rout *et al*., 1992) and somatic embryogenesis (Rout *et al*., 2000). In relation with the work carried out in tissue culture, long term cultures were achieved. This *in vitro* germplasm, maintained through reduced growth conditions for more than a decade, turned out to be a successful mode of preservation when it was used as source material for micropropagation, obtaining complete plant regeneration with phenotypic and chromosomal stability (Chaudhuri & Jha, 2008). However, Yoshimatsu & Shimomura (1994) pointed out an important limitation to this clonal propagation. They found that field-cultivation of "ipecacuana" plants is restricted to a tropical or subtropical region because the plants were significantly damaged when the monthly average of minimum temperatures was below 10° C.

As an alternative source of active principles, production of *in vitro* alkaloids via root culture (Teshima *et al*., 1988; Yoshimatsu & Shimomura, 1991; Karuppusamy, 2009) and via callus culture (Jha *et al*., 1988; Teshima *et al*., 1988; Tripathi & Tripathi, 2003) has been reported. Roots cultured in solid and liquid medium yielded similar content of emetine and cephaeline as the roots from a mother plant grown in greenhouse conditions (Teshima *et al*., 1988).

Furthermore, transformed cultures via *Agrobacterium rhizogenes* have been established (Yoshimatsu *et al*., 2003). The yield of cephaeline reported by these authors in transformed roots was over twice that in the non-transformed roots of similar age reported previously by Yoshimatsu & Shimomura (1991). However, when Yoshimatsu *et al*. (2003) regenerated plants from transformed roots, they found that the alkaloid contents in the transformed plants, both in the leaves and roots, were lower than those of non-transformed plants. Thus, these authors proposed the transformed root cultures as a promising tool for an alternative source of ipecac alkaloids and for biosynthetic and transport studies of *ipecac* alkaloids.

Finally, the use of ISSRs in this species was reported. Rossi *et al*. (2009) found high genetic differentiation between Amazonian and Atlantic "ipecacuanas". De Oliveira *et al*. (2010) applied these molecular markers to assess the implications of clonality for the *in situ* genetic conservation of "ipecacuana" to establish efficient strategies for the conservation of this species.

12) ***Cecropia pachystachya* Trécul**, synonymous: *Cecropia adenopus* Mart. ex Miq. Cecropiaceae ("ambaí", "ambay", "amba-hu", "palo lija"). The leaves of "ambay" have a long tradition of medicinal use in the treatment of respiratory diseases, mainly as an antitussive and expectorant (Argentina-Paraguay-Brazil) (Arias Toledo, 2009; Alonso & Desmarchelier, 2015). It is accepted in herbal medicine for treating coughs and asthma, and as cardiotonic and diuretic, but it also used as a dietary supplement (Gupta, 1995).

The pharmaceutical industry combines it with guayacol to prepare tablets (Laboratorios Weltrap). Recently, "ambay" has been introduced in the Primary Health Care System in the province of Misiones, Argentina (Alonso & Desmarchelier, 2015).

"Ambay" is harvested from the wild (Alonso & Desmarchelier, 2015). These authors also indicate that this species can be found as cultivated, but the provided information is very scarce and vague and, in fact, there are no references about the location of these cultivations. Besides, they include requirements of culture and forms of reproduction although no reference is reported. Despite the constant demand for its active principles H (ambain and ambainin) and C (cecropine and cecropinine), we did not find reports in the literature concerning the use of any biotechnique, such as propagation, breeding or genetic diversity studies.

13) ***Equisetum giganteum* L.** and 14) ***E. bogotense* Kunth.** Equisetaceae ("cavalinho gigante", "cola de caballo"). Although they are different, they share the same vernacular names and applications in traditional medicine. The infusions prepared with the aerial parts are used as diuretic (Menseguez *et al*., 2007). In addition, this species was found as a constituent of aphrodisiac mixtures (van Andel *et al*., 2012).

Both species possess interesting potential for the pharmaceutical industry, and *E. giganteum*, whose diuretic activity is known (Pérez Gutiérrez *et al*., 1985; Cáceres *et al*., 1987), is frequently employed to replace *E. arvense*, being extensively commercialized as a dietary supplement (Francescato *et al*., 2013). In addition, *E. giganteum* was proposed as a promising alternative for the treatment/prevention of dental stomatitis and oral candidiasis (Alavarce *et al*., 2015), and as antiinflammatory in rheumatoid arthritis and other inflammatory afflictions (Farinon *et al*., 2013).

No bibliographic references were found concerning domestication and/or cultivation of either species. There are indications of over-harvesting of *E. giganteum* in Argentina, which may endanger the species (Martínez, 2005; Alonso & Desmarchelier, 2015). However, Alonso & Desmarchelier (2015) also indicate that *E. giganteum* is cultivated as ornamental around lakes and lagoons. In the case of *E. bogotense*, Rodriguez *et al*. (1994) have reported that since very little has been done on cultivation in Chile, the plant is usually gathered in nature.

Only one report on the micropropagation of *E. bogotense* was found (Rodríguez *et al*., 1994), but no further techniques were used for breeding. For *E. giganteum*, no references were found. However, there have been studies regarding its secondary metabolism and its products (Alavarce *et al*., 2015) and also on the taxonomical relations of the genus *Equisetum* through the sequencing of chloroplast DNA and by “fingerprints” with ISSRs (Des Marais *et al*., 2003; Brune *et al*., 2008, respectively).

15) ***Eremanthus erythropappus* (DC.) MacLeisch**, synonymous: *Vanillosmopsis erythropappa* (DC.) Sch.Bip., *Albertinia erythropappa* DC. Asteraceae (“candeia”). This species is used in folk medicine as a scarring agent and in the treatment of infections and stomach ulcers (Silvério, 2004). Currently, the main product of interest is the oil, rich in α-bisabolol, extracted from the whole plant. This active principle is widely used in the pharmaceutical and cosmetic industries (Rosal *et al*., 2007).

The tree is under threat because of intensive exploitation (Souza *et al*., 2007) and because its natural environment is constantly shrinking, mainly due to the accelerated anthropic action (Vieira & Martins, 2000; Pereira & Gama, 2010).

Since sustainable supply of “candeia” oil can no longer be guaranteed, the Symrise Company has decided to stop harvesting natural bisabolol from the “candeia” tree. This company focuses now on the production of highly pure nature-identical bisabolol: Dragosantol® and Dragosantol® 100 (Symrise, 2011).

Efforts to multiply and preserve this valuable species through the application of biotechniques, such as micropropagation, have been made (Rosal *et al*., 2007; Prudente *et al*., 2016). However, both references only describe an established protocol to multiply this species, since *ex vitro* germination rate of the seeds is considered low. In this context, the objective was to establish a protocol for *in vitro* germination and multiplication, aiming at the rapid multiplication of the species. We could not find any reports concerning the use of *in vitro* culture to produce the active principles of interest. Concerning the use of molecular markers, RAPDs have been applied in order to study its genetic diversity (Moura, 2005; Estopa *et al*., 2006; Freitas *et al*., 2008).

16) ***Fabiana imbricata* Ruiz & Pav.** Solanaceae (“palo pichi”, “pichi”, “pichi romero”). The infusion obtained from the bark and stems of this shrub is used in traditional medicine as a digestive and diuretic agent (Muñoz *et al*., 2001; Navas, 2001; Schmeda-Hirschmann *et al*., 2004). In Chilean folk medicine, this infusion is also used to treat *Fasciola hepatica* infections in goats and sheep (Navas, 2001). This species is registered in the Homeopathic German Pharmacopoeia. In other countries, this shrub is used as an ornamental plant in gardens (Grüneberg, 1995).

The health-promoting properties reported for the infusions of *F. imbricata* can be associated with the presence of rutin, scopoletin, 3-O-caffeoylquinic acid, quercetin together with its derivatives (Reyes *et al*., 2005; Quispe *et al*., 2012).

This species is collected from the wild and, in this context, a few attempts have been made to domesticate and cultivate this species (Schmeda-Hirschmann *et al*., 2004; Fischer *et al*., 2011).

Concerning the application of biotechniques, an *in vitro* propagation system leading to the formation of shoots, calli, roots, cell suspensions and plantlets has been developed (Razmilic *et al*., 1994; Schmeda-Hirschmann *et al*., 2004). Schmeda-Hirschmann *et al*. (2004) evaluated conditions (temporary immersion system, morphogenesis and maintenance of cell suspensions) for the regeneration of different organs of *F. imbricata* grown under *in vitro* conditions as an alternative for active compound production and regeneration of selected plants. Their results showed an important variation in the production of the metabolites, but there is no information on the secondary metabolite production of *in vitro* cultures and its variation according to explant-type and growth regulators.

