Conservation of native plants in the seed base Bank of Chile

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Abstract
Despite the value of Chilean flora, 46% of the species, currently evaluated, are critically endangered or endangered, so ex situ conservation measures are key to their preservation. We analyzed the current state and conservation gaps of the native species preserved in the Seed Base Bank (SBB) of the Instituto de Investigaciones Agropecuarias (INIA), the main curator of Chile’s plant genetic resources. Our analysis showed that 3,040 seed accessions of native species are preserved in the SBB, corresponding to 1,256 species, which represents 26.9% of the Chilean flora. The area with the largest number of accessions collected corresponds to the northern and central parts of the country, indicating that there has been a bias in terms of concentrating efforts on the continental regions and ignoring other important geographical regions of Chile, such as those located in the insular territory. A quarter of the threatened plants belonging to the Chilean flora are not currently conserved in the SBB, as they are vascular species without seeds, which cannot be protected in the long term under the traditional conditions of a seed bank. Additional collection and research efforts are required to increase the collection of native species in the SBB, especially threatened and endemic species.

KEYWORDS
accession, Chilean flora, endemism, ex situ conservation, gap analysis, germplasm, seed collection, threatened species

1 INTRODUCTION

Ensuring the stability of biodiversity in natural ecosystems is a key objective in conservation. Nevertheless, maintaining such biodiversity is unlikely due to the strong human pressure on natural ecosystems and exacerbation of these pressures through the action of climate change (León-Lobos, Barra-Bucarel, & Ortega-Klose, 2018; Li & Pritchard, 2009; Potter et al., 2017). This scenario raises the need to evaluate and implement other conservation measures that complement traditional approaches. A different method is ex situ conservation, which protects the components of biological diversity (populations, species, and genes) outside environments where they grow naturally. For plant species this includes collections in botanic gardens and arboreta, the cryopreservation of seeds, embryos or other tissues in liquid nitrogen, and the storage of genetic material (e.g., seeds, cuttings, bulbs, tubers, pollen) in germplasm banks (Abeli et al., 2020; Li & Pritchard, 2009). Ex situ conservation in germplasm banks not only conserves the genetic diversity pool of plant species, but also facilitates...
its reintroduction to natural habitat, preventing genetic erosion and counteracting the rate of loss of individuals. (Abeli et al., 2020; Menges, Guerrant, & Hamzé, 2004). Therefore, ex situ conservation in germplasm banks is an essential tool and has a key role in conserving the diversity of wild plant species.

The geographical singularity of Chile, which has been created by the natural barriers that surround the country, means that it is usually considered as a biogeographic island. In addition to the continental territory (17°S at 56°S and 67°W at 75°W), Chile possesses insular territories comprising Rapa Nui (27°7′S, 109°22′W), the Desventuradas (26°19′S, 80°00′W), and Juan Fernández (33°40′S, 79°00′W) islands. These conditions of biogeographic isolation have contributed to the formation of a unique flora composed of a high percentage of endemic species. In fact, 4,655 native vascular plant species have been recorded in Chile, of which 46% grow exclusively in its territory (Rodríguez et al., 2018), including 4 endemic families and 83 endemic genera. Thus, the Chilean flora harbors the second highest percentage of endemicity in South America after Brazil (Forzza et al., 2012; Scherson, Albornoz, Moreira-Muñoz, & Urbina-Casanova, 2014). While flora and vegetation constitute the basis for goods and services that underpin the economy and culture of a nation, their loss or destruction transcends human well-being in a negative way (Millennium Ecosystem Assessment, 2005). Continental Chile has a remarkable diversity of endemic plants that ranges from the Atacama Desert (ca. 25°5′S) to Patagonia (ca. 45°S), but it is threatened by fires, overgrazing, habitat degradation, invasive species, and expansion of forestry (Armesto, Rozzi, Smith-Ramírez, & Arroyo, 1998; Echeverría et al., 2006). For example, the vegetation of the Juan Fernández Islands of Insular Chile, which possesses the greatest number of endemic species by surface area relative to other islands of the planet (Stuessy, Crawford, López-Sepúlveda, Baeza, & Ruiz, 2017), is nowadays subjected to selective cutting, invasive species and fires (Cuevas & Van Leersum, 2001; Penneckamp, 2018; Vargas, Reif, & Faúndez, 2011). As a result, 46% of the Chilean vascular plants currently evaluated by the National Wild Species Classification (Reglamento para la Clasificación de Especies [RCE] in Spanish, www.mma.gob.cl), are endangered or critically endangered. This situation reaffirms the urgency of implementing measures to protect the Chilean vascular flora, a unique global heritage that is recognized among the 34 global biodiversity hotspots defined by the Global 200 initiative of the World Wide Fund for Nature and the World Bank (Mittermeier, Turner, Larsen, Brooks, & Gascon, 2011).

