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Article in *Plant Ecology & Diversity* · June 2012

DOI: 10.1080/17550874.2012.713402

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The role of ex situ seed banks in the conservation of plant diversity and in ecological restoration in Latin America

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(Received 10 February 2011; final version received 16 July 2012)

Background: Over half of the world's primary forest and a quarter of the world's plant species are found in Latin America. As a result of the limited protected areas and significant degradation of terrestrial ecosystems, seed banks provide an efficient component of integrated plant conservation strategies, chiefly due to their relatively low cost and massive storage capacity for genetic resources.

Aims: We analyse the role that ex situ seed banks play in the conservation and reintroduction of threatened species, and in supporting ecological restoration programmes in the region.

Methods: An analysis of the second National Reports on the state of the World's Plant Genetic Resources for Food and Agriculture showed that most countries in the region had the capacity for seed conservation. Using three case studies from seed conservation programmes in Mexico, Brazilian Amazonia and Chile, together with the global Millennium Seed Bank Partnership, we review the status and potential of these initiatives for conservation of plant diversity.

Conclusion: The collection, conservation and use of seeds from arid and semi-arid biomes is highly effective; however the higher frequency of recalcitrant seeds in humid tropical forests requires a greater investment in research to underpin large-scale seed banking. In order to safeguard native species and provide adequate diversity of seeds for habitat restoration programmes, we anticipate the need to strengthen the capacity of the region's seed banks for preservation, research and propagation of native species.

Keywords: desiccation-tolerance; gene bank; genetic diversity; germination; germplasm; plant conservation; threatened species

Introduction

The majority of plant species are capable of surviving as seed for many decades in dry, cold conditions. This capacity enables plants to withstand extreme environments and permits wide seed dispersal through time and space (Priestley 1986; Kermodé 1995), increasing the probability of seed survival and the establishment of new plants. The ability of seeds to survive in storage (i.e. longevity) has been successfully applied by gene banks worldwide for the preservation and exchange of crop genetic resources threatened by genetic vulnerability. In the second report on the State of the World's Plant Genetic Resources (FAO 2010), genetic vulnerability was a concern for 60 countries, of which, for example, Costa Rica reported that *Phaseolus* and *Sechium* species were subject to severe genetic erosion. One of the responses by gene banks worldwide to this threat has been a 20% growth in the number of ex situ crop collections between 1996 and 2008, either through new collecting and/or through duplication of existing collections.

Seed banks are valuable for preservation of genetic diversity of non-crop plants threatened by ecosystem and land use changes, overexploitation, invasive alien species, pollution and climate change (Millennium Ecosystem

Assessment 2005). Although the underlying causes of biodiversity loss recognised by the Global Biodiversity Outlook (Secretariat of the Convention on Biological Diversity 2010a) need to be addressed, seed banking is a necessary and cost-effective complement to in situ conservation of wild plants, and it provides a vital source of material to assist in ecological restoration of damaged and degraded habitats (Maunder et al. 2004). For example, Thuiller (2007) estimated that the climate profile for species may move the equivalent of 500 km towards the poles or 500 m to higher elevation over the next century – although such estimates, based on temperature niches, do not take into account the adaptive capacity of species. Given the urbanisation and fragmentation of native habitats, active translocation may be required for many species. Seeds for translocation should be provided by ex situ seed banks.

Using three case studies from seed conservation programmes in Mexico, Brazilian Amazonia and Chile, together with the global Millennium Seed Bank Partnership (MSB Partnership), we review the status and potential of initiatives for conservation of plant diversity and its application in ecological restoration across the continent.

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The biology of seed banking

Based on their tolerance to desiccation, seeds can be divided into two main groups: orthodox (desiccation tolerant) and recalcitrant (desiccation sensitive). Orthodox seeds survive drying to below ca. 7% moisture content and tolerate subsequent rehydration without significant loss of viability (Roberts 1973). Their longevity increases in a predictable way as seed moisture content and storage temperature are reduced (Ellis and Roberts 1980). Consequently, orthodox seeds are suitable for storage in most seed banking facilities. In contrast, recalcitrant seeds cannot tolerate removal of bound water without viability loss and show signs of dehydration stress when 'free' water is being removed (Pritchard and Manger 1998; Pammenter and Berjak 1999). Recalcitrant seeds cannot therefore be dried and stored in conventional seed bank facilities. Some tropical fruits show a type of seed storage behaviour intermediate between orthodox and recalcitrant seed such that conventional gene bank storage at -20°C can be harmful (Hong and Ellis 1996).