17) ***Justicia pectoralis* Jacq.** Acanthaceae (“amansatoros”, “anador”, “curia”, “té criollo”, “tilo”). The decoction of the fresh or dry leaves is used generally as a neurosedative (Fernández *et al*., 1989; Rodríguez *et al*., 1989). The plant is also employed as an expectorant, antiasthmatic and analgesic (Lino *et al*., 1997). In addition, it is traditionally used in menopause and premenstrual syndrome due to its estrogenic and progestogenic activity (Locklear *et al*., 2010).

One of the most representative compounds of this species is justicidine B. This is a potent cytotoxic substance on several cell lines, especially chronic myeloid and chronic lymphoid leukemia. This property makes justicidin B a potential onchopharmacological agent (Hemmati & Seradj, 2016).

The properties of J. pectoralis make it a promising commercial species, and the Cuban pharmaceutical industry is actively developing technological processes to obtain pharmaceutical quality products from the natural compounds of this species. In this sense, medicinal syrups (Martín *et al*., 2011) and tablets (Rodríguez *et al*., 2015) have been obtained.

Despite all the pharmacological progress with this species and the pharmaceutically valuable characteristics of justicidin B, we could only find one recent report on micropropagation (Oliveira Freitas *et al*., 2016). Although this work is very relevant since it is the first one related to *J. pectoralis* tissue culture, it refers only to the evaluation of different basal media and growth regulators, and it does not show results in terms of the acclimation phase and transfer to soil. Furthermore, it does not evaluate the production of neither justicidin nor other principles. This is disturbing because in the bibliography regarding the production of justicidin B using biotechnology, this principle is not currently being obtained from this species. *J. pectoralis* is also cultivated as an ornamental for urban woodland.

18) ***Larrea divaricata* Cav.** Zygophyllaceae (“jarilla del cerro”, “jarilla hembra”). Together with another species of this genus, *L. cuneifolia* Cav., they constitute the “jarillales”, which are frequently considered desertification indicators. Both are regarded as anthropic species, since a terrain dominated by “jarillas” indicates that the area was subjected to intense grazing for long periods of time or suffered periodic fires (Femenia, 2014).

Several preparations are obtained from the *L. divaricata* plant, especially from its leaves and stems. Traditionally, the leaves´ infusion is recommended for the treatment of stomach disorders, arthritis, rheumatism, fever, bronchitis and other respiratory ailments (Barboza *et al*., 2009; Palacio *et al*., 2012).

The species is reputed to have antiinflammatory, antitumoral, antiviral and antimicrobial properties. These properties have been attributed to its most frequent biologically active compound: nor-dihydroguaiaretic acid (NDGA) (Anesini *et al*., 1998; Anesini *et al*., 1999; Konigheim *et al*., 2005; Svetaz *et al*., 2013). In addition, its abundant flavonoids, mainly quercetin, kaempferol, ferulic acid and coumaric acid, are considered anticancer agents, and there is a possible synergism among the compounds (Palacio *et al*., 2012).

Concerning its essential oil, α-thujene, myrcene, limonene, (E)-ocimenone, thymyl acetate, γ-elemene, spathulenol, β-oplopenone, α-cadinol and the eudesmol isomers are the main constituents (Barboza *et al*., 2009). However, it yielded very low amounts of essential oils. In contrast, high yields of alcoholic resinoids were obtained, which suggest that this species could be used as a natural fixative in perfumery (Juárez *et al*., 2016).

Commercially, two types of shampoo can be found: antidandruff and to treat alopecia. Both were developed by CONICET researchers from extracts of “jarilla” and “café” (EcoHair, 2013).

No references were found concerning the cultivation of this species, nor its conservation status. Nevertheless, it has been propagated through *in vitro* culture techniques such as micropropagation, indirect regeneration through calli and cell cultures (Palacio *et al*., 2008; Palacio *et al*., 2012).

Despite the growing potential of clinical uses for NDGA and its derivatives, its production in *L. divaricata* plant cell cultures has only been carried out in the last six years (Palacio *et al*., 2011; Palacio *et al*., 2012). In this sense, studies have evaluated the optimal conditions for the *in vitro* growth of calli and cell suspensions, as well as cell growth kinetics and the production of active principles. Calli, both with and without organogenesis, produced NDGA and quercetin. NDGA, together with p-coumaric acid, ferulic acid and sinapyl alcohol, was also produced by the cell suspension cultures (Palacio *et al*., 2012).

These authors demonstrated that the capacity of undifferentiated tissues to form phenolic compounds (NDGA and quercetin) is very limited, but when the calli underwent organogenesis, developing mainly adventitious shoots, the phenolic compound production significantly increased. This data indicated that the content of NDGA and quercetin increased with differentiation. However, plantlets regenerated from adventitious shoots of *L. divaricata* calli did not show the same phenolic pattern as wild plants, with lower levels of NDGA and quercetin.

19) ***Lepidium meyenii*** **Walp.** Brassicaceae ("ayak chichira", "ayak willku", "maca", "maca-maca", "maino"). This species has been domesticated in the Puna agro-ecological zone of central Peruvian Andes, where it is cultivated for its edible underground organs (called "roots") (Wang *et al*., 2007).

"Maca" has been consumed for many years and it is considered as an adaptogen because of its special properties (nutritional and energizing), since it provides the energy and endurance needed for daily activities (there is a large bibliography about it). Recently, "maca" has been treated as a plant with added value and many people in the world are using it as a tonifier and powerful revitalizing agent (Polzerová *et al*., 2011; Sifuentes-Penagos *et al*., 2015). In addition, it is highly demanded as a dietary supplement, because of its nutrient content (Ronceros *et al*., 2005). Also "maca" is employed traditionally to improve sexuality and fertility (Sifuentes-Penagos *et al*., 2015), properties that have been demonstrated in studies not only in male rats and mice but also in men (Gonzales *et al*., 2001; Gonzales *et al*., 2002).

Due to its aphrodisiac effects, its consumption is gradually increasing. However, the source of available "maca" seeds is restricted, because the growth conditions for its cultivation are not favorable in certain environments (Wang *et al*., 2007; Polzerová *et al*., 2011). In this context, "maca" has been subjected to the application of tissue and organ culture techniques with the aim of producing an adequate number of plantlets for cultivation. There are reports on its *in vitro* propagation through micropropagation by shoots derived from calli (Cheng *et al*., 2004; Wang *et al*., 2007), but hyperhydricity was frequent. On the other hand, Polzerová *et al*., (2011) established an effective multiplication system through direct organogenesis from nodal segments (axillary buds) with good regeneration rates, high rooting efficiency and no hyperhydricity. This system was able to produce a large number of uniform plants. These reports showed an auspicious scenario for "maca" propagation and for obtaining clonal plants from selected material. Besides, the use of microsatellites on chloroplastid DNA was reported (Hasan et al., 2010). Furthermore, "maca" has been used for the creation of somatic hybrids with Brassica oleracea L. (Ryschka et al., 2003).

20) ***Lippia alba*** **(Mill.) N. E. Br. ex Britton & P.Wilson.** Verbenaceae ("herva cidreira", "pronto alivio", "salvia de campo", "salvia de monte", "salvia morada"). It is used extensively in traditional medicine, mainly to treat digestive and respiratory disorders, but it also has sedative, analgesic, antipyretic and antiinflammatory properties (Pascual *et al*., 2001; Hennebelle *et al*., 2008a). This species is characterized by the variability in the chemical composition of the essential oils. There are several chemotypes – at least seven described – which differ in the main components of the essential oils. These are: chemotype I (citral, linalool and β-caryophyllene, as the main constituents), chemotype II (tagetenone), chemotype III (limonene and carvone or related monoterpenic ketones), chemotype IV (myrcene), chemotype V (γ-terpinene), chemotype VI (camphor-1,8-cineole) and chemotype VII (estragole) (Hennebelle *et al*., 2008a; Ricciardi *et al*., 2009; López *et al*., 2011).

The essential oils, either extracts or their constituents, have been claimed to possess antispasmodic, antiinflammatory, antiviral, antibacterial, antifungal and antiparasitic activities (Ruffa *et al*., 2004; Ara *et al*., 2009; Ocazionez *et al*., 2010; Blanco *et al*., 2013; Gómez *et al*., 2013). Among the non-volatiles, flavonoids and iridoids have been detected, and these are apparently responsible for the sedative effects of the species (Hennebelle *et al*., 2008b).

Although in Argentina there have been attempts to domesticate some of the chemotypes described above (Ringuelet & Cerimele, 2010), the preferred choice of exploitation is the collection of wild specimens (Alonso & Desmarchelier, 2015). These last authorts also indicate data on cultivation, although there is no reference report. Cultivated specimens can be found mainly for ornamental and culinary uses, particularly in Central America, Mexico, Colombia and Brazil (Urdaneta & Kanter, 1996). Asexual propagation methods are preferred for this species (Pimenta *et al*., 2007; Seaforth *et al*., 2008; Herrera-Moreno *et al*., 2013) due to the low germination of seeds of *L. alba* (Gupta *et al*., 2001).