Evaluation of the ex situ conservation of Chilean plants is poorly represented in the literature. Seguel (2008) described that 12% of wild species are found in germplasm banks, which conserves different types of species (Salazar, León, Rosas, & Muñoz, 2006). For example, the Instituto Forestal (INFOR) conserves in vitro germplasm of southern beeches (*Nothofagus* spp.) and exotic species (Gutiérrez & Koch, 2015), and the germplasm bank of the Servicio Agrícola y Ganadero (SAG) in Magallanes comprises 9,200 species accessions of herbs, shrubs and trees (Niculcar, Latorre, & Vidal, 2015). In contrast, the Instituto de Investigaciones Agropecuarias (INIA), designated as the national curator of Chilean plant genetic resources in the year 1996, undertakes long-term preservation of orthodox seeds of Chilean native plants in its Seed Base Bank (SBB) and it also provides informative public online access of their collections to the public (León-Lobos et al., 2003; León-Lobos, Way, Sandoval, & Pritchard, 2010).

The main objective of our work is to record and report the current state of ex situ conservation of Chilean seed-bearing native species in the INIA SBB and identify the main challenges it faces to improve the current state of seed collection.

## METHODS

### 2.1 Current ex situ conservation state of the Chilean seed-bearing flora in the SBB

#### 2.1.1 Collection database

Seed collection of the native species preserved in the SBB is a result of 37 initiatives (conservation projects and/or donations) undertaken across Chile during the period 2001–2019. Every collection is considered an accession, it is a sample of seeds collected in a given population and time, and is representative of the population diversity. We organized the information about the accessions collected by each initiative into a database that included taxonomic and functional data (family, genera, species, origin, way of life), collection of the seeds (e.g., geographic location, date, collector), seed management and storage (e.g., processing, humidity, weight, number of seeds), the initiatives that allowed preservation of the seeds, and the physical place where the collections are stored and/or supported. We carried out a nomenclatural update based on the Catalog of Vascular Plants of Chile (Rodríguez et al., 2018), for accesses with unidentified species and old or erroneous scientific names. The accessions that could not be identified at a species level were only considered at a genera level in the analysis.


2.1.2 | Conservation status

We classified native species according to their conservation status assigned in the RCE, including all updates. We classified species in the five following categories: critically endangered (CR), endangered (EN), vulnerable (VU), near threatened (NT) and least concern (LC), corresponding to current categories on the Red List of the International Union for Conservation of Nature (IUCN). Finally, we considered all species that were not yet classified according to the RCE as being not evaluated (NE). In this analysis, we also considered species that had more than one conservation category under the RCE (i.e., intraspecific taxa and geographically isolated populations).

2.1.3 | Geographical distribution

In order to determine the geographical origin of the native species conserved in the SBB, we determined the number of species and accessions collected in each administrative region of Chile based on the updated database. We considered oceanic islands as separate territorial units from the Valparaíso Region, to which they belong administratively, due to the high levels of endemism. This analysis was made by administrative region aiming to create future ex situ conservation strategies and facilitate decisions in environmental policies. We carried out a species richness spatial analysis of the accessions to analyze the geographic concentration of the collections, determining the species collected in $24 \times 24$ arcmin grids (ca. 44 $\times$ 44 km) using DIVA-GIS software V. 7.5.0 (Hijmans, Guarino, Cruz, & Rojas, 2001).

2.1.4 | Contributions of conservation initiatives

We identified for each accession the initiatives that have allowed preservation of native species in the SSB. We classified this information into four categories: international projects, private national projects, public national projects, and donations. We considered international projects as those financed by foreign sources, whereas private projects included collections conserved by companies that were required by Chilean environmental regulations to conserve in a seed bank the affected species as compensation measures. Public projects corresponded to research initiatives awarded and financed by the Chilean State. Finally, we also considered donations to the SBB collection.