Desiccation tolerance is acquired continuously during development and maturation of orthodox seeds on the mother plant as a normal terminal event in their development (Kermode 1995, 1997). Seeds undergo a gradual transition from a desiccation-intolerant to desiccation-tolerant state (Hong and Ellis 1992; Sun and Leopold 1993; Ellis and Hong 1994; Vertucci and Farrant 1995). In general, desiccation tolerance is acquired in the early or mid-point in the seed development process. Together with the ability to germinate, desiccation tolerance increases to a maximum at the end of the maturation phase, before seed shedding, at which stage potential seed longevity also reaches its highest level. It is therefore recommended to harvest seed for conservation during the period of natural dispersal, because potential seed longevity declines thereafter (Way 2003).

Seeds of around 88% of higher plant species show orthodox storage behaviour (Hong et al. 1998), and species with these seeds tend to be found in environments subject to occasional or seasonal drought (Roberts and King 1980), such as temperate, Mediterranean, desert and savannah ecosystems. Recalcitrant seeds, in contrast, tend to be found in species from humid and warm habitats where infrequent or little variation in environmental conditions occurs, such as tropical and aquatic ecosystems (Roberts and King 1980; Hong and Ellis 1996). Intermediate seeds have been found in species occurring in both dry and humid environments (Ellis et al. 1990; Hong et al. 1998).

The prediction of seed storability in the field is still challenging. Hong and Ellis (1996) concluded that orthodox seed storage behaviour tended to be present in species that produced achenes, many-seeded berries, many-seeded dehiscent capsules, many dry-seeded pods, many dry-seeded follicles, siliques and utricles. However, all three categories of seed storage behaviour can be found in species with drupes (with four seeds), pods with five large seeds or both containing many arillate seeds, berries (with 10 seeds), capsules (with five seeds) and single-seeded nuts. In terms of moisture content, those species in which seed is shed or

harvested at around 20% moisture content or below are very likely to show orthodox behaviour (Hong and Ellis 1996), but not all species that shed seed at $> 60\%$ moisture content are likely to show recalcitrant storage behaviour.

Collectors of seed from wild species need to assess the frequency of empty or insect-infested seeds, which can be a problem in families including Asteraceae and Poaceae. Physical quality assessment is achieved at large seed banks such as the MSB Kew by X-ray imaging, which gives excellent detail of seed structure (Linnington et al. 1995), but in the field a simple cut-test of a sample of seeds is usually effective for assessing all but the smallest seeds.

Although the distribution patterns of wild plants can be very variable, careful seed sampling from at least 50 individual plants randomly across the extent of a population is estimated to capture 95% of the non-rare allelic diversity present in most plant species (Brown and Briggs 1991), and more intensive sampling will ensure that the allelic frequencies characteristic of the population are reflected in the sample. The preserved seed collection is therefore capable of re-establishing the source population in the event of catastrophic loss, and in most out-crossing species is also representative of the genetic diversity of the species. As the intermediary between the original field collecting event and many ultimate users, seed banks have a responsibility to conserve this diversity by operating to applicable collecting and curation standards (e.g. Rao et al. 2006; ENSCONET 2009) which include maintaining essential data associated with the collection or regeneration event, and holding data from the research, testing, trialling and propagation undertaken with the material.

Threats to plant diversity of Latin America

Latin America together with the Caribbean contains around a quarter of the total world flora and over half of the world's primary forests (FAO 2011). It includes the Mesoamerican and Andean centres of crop diversity defined by Vavilov (Hawkes et al. 2000) and eight of the 34 biodiversity hotspots recognised worldwide (Mittermeier et al. 2004). These hotspots contain not just a diverse flora but also display high levels of endemism; for example a total of 3893 species of native vascular plants are found within the Chilean hotspot, of which 50.3% are endemic (Arroyo et al. 2004).