Concerning the use of biotechnology, for this ethnobotanical useful species, only two reports on its *in vitro* propagation have been found (Gupta *et al*., 2001; JibinaBai *et al*., 2014). Based on the conclusion drawn by Gupta *et al*. (2001), who observed that the phenotype and oil profile of the micropropagated plants were identical to the normal vegetatively propagated ones, the micropropagation protocol adjusted for *L. alba* ensures the cloning of selected genotypes. On the other hand, the interrelation between different accessions and chemotypes of *L. alba* have been studied through ISSRs and RAPDs (Manica-Cattani *et al*., 2009; Pierre *et al*., 2011). Also, cpDNA, ITSs and ISSRs were used in this species within a molecular study of the genus Lippia (Suárez González *et al*., 2007).

21) ***Lippia integrifolia* (Griseb.) Hieron.** Verbenaceae ("incahierba", "incayuyo", "poleo", "pulco", "té del inca", "yerba del inca"). Infusions or decoctions prepared with the aerial parts (leaves and flowers) of "incayuyo" are used traditionally for the treatment of gastrointestinal disorders or ailments. Its use as diuretic, emmenagogue and for the treatment of coughs and colds has also been documented (Iannicelli *et al*., 2016a). In Argentina, "incayuyo" is used in the preparation of bitters ("amargos serranos") and compounded herbs (Juliani *et al*., 2007), and it is included in the Argentine Food Codex for preparation of beverages intended for infusions or macerations (Alonso & Desmarchelier, 2015). Because of the variable composition of the essential oil of *L. integrifolia*, five different chemotypes were described in Argentina. These are the chemotypes: trans-davanone, lippifolienone, β-davanone-2-ol, spathulenol/bicyclogermacrene/β-caryophyllene and trans-nerolidol (Marcial *et al*., 2016). The essential oils not only confer it its digestive properties, but they also have antioxidant (Barbieri *et al*., 2015) and mosquito repellent (against *Aedes aegypti*) activities (Gleiser *et al*., 2011). Among the non-volatiles, the main compound is verbascoside (Marcial *et al*., 2014), which is apparently responsible for antiinflammatory effects on stomach cells and antiadhesive properties against *Helicobacter pylori* (Marcial *et al*., 2014). Recently, his species was macropropagated, and also introduced and multiplied *in vitro* (Iannicelli et al., 2016b). A new variety was obtained by *in vitro* polyploidization (Iannicelli *et al*., 2016a), with a higher yield in essential oils. In autotetraploids there was an increase in the production of essential oils and also quantitative differences in the proportions of some of the compounds. This was the first variety of "incayuyo" registered in Argentina. In addition, the evaluation of plants regenerated through *in vitro* culture in natural conditions has been reported, and this paves the way to future assays related to re-population (Iannicelli *et al*., 2016a). Furthermore, the ISSR technique to study the *in vitro* genetic variability has been described (Iannicelli *et al*., 2016b), and *in vitro* regenerated plants showed polymorphisms with respect to the field mother plant. Also, plants derived from *in vitro* culture showed quantitative differences in some compounds of their essential oils, but no differences in their yields were detected (Iannicelli *et al*., 2016a).

From an ecological standpoint, some of the populations are in risk because the species is not cultivated and, at the same time, it is in high demand from the liquor and herbal ("yerbatera") industries to prepare bitters and compounded herbs for infusions (Elechosa, 2009). Besides, it represents a high portion of the income of families that have been harvesting it from the wild and commercializing it for more than 20 years. In view of this situation, the Instituto Nacional de Tecnología Agropecuaria (INTA) in Argentina has started to promote sustainable harvest techniques to help "maintain the populations and plan the harvests´ collection" (INTA Informa, 2015).

22) ***Lippia turbinata* Griseb.** Verbenaceae ("poleo", "té del país", "manzanillo"). In traditional medicine, the stems and leaves are used to prepare infusions (té) that have digestive, diuretic and stimulant properties (Pascual *et al*., 2001; Arias Toledo, 2009; Elechosa, 2009). "Poleo" is part of bitters and compounded herbs (Juliani *et al*., 2007), and it is usually consumed, together with other herbs, in "mate" (Menseguez *et al*., 2007).

Analysis of the essential oils revealed the presence of limonene, piperitone oxide, bornyl acetate and 1,8-cineol although some authors report as the main constituents α-thujone, carvone, limonene, β-caryophyllene oxide, bornyl acetate, spathulenol, germacrene-D and bicyclogermacrene (Pascual *et al*., 2001; Barbieri *et al*., 2015).

There is a high demand for this species and it is under pressure because the plants are gathered by unsustainable wild harvesting (Martínez, 2005;

Elechosa, 2009). Alonso & Desmarchelier (2015) describe its cultivation requirements and the forms of reproduction; however, no studies are reported concerning its real cultivation.

Only one report on its micropropagation has been found (Ortiz *et al*., 2007), and it describes a protocol for indirect organogenesis through calli culture.

23) ***Maytenus laevis* Reissek**. Celastraceae ("chuchuguaza", "chuchuguasi capinuri", "chuchuhuasha", "chuchuhuasi", "chuchuhuazo"). There is a certain confusion regarding this species because, in the extensive ethnobotanical literature available, many different plants; e.g.: *M. krukovii* A.C. Sm. and *M. macrocarpa* (Ruiz & Pav.) Briq. have received this vernacular name. The bark, roots, and leaves of these different plants also share pharmacological properties, such as antiinflammatory activity for the treatment of rheumatism and arthritis. The "chuchuhuasi" has also been used to treat tuberculosis, bronchitis, stomach aches and fevers. In traditional medicine, chewing the bark of "chuchuhuasi" is recommended to treat diarrhea, arthritis and menstrual problems (González *et al*., 1982).

We found numerous references regarding the pharmacological properties of this species and the development of phytopharmaceuticals (Lucero & Dehesa, 2011), and there are even websites that offer products containing the active principles of this plant, but we could not find any reports on its ecological status, nor on its cultivation or the application of biotechniques. This situation would place this species at serious risk of extinction.

24) ***Minthostachys mollis* (Benth.) Griseb**. Lamiaceae ("muña", "peperina", "toronjil"). The main use of "peperina" is as a digestive, particularly in infusions or compounded digestive herbs (Juliani *et al*., 2007). However, ethnobotanists have documented a surprisingly wide array of traditional uses for this species. These include uses as a hypotensive, for antimycotic and antiparasitic purposes, against diarrhea, colics, and respiratory illnesses (bronchitis, asthma), headaches, and to induce menstruation. Besides, it is used in the formulation of "yerba compuesta", for the production of liqueur and as a condiment (Cortella & Pochettino, 1999; Schmidt-Lebuhn, 2008; Arias Toledo, 2009). The leaves of "peperina" are registered in the 5th Edition of the Argentine National Pharmacopoeia (Alonso & Desmarchelier, 2015).

Besides its uses as digestive, the essential oils of "peperina" have great potential for the development of products such as toothpastes and mouthwashes (Huari Guerrero, 2014) due to its proven antibacterial and antimycotic activities (Carhuapoma *et al*., 2009; Zapata *et al*., 2009).

Regarding the active principles, the composition of its essential oils is well known, and the diverse aromas detected in the populations analyzed are due to a large chemical variety of compounds. For example, in Argentina, 13 different chemotypes were detected and these were differentiated according to regions. The mentone-pulegone chemotype is the one that confers the typical aroma required by the market for this species. The rest of the chemotypes have linalool, carvacryl acetate, carvacrol, dihydrocarvone, carvone, limonene and menthyl acetate, as the main constituents (Elechosa, 2009).

Traditionally, this species has been exploited through unsustainable, wild harvesting practices, particularly in the central region of Argentina (Ojeda *et al*., 2001; Bustos & Bonino, 2005). Although there have been initiatives to domesticate and cultivate this species, these have encountered difficulties because "peperina" is highly demanded by the herbal industry and the market. The situation is further complicated because many "peperina" populations, at least in Argentina and according to some authors, are in serious risk of extinction (Martínez, 2005; Origlia, 2015). Concerning its cultivation, the most interesting study is that of Ojeda *et al*. (2004) who tested the best form of cultivation. These authors found significant differences in plant mortality, productivity and essential oil yield between the regimes, but also that some populations were better adapted to cropping than others. Furthermore, we found references pertaining to the development of cultivation in the work of Alonso & Desmarchelier (2015).