2.2 | Current challenges for Chilean seed-bearing flora conservation in the SBB

2.2.1 | Native flora representativeness by region

To determine the representativeness of the collected native species according to the Chilean administrative region (Rodríguez et al., 2018), we calculated an effort index ($I$) (Equation (1)). This index measured the average between the collected native species that are present in a region, and the species collected in the same region, multiplied by an inverse coefficient of the number of species known in the regional flora (Rodríguez et al., 2018). We calculated this index as follows:

$$I = \frac{C_i + A_i}{2 \times N_i}$$

where $C_i$ is the number of species naturally distributed in a region $i$ that are conserved in the SBB, independent of the region of origin of their accessions (according to Rodríguez et al., 2018); $A_i$ is the number of species collected in the region $i$ that have been conserved in the SBB and whose accessions have been strictly collected in the same region; and $N_i$ is the total number of species of the region $i$. This index has values from 0 to 1, and is close to 0 when the species of a region have a low representation in the SBB collection ($I = 0$ means there are no species of the region conserved, independent of the accession origin) and close to 1 when there is a high representation in the collection ($I = 1$ means that all the species of the region are represented by an accession collected in the same region).

2.2.2 | Species conservation gaps and poorly represented regions

We analyzed the information of the SBB collection to determine which native species were not currently preserved in the seed bank. The analysis allowed identification of gaps in the seed bank’s collection from the perspective of species and regional distribution. We compared regional richness with the SBB database, determining the number of species whose seeds have not been collected and preserved in the seed bank (species conservation gaps, Equation (2)).

$$S_i = N_i - C_i$$
where \( S_i \) represents the number of species in the region \( i \) that do not have any accessions conserved in the seed bank. Similarly, in order to identify the least represented regions in Chile, the proportion of native species per region that were not preserved in the SBB \( (R_i) \) was then determined relative to the total number of species that exists in that region (Equation (3)).

\[
R_i \% = 100 \times \frac{S_i}{N_i}
\]  

### 2.2.3 Non-conserved threatened species

The Global Strategy for Plant Conservation (GSPC)’s Target 8 includes that at least 75% of threatened plant species should be conserved in ex situ collections by the year 2020. This accomplishment is especially relevant in a biodiversity hotspot as Chile, in which there is a combination of high endemcity and threats. In order to assess this target, we determined the total number of threatened species (VU, EN, CR) for Chile and each administrative region that were conserved in the seed bank based on the Chilean RCE. This analysis only included threatened plant species with seeds.

### RESULTS

#### 3.1 Current ex situ conservation state of the Chilean seed-bearing flora in the SBB

##### 3.1.1 Native species collection

The SBB of INIA currently stores 3,040 seed accessions of Chilean native species, of which 93% (2,813 accessions) are identified to species level. The total number included at least 1,343 intraspecific taxa belonging to 1,256 species, which represents 26.9% of the total Chilean flora. Of the total, 59% were Chilean endemics. The native species conserved in the SBB were distributed across 118 families and 405 genera. Relative to seed number, we found that the majority of the SBB collection (78%) surpassed 2,000 seeds per accession (Figure 1).

##### 3.1.2 Conservation status

Most of the native species preserved in the SBB (84.6%) have not been evaluated under the RCE process, so there is no up-to-date information on their conservation status. Of the categorized proportion, 11% of the species were under some degree of threat, which includes 10 critically endangered species, 61 endangered, and 66 vulnerable. In this sense, it is also important to highlight that 32% of threatened Chilean flora, currently evaluated by the RCE process, is conserved in our Seed Bank. It means that we are far from meeting the GSPC’s Target 8. Finally, 2.4% of species in the remaining collection were classified as near threatened and 2.1% of least concern (Figure 2).

##### 3.1.3 Geographical distribution

The regions of the country with highest number of collected accessions and species were the north and center ones (between Arica y Parinacota to Maule Regions), which represented 85% of the total collection in the SBB (Table 1). On the other hand, the lowest number of collected accessions were found in southern Chile.
In the insular territory the number of accessions and species was significantly lower than in the continental territory, because there were only two accessions of wild potato (Solanum fernandezianum) conserved from the Juan Fernández Islands, which represented 0.07% of the total collection. Finally, in terms of distribution of the collections, the zones identified with the highest numbers of collections were the north and central zones (Figure 3a). On the other hand, geographic zones including the insular territory and the southern-western end of Chile had the lowest values for the number of conserved species.