Although pre-Columbian inhabitants of the region modified the environment for agriculture and management of grazing and other resources, progressive destruction and degradation of the natural vegetation has increased dramatically since the arrival of European settlers in the sixteenth century. The original plant communities were cleared to meet demands for agriculture, grazing, timber, firewood, minerals and urbanisation. Forest area declined in Central and South America after settlement, with the leading cause of deforestation being the conversion of forest land to agriculture. For example, more than 90% of the original Atlantic rainforest has been converted to agriculture or urban areas, and recent analysis by the Sampled Red List

Index (RBG Kew 2010a) estimated that around one-third of the world's threatened plant species were in Latin America and the Caribbean. Although land use changes have been the dominant threat to plants for many decades, the impact of invasive species and global climate change is expected to increase. For example, the moth *Cactoblastis cactorum* Berg. which was once valued for controlling invasive cacti in Australia, has been spreading along the southern USA towards Mexico, where it now threatens 51 endemic cactus species and could impact a major horticulture industry (Rose et al. 2010).

Global initiatives to conserve plant diversity

The Global Strategy for Plant Conservation (GSPC) adopted by the Conference of the Parties to the CBD in 2002 has promoted unparalleled cooperation between botanical organisations worldwide in its implementation. Such collaboration led in 2009 to the production of the Plant Conservation Report (Secretariat of the Convention on Biological Diversity 2009) concluding that one-third of plant species were threatened with extinction. In 2010, the consortium of organisations within the Partnership for Plant Conservation was given a renewed mandate from the Tenth Conference of the Parties to the CBD in Nagoya, Japan to work to more ambitious targets by 2020. In common with most of the aims, Target 8: "At least 75 per cent of threatened plant species in ex-situ collections, preferably in the country of origin, and at least 20 per cent available for recovery and restoration programmes" will be achieved only by efficient investment of resources and by extensive collaboration at regional and international level.

Critical to the prioritisation of ex situ efforts is the knowledge of the distribution and status of plant species as recognised by GSPC targets 1 and 2, the foundation for fulfilling Target 8. The compilation of a provisional global plant species list (The Plant List 2010) for around 300,000 of the estimated 380,000 plant species is therefore a major achievement which is mirrored at national level by checklists such as the Ecuador red list (Valencia et al. 2000) and Brazil list (Forzza et al. 2010). Importantly, the increased availability of digital specimen data and GIS mapping tools are facilitating gap analyses to prioritise collecting efforts. For example, Ramírez-Villegas et al. (2010) used data from nine gene banks and 26 herbaria in order to identify priority collection zones for wild relatives of the crop *Phaseolus* in the Americas.

Case studies

Within the Latin America and Caribbean region, seed banks from international, national and non-governmental sectors have responded differently to threats to plant diversity and to demands from user-groups. The following examples demonstrate strategies used by seed banks from different sectors for the conservation of plant diversity in Latin America.

Application of seed banks in Chile

The central-north zone of Chile is acknowledged as one of the 25 centres of global biodiversity (Myers et al. 2000); this has been subsequently broadened to include the temperate rain forest of southern South America and the Juan Fernandez Archipelago (Arroyo et al. 2004). Due to the great plant diversity of the Chilean hotspot in situ biodiversity conservation has been an important strategy, resulting in around 19% of the country's surface benefiting from some protection within National Parks and Natural Reserves (Rovira et al. 2006). However, despite these measures there are still important ecosystems and plant communities that are not included within the national in situ Conservation System (Pliscoff and Fuentes-Castillo 2008). Over 80% of the protected areas are concentrated in the south of the country (43° 38' S to 56° 30' S), with very little protection in central and northern Chile (Armesto et al. 1998, Squeo et al. 2012). In addition, a significant proportion of the endemic species in Chile are either rare or of very restricted distribution within the country. Around 31% of Chilean endemic flora in the central-north of the country is restricted to one and 27% to two administrative regions (León-Lobos et al. 2011). Faced with this situation, it has been vital to develop and strengthen actions that can preserve the genetic diversity of endemic and threatened species as part of an integrated conservation strategy.