Concerning the use of biotechniques, this species has been successfully cultivated *in vitro*. Chebel *et al*. (1998) micropropagated plantlets which were then successfully transferred to soil. When their essential oils were analyzed, little differences were found in the composition compared to the plant grown in the wild. Continuing the work of Ojeda *et al*. (2004), shoots of a cultured clone were used to optimize experimental conditions for rapid *in vitro* multiplication and transfer to soil of selected "peperina" clones. These results will allow the multiplication of selected clones to use them for breeding programs or for homogeneous commercial productions, with verified quality (Bima *et al*., 2006). In addition, we found a reference mentioning the use of expressed sequence tag - Inter-Simple Sequence Repeat (EST-ISSR) to study genetic diversity in populations (Bonafede *et al*., 2014), in which the authors report that, despite the excessive exploitation of these populations, there are still diversity hubs that could be used for conservation or breeding. The use of AFLP in the genus *Minthostachys* has been reported by Schmidt-Lebuhn (2008) in a work that supplements field and morphological studies within the framework of a taxonomic and systematic revision. Besides, this work suggests that *M. mollis*, considered as the most complicated species, is genetically very heterogeneous and, according to their geographical origin, exhibits a high discrepancy with the pattern of morphological variation.

Despite the fragile ecological status of this species, it is important to highlight the work carried out by the group of the National University of Córdoba (Argentina) in terms of the domestication and improvement of "peperina" (Ojeda *et al*., 2004; Ordóñez *et al*., 2006; Rodríguez Pardina *et al*., 2013). One of the most important result obtained by the group of Dr. Marta Ojeda from the above mentioned University is the development of a variety of "peperina" registered in the Argentine National Institute for Seeds (INASE) in order to facilitate its industrial cultivation (Carrizo, 2017; Tolchinsky, 2017). Currently, this new variety is being cultivated in fields in the province of Cordoba, Argentina. However, we could not find information about its commercialization. Finally, it is important to mention a website that compiles most of the existing references on "peperina" (Phylobotanist, 2012).

25) ***Momordica charantia* L.** Cucurbitaceae ("balsamina", "cundeamor", "melón amargo", "pepinillo", "pepino cimarrón", "tomaco"). In traditional medicine, fruits and seeds of *M. charantia* are considered as a powerful antidiabetic, but they also have numerous applications, such as anti-HIV, antiparasitic, emmenagogue, antihelmintic, antimycotic, antirheumatic, antihipertensive, antimalarial, carminative, choleretic, antipyretic and abortive (Chauhan *et al*., 2010; Ananya & Sarmistha, 2010; van Andel *et al.*, 2014).

Among the compounds isolated from different organs of the plant, we can mention a mixture of steroidal saponins known as charantins, insulin-like peptides and alkaloids that are responsible for the hypoglycemic activity of this species (Ananya & Sarmistha, 2010).

In the field of the biotechnology, several protocols have been established with different strategies and explants in tissue culture and micropropagation. For example, direct and indirect regeneration from leaves, apices, nodal segments, cotyledonary nodes and embryogenic suspension culture have been reported. Agarwal (2015) has reviewed these large applications of *in vitro* culture techniques, describing methodologies and results. However, in this review there is no information concerning the effects on the production of secondary metabolites. In fact, despite the existence of a large number of references, only a few have as their objective the production and evaluation of the active principles. In this sense, Agarwal & Kamal (2007) reported the use of callus cultures and different *in vitro* developmental stages for the production of flavonoids. *Agrobacterium rhizogenes*–mediated hairy root induction to produce secondary metabolites was reported. Swarna & Ravindhran (2012) obtained transformed hairy roots to produce charantin. The authors informed the detection of this compound but there were no references about the amounts obtained. Also, Thiruvengadam *et al*. (2014) established a protocol for the production of phenolic compounds from hairy root cultures. The flavonoids (catechin, myricetin and quercetin) and the phenolic acids (gallic acid, chlorogenic acid, gentisic acid and salicylic acid) levels were highly increased in hairy roots compared to untransformed control roots; consequently, there was an increase in both the antioxidant and antimicrobial activities in the hairy roots. Furthermore, different types of molecular markers have been used. Ananya & Sarmistha (2010) reviewed the use of RAPD and Sequence Characterized Amplified Region (SCAR) to study the genetic diversity of the species. The authors suggest that both molecular markers could be used to explore the genes associated with medicinal properties of *M. charantia*. Besides, the efficiency of RAPD, ISSR and AFLP in detecting genetic diversity was evaluated (Singh *et al*., 2007; Behera *et al.*, 2008a; Behera *et al*., 2008b) and internal transcribed spacer 2 (ITS2) was used as an identifying tool (Michel *et al*., 2016).

This species is commonly cultivated in South

America and the Caribbean, Asia and Africa (Basch *et al*., 2003; Portillo, 2009). In Argentina it is considered as an edible weed (Rapoport *et al*., 2009). Also, in Mexico, the National Commission for the Knowledge and Use of Biodiversity (CONABIO) considers it a weed (CONABIO, 2009).

There are products already in the market such as of the immune system strengthening capsules in Spain (Thaivita, 2018) and capsules and extracts with hypoglycemic activity in Mexico (Tecnobotanica de México, 2018).

26) ***Passiflora caerulea* L.** Passifloraceae (“burucuyá”, “maracuyá”, “mburucuyá”: names derived from the guaraní language; “flor de la pasión", “pasionaria”, “pasionaria azul”). It is widely employed in traditional medicine, where all the parts of the plant are used. The infusion prepared with the leaves and flowers is traditionally consumed for its sedative and anxiolytic properties, in the treatment of insomnia. In ethnomedicine, other uses are also reported for its aerial parts, as diuretic, antitussive and antiasthmatic and spasmolytic, as eupeptic (by decoction of its fruits), antiscurvy, urinary antiinfectant, and its root is used as anthelmintic, menstrual cycle regulator and contraceptive (Alonso, 2004; Menseguez *et al*., 2007; Arias Toledo, 2009). Furthermore, the ornamental value of the flowers is highly appreciated.

Its use as a sedative and anxiolytic is mainly due to the presence of chrysin, which is similar to diazepan, although its effect is ten times lower and it has no myorelaxant properties (Medina *et al*., 1990; Wolfman *et al*., 1994).

In Argentina, *P. caerulea* is highly demanded by consumers and it is obtained by extractive wild harvesting, causing a marked decrease in its relative abundance decreased in the last years (Martínez, 2005; Mendiondo & Garcia, 2006). Conventional propagation displays certain problems concerning the percentage of seed germination and growth rates. Considering the economic importance and the conservation problems of *P. caerulea*, Mendiondo & Garcia (2006) examined the seed emergence pattern from the standpoint of either *in situ* or *ex situ* plant preservation and propagation. These authors informed low levels of seed emergence and slow growth rates.

Since conventional propagation of this species seems to be a difficult task, the application of biotechniques has been considered for its conservation. Regarding the use of tissue culture, *P. caerulea* has been extensively studied and there are numerous papers dealing with its *in vitro* culture through different methodologies, including micropropagation and regeneration from calli (Carnier Dornelas & Carneiro Vieira, 1994; Severin *et al*., 2011; Ozarowski & Thiem, 2013; Rathod *et al*., 2014). Using leaf, nodal or internodal segments, regeneration of shoots was obtained with and without callus development and the plantlets were successfully transferred to *ex vitro* conditions. In this sense and considering the problems with conventional propagation, Rathod *et al*. (2014) reported that a complete plant along with shoots and roots can be developed in around 54 days, which may take about two seasons or sometimes a year for a seed to germinate.

On the other hand, there is a lack of systematic phytochemical evaluation in *in vitro* clones for the detection of flavonoids, phenolic acids and alkaloids in regenerated plantlets. Only a few reports have confirmed the occurrence of secondary metabolites in plantlets, showing the presence of apigenin, luteolin, vitexin, isovitexin, rutin, hyperoside, chlorogenic and rosmarinic acids (Busilacchi *et al*., 2008; Ozarowski & Thiem, 2013). It is important to highlight a specific reference in which micropropagated plants of *P. caerulea* were evaluated in the field, and it was observed that there were no qualitative differences in the secondary metabolites found in the plant from where the explants were originally obtained (mother plant) compared to the ones obtained by *in vitro* culture (Busilacchi *et al*., 2008). Although in other species of the genus *Passiflora* the use of transformation methods and polyploidization were reported, in *P. caerulea* no references were found regarding the application of these biotechniques. Genetic marker techniques, such as RAPDs, to study genetic diversity among different species have also been used (Fajardo *et al*., 1998; Crochemore *et al*., 2003). Crochemore *et al*. (2003) generated fingerprints that would allow the identification of improved varieties/populations.

There has been remarkable progress in the breeding, cultivation and production species of this genus with great importance as food, such as *P. edulis* Sims or *P. alata* Curtis (Cerqueira-Silva *et al*., 2014). However, the species with medicinal uses, such as *P. caerulea*, are still exploited through collection of wild specimens. This not only results in conservation problems, as in the case of *P. caerulea*, but also produces batches of raw material with high variability and low quality (Severin *et al*., 2011).