### Table 1

| Regions                  | Accessions (no) | Species (no) |
|--------------------------|-----------------|--------------|
| Arica y Parinacota       | 93              | 76           |
| Tarapacá                 | 182             | 64           |
| Antofagasta              | 217             | 150          |
| Atacama                  | 422             | 198          |
| Coquimbo                 | 826             | 333          |
| Valparaíso (continental) | 318             | 232          |
| Metropolitana            | 210             | 160          |
| Libertador Bernardo O’Higgins | 115         | 71           |
| Maule                    | 200             | 157          |
| Ñuble                    | 47              | 36           |
| Biobío                   | 65              | 53           |
| Araucanía                | 114             | 80           |
| Los Ríos                 | 87              | 61           |
| Los Lagos                | 36              | 26           |
| Aysén                    | 10              | 3            |
| Magallanes y Antártica   | 69              | 9            |
| Rapa Nui Island          | 0               | 0            |
| Juan Fernández Islands   | 2               | 1            |
| Desventuradas Islands    | 0               | 0            |

(between Ñuble to Magallanes Regions). In the same way, in the insular territory the number of accessions and species was significantly lower than in the continental territory, because there were only two accessions of wild potato (Solanum fernandezianum) conserved from the Juan Fernández Islands, which represented 0.07% of the total collection. Finally, in terms of distribution of the collections, the zones identified with the highest numbers of collections were the north and central zones (Figure 3a). On the other hand, geographic zones including the insular territory and the southern-western end of Chile had the lowest values for the number of conserved species.

### 3.2 Current challenges for Chilean seed-bearing flora conservation in the SBB

#### 3.2.1 Native flora representativeness by region

According to the $I$ index values for each region (Figure 3b), the continental regions of Chile located between latitudes 17°S and 36°S (Arica y Parinacota to Maule regions) had an $I > 0.20$, but this decreased in regions located between latitudes 36°S and 55°S (Ñuble to Magallanes regions). This indicates that in the north and central regions of Chile there have been greater efforts towards collecting native species than in the south of the country. The Coquimbo and Atacama regions had the highest of all (>0.3), which contrast with the islands, specifically in Rapa and the Juan Fernández Islands, where the collection of accessions from the oceanic territories themselves has been rare ($I < 0.05$).

#### 3.2.2 Species conservation gaps and poorly represented regions

In terms of conservation gaps, we found that the central zone of Chile, between the Coquimbo and Araucanía Regions, has a high number of native species that are not preserved in the SBB (Figure 3c). Among these areas, the Metropolitana Region has the highest number of species that are not represented in the SBB, totaling 949 species. Nevertheless, these results change when the numbers of absent species are relativized to the regional total species number (Figure 3d). In this sense, the poorly represented regions are the extreme south of continental Chile and the insular territories. Among these it is worth mentioning the Magallanes Region, Rapa Nui, and the Juan Fernández islands, which are places where the regional proportions of species that have not been collected are close to 100%.

#### 3.2.3 Non-conserved threatened species

Remarkably, the Juan Fernández Islands have the highest number of threatened species (95 species) not collected and preserved in the SBB (Figure 4). This is followed by the Atacama, Antofagasta, Coquimbo and Valparaíso Regions, among which the number of non-conserved threatened species ranges from 45 to 73. Furthermore, in the southern extreme of continental Chile (Aysén and Magallanes Regions) and the insular territories of Rapa Nui and the Desventuradas Islands, there have been no collections undertaken of any threatened species.
FIGURE 3  Native species preserved in the seed bank and conservation gaps according to the Chilean region: (a) Number of species collected, (b) Collect Effort Index, (c) Non-conserved species, (d) Proportion of non-conserved species. AYP, Arica y Parinacota; TAR, Tarapacá; ANT, Antofagasta; ATA, Atacama; COQ, Coquimbo; VAL, Valparaíso; RME, Metropolitana; LBO, Libertador Bernardo O’Higgins; MAU, Maule; BIO, Biobío; ARA, Araucanía; LRI, Los Ríos; LLA, Los Lagos; AYS, Aysén; MAG, Magallanes y la Antártica Chilena; JFE, Juan Fernández Islands; IPA, Rapa Nui; IDE, Desventuradas Islands
populations within these territories, despite the low numbers of threatened species compared to the rest of Chile.

4 | DISCUSSION

4.1 | Current state of the ex situ conservation of Chilean seed-bearing Flora in the SBB

After 18 years of collecting native seeds (2001–2019), the SBB of INIA conserves the seeds of 26.9% of the Chilean flora. The diversity in the SBB is not only represented by different species, but also by different populations of the natural distribution of an individual species, which means that conservation also considers genetic variability (Potter et al., 2017; Sharrock, 2012).

Currently, about half of the collection has more than 10,000 stored seeds per accession, which ensures conservation of a representative sample of the population, enables development of germination protocols to assess long-term sample viability, and allows distribution of accession duplicates to other seed banks (Di Sacco, Way, León-Lobos, & Suarez-Ballesteros, 2018).