Implementation of the CBD in Chile is primarily through the National Strategy for Biological Diversity (CONAMA 2003). This provides a framework for actions to preserve species and genetic diversity, including conservation status assessment, ex situ conservation and promoting sustainable use of genetic resources. Since 1990 the Instituto de Investigaciones Agropecuarias (INIA) has invested in a conservation programme for plant genetic resources, focused on safeguarding genetic diversity critical to national food and agriculture (Suzuki 1994). To date, around 54,000 accessions of germplasm of major crops have been preserved in the INIA gene bank, cereals and legumes being the main collections (Salazar et al. 2006). The National Forest Corporation (CONAF), responsible for the management of national parks and reserves, has undertaken targeted ex situ conservation actions for threatened species of the Juan Fernandez Archipelago (Ricci 2006), but seed banking for wild plant species has been limited until 2001 when INIA developed a seed conservation programme in collaboration with the Royal Botanic Gardens, Kew. The objective of this collaboration has been to preserve the genetic diversity of endemic and endangered plant species of Chile as part of the global MSB partnership. In regions not covered by up-to-date red lists (Squeo et al. 2001; 2008; Serey et al. 2007), species have been prioritised by using an index, based on their level of endemism and phylogenetic uniqueness (Guerrero et al. 2008). This has become a simple and useful tool to focus efforts on species that are most unique and most threatened, where such information is available. Priority has also been given to those species with particular use such as ornamental

or medicinal plants, together with more common species needed for restoration of degraded habitats.

By 2010, 1650 collections representing 1000 species and subspecies have been made and safely preserved in the INIA Seed Base Bank and most of them have been duplicated at the Royal Botanic Gardens, Kew, under a legally binding agreement (León-Lobos et al. 2011). Some 20% of the endemic and useful flora of Chile is now conserved in these banks. Although these figures indicate that the activities have been successful, it is difficult to quantify the precise impact on threatened species as their conservation status is often unknown. Despite that, dozens of endangered and vulnerable species are already banked, including two species declared extinct in the past, but recently rediscovered (León-Lobos et al. 2011). The activities have also made a significant contribution to knowledge about the distribution of native plants in Chile. Information from over 4500 pressed plant specimens deposited in herbaria in Chile and Kew will promote the taxonomic and biogeographic

study of rare and endemic species. As an example, herbarium data have been combined with other records to produce a flora checklist of the Atacama region and to establish the conservation status of its flora (Squeo et al. 2008). The same process is underway for Tarapacá and Antofagasta regions in the extreme north of Chile (Figure 1).

The physiology of seeds of endemic and native species from Chile, especially those from arid and semiarid zones, is an area of limited biological knowledge (Figueroa et al. 2004). The seed bank collections have enabled germination studies to be carried out for around 400 species. For example, seeds of *Placea* species (Amaryllidaceae), an endemic and rare genus of Chile, require pre-chilling to germinate (Guerrero et al. 2007). Germination trials for 290 Chilean Asteraceae species conducted at 20 °C showed that 46% of the total accessions had over 50% germination. Preliminary results from tetrazolium tests indicated that most species that had < 50% of germination had viable seeds, or in some cases had empty seeds (P. León-Lobos, unpublished).