27) ***Phyla scaberrima* (Juss. ex Pers.) Moldenke**, synonyms: *Lippia dulcis* Trevir., *Phyla dulcis* (Trevir.) Moldenke. Verbenaceae ("hierba buena", "hierba dulce", "orozús"). It is one of the species with the most number of ethnobotanical references, and there are bibliographic data dating back to the 15th century (Compadre *et al*., 1986). Although the US Pharmacopoeia has mentioned it as an expectorant since the late 19th century, in traditional medicine it is used as a sweetener, for the treatment of respiratory diseases (bronchitis, coughs and colds) and as abortive (Compadre *et al*., 1986; Pascual *et al*., 2001; Gornemann *et al*., 2008).

Its sweetening properties are due to hernandulcine (500 sweeter than sucrose, Compadre *et al*., 1986). However, the production of hernandulcine from leaves and flowers has low yields (Urrea *et al*., 2009). Furthermore, the presence of significant quantities of camphor in its essential oil, which is responsible for the medicinal use in the treatment of respiratory ailments (Gornemann *et al*., 2008), confers an unpleasant taste and restricts its use in the food industry (Urrea *et al*., 2009).

Commercial cultivation is very recent and at a small scale and it is sold falsely as "Stevia". Information about its cultivation, at least in Mexico, is nonexistent or very scarce (Fierro *et al*., 2015). The species is also used in gardens and family orchards as an ornamental.

The vegetative propagation of the species has been reported (Fierro *et al*., 2015) and diverse biotechnological tools have been used to optimize the production of hernandulcine, such as micropropagation, callus culture and cell suspensions (Urrea *et al*., 2009), and *in vitro* culture of apices in solid and liquid media (Sauerwein *et al*., 1991a). There are also reports on the use of hairy root cultures to obtain hernandulcine *in vitro* (Sauerwein *et al*., 1991b), indicating that the transformed roots can produce secondary metabolites present in the mother plant, even if those compounds are not found in the root system of the plant. Molecular markers to analyze its genetic diversity (together with other species of the genus *Lippia*) have also been used, through cpDNA ITSs and ISSRs (Suárez González *et al*., 2007).

Despite the high sweetening and medicinal power of *P. scaberrima* and the high costs of *in vivo* cultures, works dealing with the search for alternative production methods are scarce. In this sense, the most recent one refers to the generation of calli and cellular suspensions (Urrea *et al*., 2009) as preliminary stages for the study of hernandulcine production and upscaling.

28) ***Quillaja saponaria* Molina**. Quillajaceae ("quillay"). The saponins have great economic importance and they are used with pharmaceutical, industrial and agronomic purposes (Hoffmann, 1995; Vega & Prehn, 2005).

Due to the high demand for quillay bark (the main source of saponins) and changes in the use of soils where it grows, the existence of adult trees was reduced (Prehn *et al*., 2003).

This situation prompted the development of a forest management program for this species, supported by a propagation program (Copaja *et al*., 2003; Prehn *et al*., 2003). The objective of this program is to obtain a massive, efficient and economical production of "quillay" trees with a high content of good quality and low toxicity saponins. For this purpose, *in vitro* culture techniques for the multiplication of this species (micropropagation and embryogenesis) were implemented (Prehn *et al*., 2003; Vega & Prehn, 2005). Besides, there have been attempts to use *in vitro*-grown shoots as a source of extracts with biological activity (Ribera *et al*., 2008), focusing on the value of developing *in vitro* plant tissue cultures in order to obtain desirable compounds.

29) ***Stevia rebaudiana* (Bertoni) Bertoni**. Asteraceae ("stevia", "estevia", "ka'a he'ẽ"). The main use is as a natural sweetener (Durán *et al*., 2012), and there are many brands in the market that use this species. Besides, "stevia" has been suggested as hypoglycemic, antioxidant, antimicrobial, antifungal and anticancer agent (Gupta *et al*., 2013).

The main metabolites are stevioside and rebaudioside A, which are 250 to 300 times sweeter than sucrose. Both compounds are thermo and pH-stable and non-fermentable. For these reasons, "stevia" is one of the main sucrose substitutes and it also possesses potential as a health promoter (Gupta *et al*., 2013).

Due to the real and potential economic value of "stevia", there is a large quantity of publications dealing with biotechnological applications, particularly tissue culture and breeding of the species.

There are several reports on its micropropagation, with different conditions and techniques (Oviedo-Pereira *et al*., 2015). The highest multiplication rates were obtained in semisolid mediums by Mohamed (2011), Preethi *et al*. (2011), Verma *et al*. (2011), and Thiyagarajan & Venkatachalam (2012). Temporary immersion systems (TIS) were also developed for this species, but the multiplication rates were lower (Jiménez-Quesada, 2011; Noordin *et al*., 2012; Alvarenga-Venutolo & Salazar-Aguilar, 2015). However, the quality of the obtained plantlets was different. The plantlets from TIS showed higher length and biomass, and this facilitated their acclimatization (Alvarenga-Venutolo & Salazar-Aguilar, 2015). Another successful methodology used in this species is direct organogenesis using thin cell layer (TCL) (Ramírez-Mosqueda & Iglesias-Andreu, 2016). With this technique the authors have obtained high multiplication rates and vigorous shoots.

Several types of cultures were developed for the production of steviosides, such as callus, shoots, roots, cell suspensions (Rajasekaran *et al*., 2007; Dheeranapattana *et al*., 2008; Karuppusamy, 2009; Mathur & Shekhawat, 2013). Rebaudioside-A is of particular interest among the glycosides produced in the leaves of "stevia" because the most desirable flavour profile is the one with the most rebaudioside-A (Yadav *et al*., 2011). In this sense, Rajasekaran *et al*. (2007) compared the stevioside production in both *in vitro* and *ex vitro* grown plants and in its different organs. They found the highest contents of rebaudioside-A in *in vitro* leaves.

One of the strategies used to increase the production of active principles is the generation of autotetraploids (Li *et al*., 2012; Yadav *et al*., 2013). Yadav *et al*. (2013) reported the obtention of tetraploids with higher content of rebaudioside-A than diploids.

Genetic transformation protocols via *A. tumefaciens* have also been reported (Khan *et al*., 2014). Transformed plants showed a different glycoside profile, where the content of steviol was higher in the transformed plants than in mother plant, whereas the content of stevioside was higher in the mother plant. The level of rebaudioside-A was similar in both types of plants.

Genetic variability studies with ISSRs have also been performed (Garro-Monge *et al*., 2014; Khan *et al*., 2014). Garro-Monge *et al*. (2014) found polymorphisms between cultivated plants, which could be helpful for the identification of cultivars designated to the production of steviosides. Khan *et al*. (2014) reported the generation of somaclones in *in vitro*-derived plants with lower contents of steviol than the mother plant.

Finally, this species is cultivated since early 1970s and as a result of the introduction of "stevia" in Japan and their research to evaluate the potential of the species, nowadays commercial cultivation is extended/attempted in Japan, southeast Asia, and United States and there are guidelines available to develop its cultivation (Ramesh *et al*., 2006; Carrascal, 2017).

30) ***Valeriana carnosa* Sm**. Valerianaceae (ñancú lahuén, yerba del aguilucho blanco). Its underground organs (rhizomes) have similar properties as *Valeriana officinalis* L., and the mapuche ethnic, which considers it a magical plant, has used it from time immemorial as a sedative, antispasmodic, hepatoprotector, and to treat rheumatism (Estomba *et al*., 2005). It has also been cited as diuretic and digestive (Bach *et al*., 2011). Among its active principles, the major constituents are β-sesquiphellandrene, α-pinene, bornyl acetate and mainly isovaleranic acid, a compound found in the widely used euro-asian commercial species *V. officinalis*, and associated with the sedative properties of "valerianas" (Bach *et al*., 2013).

Contrary to *V. officinalis*, there are no important commercial cultivars of this species. Currently, the laboratories and herbalists´ providers gather their material from wild populations, with the expected loss of the biological resource. The species grows by forming isolated and low-number populations and the root and/or rhizome are used to induce sleep or as sedative. As a result, the whole plant is lost when used, placing this and other species of the genus *Valeriana* in risk of extinction.

Recently, attempts to introduce this species into cultivation have been reported. It is expected in the medium term that the results obtained will allow to start a rational productive economic management with social inclusion (original groups and small producers) that contemplate its conservation (Nagahama *et al*., 2016). Concerning the application of biotechnology, no references were found for this species, which is disturbing since this species is under risk.

DISCUSSION

Understanding that society at large depends to some extent on the extractive use of wild species is paramount (Hutton & Leader-Williams, 2003), but the fate of many of the natural resources in the developing world is limited by the resilience of nature and the ability of societies to manage it. Indefinite harvesting, under growing human pressures, could be the harbinger of the extinction of many species (Tarciso *et al*., 2017).