Currently, it is not possible to estimate with accuracy the real level of threat to the species conserved in the SBB because a high percentage of them (78.6%) have not been classified. The lack of species classification makes it necessary that a larger number of plant species be reviewed according to the RCE, not only to know the state of the already conserved species, but also to focus new efforts towards the most threatened ones. Currently, around 21.3% of the Chilean plants are classified according to the RCE, which is better than the global average, considering that only around 10% of the world’s plants are thought to be represented in red lists under the IUCN (Pelletier, Carstens, Tank, Sullivan, & Espíndola, 2018). In any case, the development of new tools that allow quick classification, such as indexes for instance, could be useful for this purpose.

In continental Chile, despite the lack of ex situ species conservation in the north of the central zone of the country, this area is where most collecting efforts have been done, and where most species are being conserved. This is explained because the administrative regions located in the Chilean Matorral (sensu Olson et al., 2001) contain higher species number than other regions (Rodríguez et al., 2018), which becomes this zone as one of the main biodiversity hotspots in the country (Mittermeier et al., 2011; Myers, Mittermeier, Mittermeier, da Fonseca, & Kent, 2000). Nevertheless, differences in collecting efforts also indicates that there has been a bias in conservation focus that has ignored other important geographical zones in Chile. The current collection of conserved species is a consequence of the types of projects that the SBB has worked with, and which have commonly been restricted to a group of target species. To change this limitation, more public projects and donations could be encouraged, which means broader contributions through public participation (Jorquera-Jaramillo et al., 2012).
4.2 Conservation challenges of the Chilean seed-bearing flora in the SBB

Currently, conservation efforts should focus in decreasing the gap between conserved species and those that have not been conserved, especially threatened endemic plants. Regarding the latter case, the Biosphere Reserve of the Juan Fernandez Islands is a priority site due to its high number of threatened species that are currently under-represented in the SBB. The regions of Antofagasta and Atacama are also important as they are home to a substantial proportion of threatened species that have not yet been collected for conservation. In the Atacama and Antofagasta Regions there is a relatively high diversity of species, most of which are endemics (Letelier et al., 2008; Squeo et al., 1998). Furthermore, the austral territory of Chile (the Aysén and Magallanes Regions), which comprises the Patagonian Steppe and the Magellanic Antarctic Forest, is very under-represented in the collections of the SBB. A 32% of the threatened Chilean plants, currently evaluated by the RCE, are preserved in our Seed Bank, which means that we are far from meeting the GSPC’s Target 8.

An increase in the protection of native threatened species in the collection should be complemented with the development of new methods in conservation, which would allow ex situ preservation of a new range of plants. Considering that 25% of the threatened plants in Chile have spores and not seeds (Pteridophyta), the lack of a conservation method for these species in the nation’s seed banks needs to be overcome. In this regard, Blackmore and Walter (2007) mention that one of the challenges of the GSPC is to better understand the conservation needs of these species, with cryopreservation likely to be the most effective way for long-term storage of spores (Ballesteros, Estrelles, & Ibars, 2006; Ballesteros, Estrelles, Walters, & Ibars, 2012). This technology could also allow storage of plants with recalcitrant seeds that do not withstand low levels of hydration, and therefore cannot be stored in traditional seed banks (Wyse & Dickie, 2017). Despite this physiological trait being more commonly associated with tree species from wet tropical zones (Tweddle, Dickie, Baskin, & Baskin, 2003), our team has observed several species in Chile with this trait that are components of relict ecosystems, which are commonly threatened. At the global level, Wyse, Dickie, and Willis (2018) have noted that at least 36% of species catalogued as critically endangered on the IUCN red list possess recalcitrant seeds. Hence, these authors have argued that an increase in research effort towards the development of cryopreservation as a tool for ex situ conservation of threatened species should be a post-2020 target of the GSPC.

Finally, among the general challenges that seed banks need to overcome (Díez et al., 2018) are recommendations to improve the characterization of collections, including phenotypic and genotypic information. Also of relevance is the implementation of new global portal for information access in the form of the Global Biodiversity Information Facility (GBIF; www.gbif.org), which is an important tool in the development of plant diversity research and collection management (Wyse et al., 2018).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Carolina Panitirur-De la Fuente investigation, database analyses and writing the original article; Sergio T. Ibáñez performed the index effort and gaps analyzes, writing-review and editing; Karina Martínez-Tilleria organizing the database; Mario F. León and Ana Sandoval review the article. All authors have read and agreed to the published version of the manuscript.

ETHICS STATEMENT

The authors declare no ethical issues of any kind. This study is accordant with the SCB’s Code of Ethics.

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