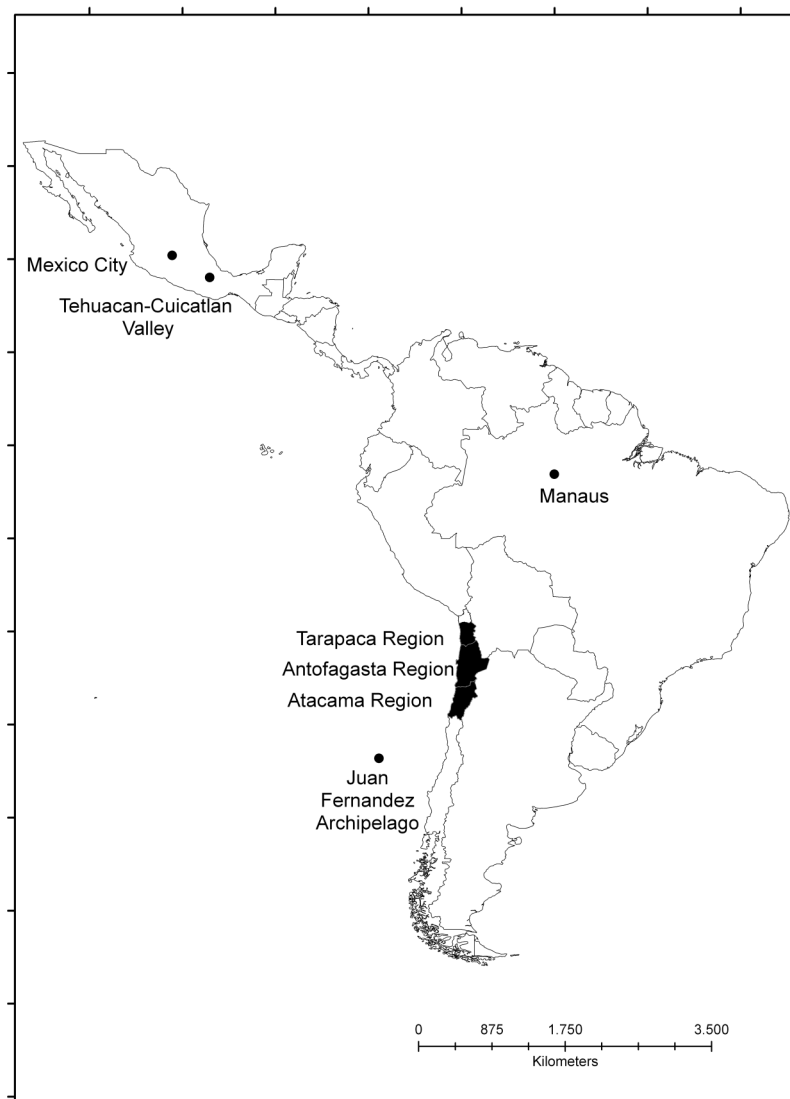


Figure 1. Localities of Chile, Brazil and Mexico where seed conservation-related projects are being carried out.

Future priorities for seed banking are the native forest species and the Juan Fernandez Archipelago species, which will require the strengthening of existing plant conservation networks, especially through building capacity for seed collecting and plant propagation. The limited resources of the recent Chilean national network of botanic gardens have limited its activities of *ex situ* conservation of threatened plants. Nevertheless, these gardens have an active role to play in plant propagation and cultivation, capacity building, display and public education. Through these collaborations and also with other organisations, it is planned to extend by the year 2020 seed banking to 45% of the Chilean flora, in support of Target 8 of the updated GSPC (Secretariat of the Convention on Biological Diversity 2010b).

Seed collections and associated field data are important resources for species reintroduction and ecological restoration initiatives. An example is the germination, propagation and establishment of *Laretia acaulis* (Cav.) Gill. et Hook, a Chilean endemic cushion plant. Restoration activities have been carried out with this species to mitigate possible impacts from mining in the high central Andes (P. León-Lobos, unpublished). In addition, research aiming to produce protocols and guidelines for restoration has been conducted on using native plant species for rehabilitation of mined sites in Chile (De la Fuente et al. 2011; Ginocchio and León-Lobos P. 2011; León-Lobos et al. 2011). As opportunities arise for habitat restoration and degraded land reclamation it is expected that seed banks will need to supply some common native species, such as early successional and keystone species for specific restoration areas. In conclusion, seed banks will have the greatest impact if their managers communicate effectively with regional partners, focus their efforts on the most important species and habitats, and maintain high technical standards.

Seed banks in Amazonian forests

The Amazon Basin is estimated to cover 5 million km² in total (Braga 1979) and it holds the highest worldwide biodiversity of the planet with around 300 species of trees (DBH = 10 cm) per hectare. Forest inventory results reported by Rankin-de-Merona et al. (1992) within the Amazon region vary from 167 to 676 trees/ha, 18 to 55 families/ha and from 61 to 698 species/ha. The number of species commercially exploited typically varies between 38 and 60 (Higuchi et al. 1985; Faraco and Coelho 1996) with as many as 157 species with potential for exploitation. To preserve these forest resources, highly valued species such as rosewood (*Aniba rosaeodora* Ducke) have been protected from felling (CITES 2010). The Department of the Environment, Amazonas State, Brazil, has forbidden felling of andiroba (*Carapa* sp.) and copaiba (*Copaiba* sp.) as these species can be exploited for products such as oils and seeds without felling. Species such as virola (*Virola surinamensis* (Rol. ex Rottb.) Warb.) and sumaúma (*Ceiba pentandra* (L.) Gaertn.) are also threatened, and seed banks could be very important for restoration and conservation programmes for species with orthodox seed.