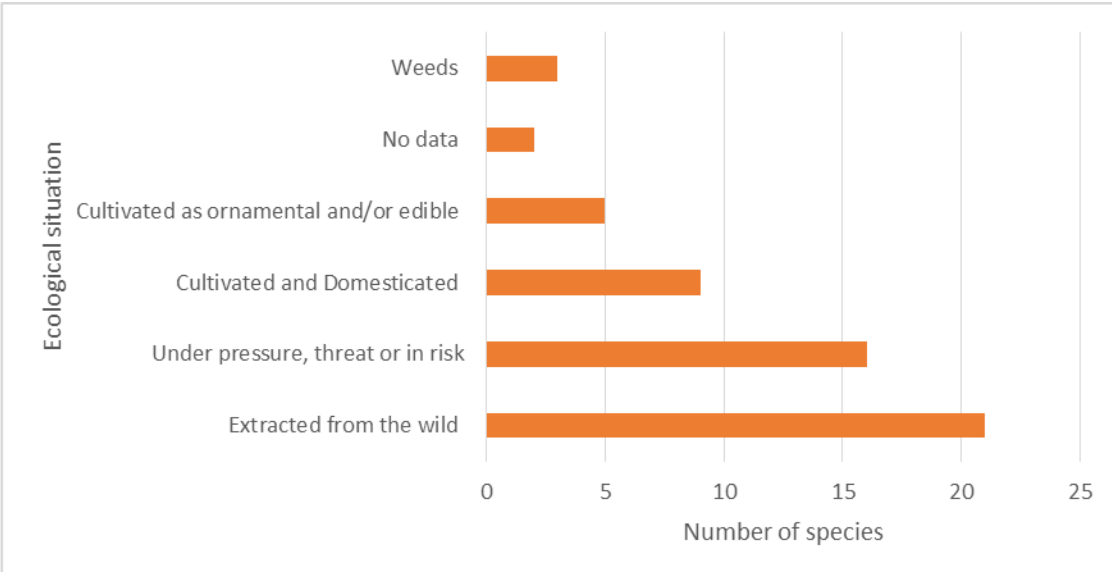

Figure No. 1
Ecological situation of the MAPs of the American continent involved in this review

In this paper, we reviewed the ecological situation of 30 species, native to America, widely employed in the pharmaceutical, cosmetic and food industries. Figure No. 1 shows that, despite their extensive use as raw materials for these industries, more than half the species analysed (n=21) are exploited directly from the environment by wild harvesting. Only four of them are extracted from the wild but also cultivated in certain countries: *Achyrocline satureioides* (Retta *et al*., 2010; Davies, 2011), *Aloysia polystachya* (Alonso & Desmarchelier, 2015; Martínez, 2005), *Carapichea ipecacuanha* (Oliveira & Martins, 1998; Ocampo, 2007) and *Lippia alba* (Ringuelet & Cerimele, 2010; Alonso & Desmarchelier, 2015).

Harvesting wild species is frequently unsustainable and a major source of biodiversity loss (Rice *et al*., 1997; Struhsaker, 1998). Thus, 17 species are in some risk of extinction, with varying degrees. Among the studied species, *Aniba rosaedora*, *Eremanthus erythropappus*, *Minthostachys mollis* and *Quillaja saponaria* are the most affected ones. Their critical situation has led to the development of alternatives for their conservation.

On the other hand, nine of the selected species are domesticated and cultivated. However, as we mentioned before, in four of them the extractive regimes are still in use. We found that, from the list of species analysed, the only five that are cultivated for their exploitation in the market are *Aloysia citriodora* (Elechosa, 2009; Alonso & Desmarchelier, 2015), *Lepidium meyenii* (Wang *et al*., 2007), *Phyla scaberrima* (Fierro *et al*., 2015), *Momordica charantia* (Portillo, 2009) and *Stevia rebaudiana* (Ramesh *et al*., 2006). Furthermore, another five species are also cultivated mainly for their ornamental purposes, and secondarily for their medicinal properties. We could find no references concerning extraction or production management for two species.

Likewise, we found that the state-of-the-art concerning the use of biotechnology in the different species was also very variable. It is well known that *in vitro* propagation methods play a vital role not only in searching for alternatives to produce desirable medicinal compounds from plants, but also in the conservation and preservation of plant biodiversity (Rao & Ravishankar, 2002; Karuppusamy, 2009; Cruz-Cruz *et al*., 2013). In this respect, we found that 77% of the selected species (23 species, Figure No. 2, Table No. 1) had been propagated through some tissue culture technique (micropropagation, cell culture, indirect regeneration, somatic embryogenesis, protoplast culture, thin cell layer and/or culture in bioreactors). Nevertheless, only in 50% of these propagated plants, tissue culture techniques were also implemented as a source for alternative production of secondary metabolites (Figure No. 2a). This indicates that, despite the many promising results reported, biotechniques are rarely used to improve the rational profit of this germplasm. For instance, there has been research dealing with production of the following compounds: essential oils in micropropagated plants of *Aloysia citriodora* and *Lippia integrifolia* (Severin *et al*., 2006; Moradi *et al*., 2014; Iannicelli *et al*., 2016b), phenolic compounds in cell culture of *Bauhinia forficata* (Lima, 2009), alkaloids (emetine and cephaeline) in root cultures of *Carapichea ipecacuanha* (Teshima *et al*., 1988; Yoshimatsu & Shimomura, 1991), NDGA and flavonoids in organogenic calli of *Larrea divaricata* (Palacio *et al*., 2012), flavonoids in callus culture of *Momordica charantia* (Agarwal & Kamal, 2007), flavonoids in plantlets of *Passiflora caerulea* (Busilacchi *et al*., 2008), steviosides in cell suspension and plantlets in *Stevia rebaudiana* (Rajasekaran *et al*., 2007; Mathur & Shekhawat, 2013). However, some of these reports are not followed through, as far as scaling-up and/or production of the metabolites of interest, and we did not find current applications of these reports as sources of active principles. This is disturbing, because there exist examples of the integration between biotechnological techniques, such as *in vitro* cultures, and sustainable exploitation for the formulation of a drug. This is the case of *Aloysia polystachya* (Aguado *et al*., 2006).

Likewise, Figure No. 2a shows that, although micropropagation protocols have been established and applied to half of the species, improved plants have not been obtained from all of them. For instance, in 27% of the species studied, the implementation of micropropagation techniques has not been developed further to introduce an improvement in the species. In many of the reports analysed, the use of *in vitro* culture is only considered in order to establish the conditions to propagate the species. Although this is necessary for conservation and/or subsequent improvement, in several of the works the continuity of the results obtained was not found. These are the cases of *Achyrocline satureioides* (Gatusso *et al*., 2007; Severin *et al*., 2008; Kotik *et al*., 2014), *Actaea racemosa* (Lata *et al*., 2002), *Aniba rosaedora* (Handa *et al*., 2005; Jardim *et al*., 2010), *Equisetum bogotense* (Rodriguez *et al*., 1994), *Eremanthus erythropappus* (Rosal *et al*., 2007; Prudente *et al*., 2016), *Justicia pectoralis* (Oliveira Freitas *et al*., 2016), *Lepidium meyenii* (Polzerová *et al*., 2011) and *Lippia turbinata* (Ortiz *et al*., 2007). Furthermore, in 23% of the species studied, we could not find any report concerning their propagation despite the fact that they are in high demand and the possibilities that it could be used in the conservation of this species. These are the cases of *Adesmia emarginata*, *Baccharis articulata* and *B. trimera*, *Cecropia pachystachya*, *Equisetum giganteum*, *Maytenus laevis* and *Valeriana carnosa*.

Biotechnological tools such as in-vitro regeneration together with genetic transformation or polyploidization are important to facilitate efforts to engineer secondary product metabolic pathways (Tripathi & Tripathi, 2003; Gómez-Galera *et al*., 2007). Genetic transformation may provide increased and efficient systems for *in-vitro* production of secondary metabolites (Tripathi & Tripathi, 2003). Polyploids are usually more valuable because they exhibit increased biomass and content of effective compounds (Dhooghe *et al*., 2011).

In this sense, Table No. 1 details that 15 species of the list were not only propagated *in vitro* but also subjected to some type of biotechnique. According to Figure No. 2b, this represents 50% of the studied species. In seven of these species (23%, Figure No. 2b), *in vitro* culture and propagation techniques were combined with transgenesis or polyploidization strategies aimed at studying and/or improving the production of secondary metabolites. Also, molecular markers were applied to them. From a biotechnological perspective, *Carapichea ipecacuanha* is a very good example to follow. In this case, biotechnology was clearly used not only to preserve the germplasm, but also to develop this valuable medicinal species. *Stevia rebaudiana* is the only species developed employing all the tools analysed in this review and its development corresponds to the importance this species has gained for the industries. In the remaining eight species

(27%, Figure No. 2b), genetic variability was studied through a combination of *in vitro* culture and the use of molecular markers. However, in some species the application of molecular markers still remains unexploited. This is the case of *Achyrocline satureioides*, in which despite the high variability expressed in important characters such as biomass productivity, morphology and architecture of the leaves and crowns, time of flowering, active principles contents and rooting percentage of the cuttings (Magalhães, 2000), we could not find any studies with molecular markers to analyze this variability at the genetic level. *Lippia turbinata* is another case in which no genetic variability studies were detected, even though the technique used for its propagation could compromise the genetic stability of the propagated material. Furthermore, although Elechosa (2009) mentioned the possible existence of different chemotypes, the extant genetic diversity is completely unknown.