The conversion of natural forest into other forms of land use has led to severe environmental degradation with impacts on biogeochemical cycles and biodiversity (Achard et al. 2002; Lamb et al. 2005). In Amazonia, in particular, cattle grazing, expansion of agricultural areas, mineral exploitation and selective felling of trees without a forest management plan has resulted in the conversion of ca. 500,000 km² of forest to unsustainable land uses in the last 20 years alone (Fearnside 2005; Malhi et al. 2008; Verweij et al. 2009).

The Amazonas Native Seed Center (CSNAM) of the Universidade Federal do Amazonas (UFAM) Manaus, established in 2001 with state and country-level government support, produces and supplies seeds for restoration programmes in permanent forest reserve areas and other restricted areas that have been damaged by exploitation of timber and minerals. The main restoration strategy is to establish agroforestry systems. This is supported by establishing seed orchards, for example, as has been done for the palms tucumã (*Astrocaryum aculeatum* G.Mey) and açai (*Euterpe oleracea* Mart.) that have been selected from 52 species of economic importance in the region. However, the scarcity of registered Seed Collection Areas (SCA) for collection of seeds for production, and the difficulty associated with training collectors (e.g. Lima Jr 2010a) and collecting in remote areas has long hindered efforts. CSNAM now has a group of trained collectors and two SCAs of 50 ha each are being established, one in the Sustainable Development Reserve of Uatumã-Itapiranga (−02° 22' 32" S; −58° 14' 2" E) and another on the Experimental Farm of the Federal University of Amazonas (−02° 39' 1" S; −60° 03' 40" E), near Manaus. CSNAM has collaborated in the Ducke Forest Reserve with the National Research Institute of Amazonia (INPA), who have recorded 60 species of economic importance that have been studied in relation to seed ecology.

Seed-handling conditions in Amazon forest are challenging because of the large variety of fruit types. Furthermore, limited information is available on species, equipment, and techniques for seed extraction, drying and cleaning. A guide to fruit/seed handling of tropical tree species is therefore in preparation to help collectors maintain seed lot viability and quality.

Seed viability tests (e.g. Lima Jr. 2010b) and germination tests need to be conducted following standard protocols. For example, seed germination tests have indicated absence of dormancy for the majority (69%) of species of economic importance studied in the Ducke Forest Reserve, Manaus. The best germination temperature for tropical tree species is between 25 °C and 30 °C, and seeds typically take around 3 months to start to germinate (Ferraz et al. 2004). An estimated 62% of the species studied have recalcitrant seed storage behaviour.

The Amazon Seed Network (Ferraz 2007) has prioritised seed collecting, botanical identification and seed storage in order to improve the supply of native seeds to users for restoration programmes. With support from the Ministry of Environment and through technical

partnerships developed with RBG Kew and CATIE in Costa Rica, CSNAM will enhance seed production and commercialisation of seeds from the Amazon region. These activities will be based on strong academic and technical support, and implemented in close cooperation with local forest dwellers as the collectors and users of the resulting materials.

Application of seed banks in the arid and semi arid regions of Mexico

The estimates of vascular plant diversity of Mexico vary between 22,000 and 31,000 species, according to different authors (Rzedowski 1991; Toledo 1993; Villaseñor 2003). Influenced by the rich cultural diversity of the country, close to 4000 Mexican plant species have been recorded as medicinal. At a global level, Mexico is the fifth most diverse country for plants and the sixth in endemism rate, with 40% of its flora being restricted to the country (Llorente-Bousquets and Ocegueda 2008).