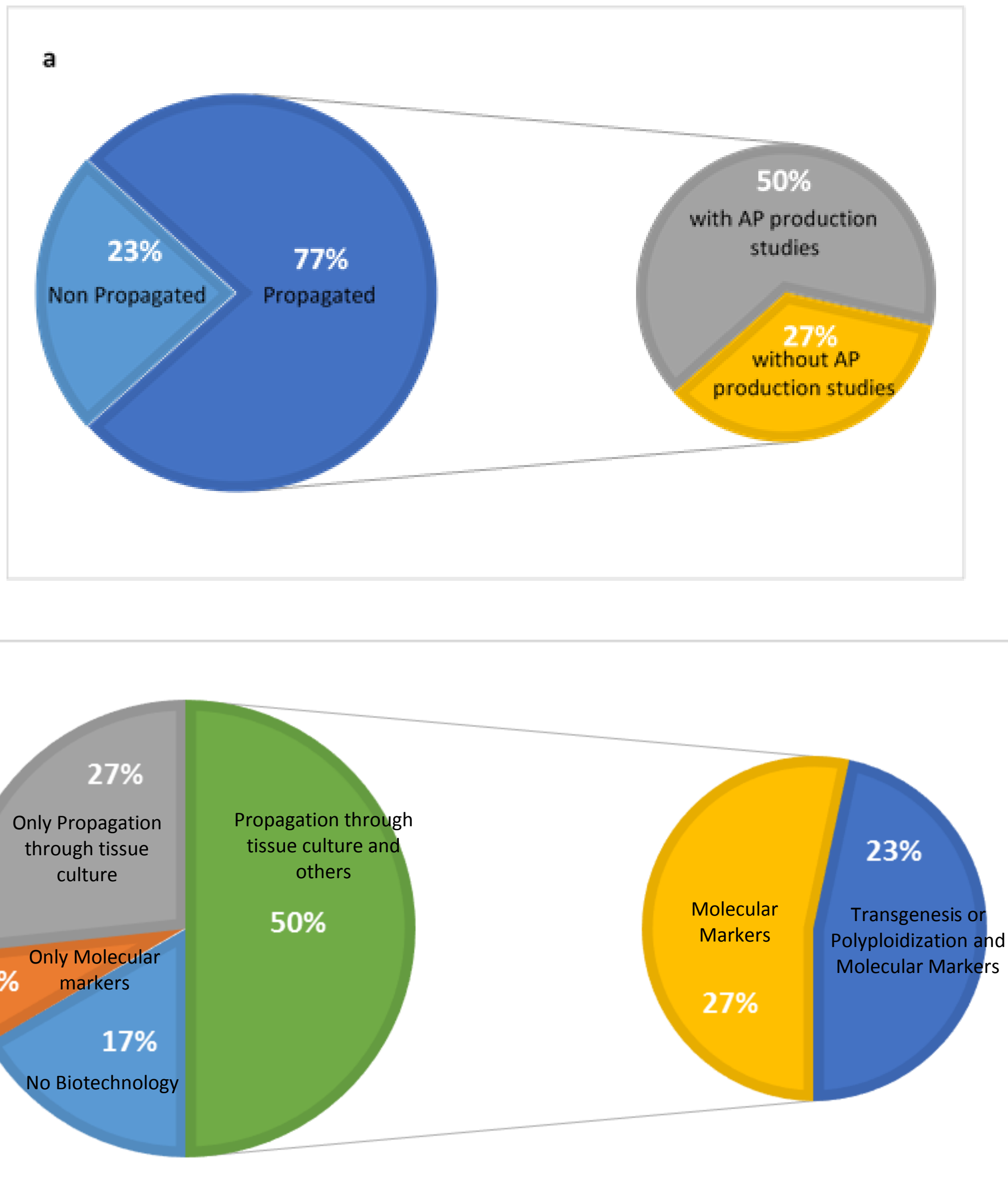

Figure No. 2
a) Application of plant propagation and active principles (AP) production studies on the MAPs of the American continent mentioned in this review. b) State-of-the-art concerning the combination of biotechnological tools in the MAPs of the American continent mentioned in this review

Species	PTC	T	P	MM	Biotec. Ref.
1) Achyrocline satureioides (Lam.) DC.	M	-	-	-	Gattuso *et al*., 2007; Severin *et al*., 2008; Kotik *et al*., 2014; Rosso *et al*., 2015
2) Actaea racemosa L.	IR H	-	-	AFLP	Lata *et al*., 2002; Zerega *et al*., 2002; Hyung *et al*., 2005
3) Adesmia emarginata Clos	-	-	-	-	-
4) Aloysia citriodora Palau	M IR	Ar	-	cpDNA ITS ISSR	Severin *et al*., 2005; Severin *et al*., 2006; Suárez González *et al*., 2007; El-Hawary *et al*., 2012; Figueroa *et al*., 2013; Moradi *et al*., 2014; Boustani *et al*., 2016;
5) Aloysia polystachya (Griseb.) Moldenke	M	-	-	-	Aguado *et al*., 2006; Burdyn *et al*., 2006
6) Aniba rosaeodora Ducke	M	-	-	SSR RAPD	Handa *et al*., 2005; Santos *et al*., 2008; Angrizani *et al*., 2013; Jardim *et al*., 2010
7) Argemone mexicana L.	M CC	At	-	RAPD	Godoy-Hernández *et al*., 2008; Ziegler & Facchini, 2008; Trujillo-Villanueva, 2010
8) Baccharis articulata (Lam.) Pers. and *9) B. trimera* (Less.) DC.	-	-	-	Isoenzymes ISSR (in 9)	Vaco, 2011; Cúneo, 2012
10) Bauhinia forficata Link subsp. pruinosa (Vogel) Fortunato & Wunderlin	M CC	-	-	-	Mello *et al*., 2000; Appezzato-da-Glória & Machado, 2004; Lima, 2009; Teixera da Silva, 2013
11) Carapichea ipecacuanha (Brot.) L. Andersson	M IR SE	Ar	-	ISSR	Jha *et al*., 1988; Teshima *et al*., 1988; Jha & Jha, 1989; Yoshimatsu & Shimomura, 1991; Rout *et al*., 1992; Yoshimatsu & Shimomura, 1994; Rout *et al*., 2000; Yoshimatsu *et al*., 2003; Tripathi & Tripathi, 2003; Chaudhuri & Jha, 2008; Rossi *et al*., 2009; De Oliveira *et al*., 2010
12) Cecropia pachystachya Trécul	-	-	-	-	-
13) Equisetum giganteum L. and *14) E. bogotense* Kunth	M (in 14)	-	-	Sequencing CpDNA ISSR	Rodriguez *et al*., 1994; Des Marais *et al*., 2003; Brune *et al*., 2008
15) Eremanthus erythropappus (DC.) MacLeisch	M	-	-	RAPD	Moura, 2005; Estopa *et al*., 2006; Rosal *et al*., 2007; Freitas *et al*., 2008; Prudente *et al*., 2016
16) Fabiana imbricata Ruiz & Pav.	M IR CC TIS	-	-	-	Razmilic *et al*., 1994; Schmeda-Hirschmann *et al*., 2004
17) Justicia pectoralis Jacq.	M	-	-	-	Oliveira Freitas *et al*., 2016
18) Larrea divaricata Cav.	M IR CC	-	-	-	Palacio *et al*., 2008; Palacio *et al*., 2011; Palacio *et al*., 2012
19) Lepidium meyenii Walp.	M IR	-	-	SSR cpDNA	Cheng *et al*., 2004; Wang *et al*., 2007; Hasan *et al*., 2010; Polzerová *et al*., 2011
20) Lippia alba (Mill.)	M	-	-	ISSR	Gupta & Khanuja, 2001; Suárez González *et al*.,

N. E. Br. ex Britton & P.Wilson	IR			RAPD CpDNA ITS	2007; Manica-Cattani *et al*., 2009; Pierre *et al*., 2011; JibinaBai *et al*., 2014
21) Lippia integrifolia (Griseb.) Hieron.	IR	-	P	ISSR	Iannicelli *et al*., 2016a; Iannicelli *et al*., 2016b
22) Lippia turbinata Griseb.	IR	-	-	-	Ortiz *et al*., 2007
23) Maytenus laevis Reissek.	-	-	-	-	-
24) Minthostachys mollis (Benth.) Griseb.	M	-	-	EST-ISSR AFLP	Chebel *et al*., 1998; Bima *et al*., 2006; Schmidt-Lebuhn, 2008; Bonafede *et al*., 2014
25) Momordica charantia L.	M IR CC SE	Ar		RAPD AFLP ISSR SCAR ITS2	Agarwal & Kamal, 2007; Singh *et al*., 2007; Behera *et al*., 2008a; Behera *et al*., 2008b; Ananya & Sarmistha, 2010; Swarna & Ravindhran, 2012; Thiruvengadam *et al*., 2014; Agarwal, 2015; Michel, 2016
26) Passiflora caerulea L.	M IR	-	-	RAPD	Carnier Dornelas & Carneiro Vieira, 1994; Fajardo *et al*., 1998; Crochemore *et al*., 2003; Busilacchi *et al*., 2008; Severin *et al*., 2011; Ozarowski & Thiema, 2013; Rathod *et al*., 2014
27) Phyla scaberrima (Juss. ex Pers.) Moldenke	M CC	Ar	-	ISSR/ cpDNA/ITS	Fierro *et al*., 2015; Sauerwein *et al*., 1991a; Sauerwein *et al*., 1991b; Suárez González *et al*., 2007; Urrea *et al*., 2009
28) Quillaja saponaria Molina	M SE	-	-	-	Prehn *et al*., 2003; Vega & Prehn, 2005; Ribera *et al*., 2008
29) Stevia rebaudiana Bertoni (Bertoni)	M IR CC TIS TCL	At	P	ISSR	Rajasekaran *et al*., 2007; Dheeranapattana *et al*., 2008; Karuppusamy, 2009; Jiménez-Quesada, 2011; Mohamed, 2011; Preethi *et al*., 2011; Verma *et al*., 2011; Li *et al*., 2012; Noordin *et al*., 2012; Thiyagarajan & Venkatachalam 2012; Mathur & Shekhawat, 2013; Yadav *et al*., 2013; Garro-Monge *et al*., 2014; Khan *et al*., 2014; Alvarenga-Venutolo & Salazar-Aguilar, 2015; Oviedo-Pereira *et al*., 2015; Ramírez-Mosqueda & Iglesias-Andreu, 2016
30) Valeriana carnosa Sm.	-	-	-	-	-

Table No. 1
Biotechnological techniques applied to MAPs of the American continent mentioned in this review. References: PTC: Propagation through tissue culture; T: Transgenesis; P: Polyploidization; MM: Molecular Markers; M: microprogation; CC: cell culture; SE: somatic embryogenesis; P: protoplasts; IR: indirect regeneration; H: hydroponics; TIS: temporary immersion systems; TCL: thin cell layer; Ar: *A. rhizogenes*; At: *A. tumefaciens*.

The use of bioreactors and the TIS are two of the most powerful tools in biotechnology. Bioreactors are the key step towards commercial production of secondary metabolites by plant biotechnology (Tripathi & Tripathi, 2003). Large-scale plant tissue culture is an attractive alternative to the traditional method of plantation, or even plant cell culture, since scaled-up and automated systems help overcome and/or minimize production costs, increase multiplication rates and reduce the amount of handling during the steps required for micropropagation (Watt, 2012; Paek *et al*., 2005).

However, TIS are not widely applied. In fact, from the list of species analysed in this review, we found that this technology was used only in two species: *Stevia rebaudiana* (Jiménez-Quesada, 2011; Noordin *et al*., 2012; Alvarenga-Venutolo & Salazar-Aguilar, 2015) and *Fabiana imbricata* (Schemeda-Hirschmann *et al*., 2004).

Finally, we found that in five species (17%, Figure No. 2b) no biotechnology was applied. These species are *Adesmia emarginata*, *Baccharis articulata*, *Cecropia pachystachya*, *Maytenus laevis* and *Valeriana carnosa*. This is an alarming and disturbing fact, because the conservation and breeding of these species is not being properly undertaken, although they are under pressure and even in risk.

Concerning the state-of-the-art in biotechnology, we must highlight the differences found among the species analysed in this review. As an example, we can compare *Carapichea ipecacuanha* or *Stevia rebaudiana* versus *Cecropia pachystachya*. For “ipecacuana” many biotechniques have been developed, including different forms of *in vitro* propagation (relevant for its conservation) and the use of hairy roots, obtained by genetic transformation with *Agrobacterium rhizogenes*, as a source of active principles. Meanwhile, in “stevia” practically all *in vitro* propagation methods have been explored. Even temporary immersion systems were developed with successful results. Concerning biotechniques, not only genetic transformation has been applied but also the obtaining of autopolyploids was described. Both techniques resulted in materials with higher content of active principles. These cases are the opposite of “ambay”, which has not been propagated and its conservation status is not known, although it is widely used and exploited by the local pharmaceutical industry.

It would be interesting to use the data presented in this review, and further additional ones, to promote programs to study native plants and their potential as cultivars for the future. Brazil developed such a program during the past decade, and they published a book where they detailed the possibilities of using many of their native plants as food, even including cookbook recipes for each one (Ferreira Kinupp & Lorenzi, 2014). In Argentina, Rapoport *et al*. (2009) have published a book on edible weeds, where many of them, native to the Southern Cone region, are medicinal as well as being classified as weeds. We mentioned the case of *Momordica charantia*, but there are many native species – weeds – with important value as medicinals such as *Alternanthera pungens* Kunth (“yerba del pollo”), *Dysphania ambrosioides* (L.) Mosyakin & Clemants (“paico”) or *Commelina erecta* L. (“Santa Lucía”) among others.

Safeguarding biodiversity is a global chore that should engage all of us. Through our systematic literature search and interviews with our colleagues, we thought that the perilous situation of the MAPs germplasm is similar in all the American continent and, perhaps, worldwide.

Stevia rebaudiana is a clear and paradigmatic example of the mismanagement and exploitation of the natural resources in our region, since Paraguay, and particularly the Guaraní population, lost the germplasm in the 70´s to the Japanese sweetening industry (Delgado Jiménez, 2016). Today, Japan is a major grower and marketer for the sweetener and has approved it for use in many food products, including cereals, teas, and soft drinks (Ramesh *et al*., 2006). The case of *Larrea divaricata* is another example of the appropriation of genetic resources, without recognizing the origin of the species and the ancestral knowledge that allowed the use of this species in folk medicine. In fact, the extraction process of NDGA from *L. divaricata* was developed and patented by the University of Minnesota and produced in a commercial scale (Pardo, 1965). However, this patent has expired.

The sustainable use and exploitation of aromatic/medicinal native species is a multi-disciplinary task that requires the collaboration of ethnobotanists, taxonomists, ecophysiologists, chemists, pharmacologists, agronomists, biotechnologists, together with the social work of extensionists, economists and politicians. In this scenario, the countries in our region, through a network of laboratories, should collaborate in studying, developing and proposing a rational and sustainable exploitation of the germplasm at risk.

This coordinated and concerted action will enable the reversal of the status of many of these species. In this context, biotechnology can play a relevant role, ranging from propagation methods to the analysis of the genetic variability available in the germplasm to be employed in breeding programs.

In addition, it has been demonstrated that biotechnological methods are reliable and safe and contribute to the conservation of biodiversity. They also provide high value products, such as pharmaceuticals, cosmetics, dietary supplements and other industrial products.

The strategy proposed by our team, which is constituted by pharmacists, agronomists, chemists, biologists and biochemists, is to obtain improved genotypes of the wild plants and offer them to the harvesters/producers. Since we can use INTA´s structure, we can work together with extensionists and take advantage of the social programs carried out by INTA and the National Universities involved, turning an extractivist model into a productive one. This is impossible without a State initiative to standarize and regulate the extraction from the wild, according to good extractive practices.

With respect to the technological aspects, in our research group we proposed the use of polyploidization-nature´s own way of accelerating evolutionary processes-to develop MAPs´ germplasm rapidly. In agreement with the literature, in previous studies with ornamental/medicinal species, we observed an increase in the size of organs when we applied this strategy (Escandón *et al*., 2005, 2006; González Roca *et al*., 2015; Iannicelli *et al*., 2016a). Since there were also reports of polyploidization on a number of MAPs, we adopted this strategy for species at risk, starting with *Lippia integrifolia*, and, after 4 years, we registered a new variety of "incayuyo", Tawa INTA (Register number: 15679. Date: 2015/07/06), at the National Institute of Seed (INASE) of the Argentine Republic. This development was declared of interest by Argentina´s Honorable National House of Representatives in 2015.

CONCLUSIONS

As a starting point, it is essential to understand our knowledge with respect to the biology and the propagation of a certain species. From a biotechnological viewpoint, and taking into account a few MAPs, we can observe that there is still a great deal to do in the field and that rational and systematic work has been undertaken only with a few species. This is probably due to the economically valuable active principles of those species and the publicity surrounding them.

This is a major hindrance, since this type of research requires abundant funding and MAPs are not a priority in Western society, except when their illicit exploitation yields good dividends. Consequently, in an effort to continue working with the native species in risk of extinction, we combine this line of work with other more "profitable" ones, precluding the necessary concentration on species that need to be studied urgently.

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