The complex biological mosaic that is found in the Mexican drylands is subject to degradation processes that are the result of various human activities, often carried out without planning or prior knowledge of the biota inhabiting different areas. Inadequate management has been a cause of major genetic erosion and loss of biodiversity. Approximately 67% of the drylands of Mexico have been drastically altered (Challenger 1998). The species distribution ranges of various wild plant species have been reduced or fragmented, and many species are now endangered. The IUCN red list (IUCN 2004) includes a total of 314 Mexican plant species at risk, whereas the national threatened list includes 981 plant species in various risk categories (SEMARNAT 2002). In addition, Villaseñor and Espinosa-García (2004) have recorded the presence of 618 exotic plant species, and with the impacts of globalisation it is probable that this number will increase each year.

Mexican National Strategies have been developed for biodiversity, plant conservation and invasive species. The National Strategy of Biodiversity is based on inventories, workshops and data from projects and scientific research activities (CONABIO 2000). This Strategy has four basic lines that include a total of 22 groups of actions that are focused on the conservation of the biodiversity. This document is the backbone of any public environmental policy in the country. Within its framework, Mexico has been gradually developing Conservation Strategies at the state level. The National Strategy for Invasive Species has been developed in order to set well-defined objectives for preventing, controlling and eradicating invasive species (Koleff et al. 2010). The Mexican National Strategy for Plant Conservation is currently being developed in support of the implementation of the GSPC (Secretariat of the Convention on Biological Diversity 2010b). Two of the three phases of this Strategy have already been completed (Anonymous 2008). Within this framework, the commitments and actions of the Seed Bank

of the Facultad de Estudios Superiores, Iztacala (FESI) of the National University of Mexico (UNAM) have been established.

The objectives of the FESI Seed Bank are to: (1) undertake long-term storage and conservation of seeds from wild plant species from the arid and semiarid areas of Mexico, especially of species that are endemic, narrowly distributed, rare, threatened, or are wild relatives of economically important species, in accordance with international standards; (2) generate and compile morphological and physiological seed data to enable effective seed banking; and (3) promote a continuous capacity-building programme for students and technicians, for undertaking field and laboratory work to high standards.

A field collection and survey has followed the Priority Terrestrial Regions of Mexico (Arriaga et al. 2000) and has been assisted by extensive analysis of herbarium specimen data for target regions. The laboratory work for documenting the basic morphological and physiological seed features follows international standards (ISTA 1999; Engels and Visser 2003). Most of the FESI Seed Bank activities have been in collaboration with the Royal Botanic Gardens, Kew, and the National Commission of Biodiversity (CONABIO). The FESI Seed Bank now has the basic infrastructure and equipment needed to provide long-term storage and conservation of seeds to international standards, and the collections are recognised as one of the long-term biological collections of Mexico.

The FESI Seed Bank holds 1980 accessions, belonging to 130 botanical families and 1174 vascular plant species (endemic or with a restricted distribution pattern, some rare or threatened species, and some close relatives of economically important plants). The most represented families stored are: Asteraceae, Agavaceae, Cactaceae, Caesalpinaceae, Fabaceae, Euphorbiaceae, Malvaceae, Mimosaceae, Poaceae, and Solanaceae. As part of seed bank protocols, germination tests have been conducted and protocols defined for 850 species (P. Dávila, unpublished).

The information generated by the staff of FESI Seed Bank and collaborators has provided a platform for the development of a diversity of research activities (Table 1). In addition to supporting the compilation of a seed catalogue of the columnar Cactaceae of Mexico, and studies of seed physiology and germination closely linked to the routine running of the seed bank, studies have extended into seed ecology, phytochemistry, conservation assessment, reproductive biology, genetic analysis, seedling establishment, global change and the conservation of useful plants. These studies have resulted in publications ranging from undergraduate theses to articles in high-impact journals, and provide a strong rationale for continuing to maintain and develop the wild species collections held in the FESI seed bank.

In 2011, a new phase of the Seed Bank programme started. Although the former activities, such as those described above, will continue, there will be an emphasis on the collection and study of useful plants and tree species of the arid and semiarid areas of Mexico.

Table 1. The research projects developed and their publications related within the framework of the FESI Seed Bank, Iztacala, Mexico.

Title of project	Related publications
Determination of the physiological and genetic parameters related to the ex situ conservation of <i>Beaucarnea gracilis</i> and <i>Hechtia podantha</i>	● In preparation
Ex situ and in situ conservation of two columnar Cactaceae that are endemic to Mexico (<i>Polaskia chende</i> and <i>P. chichipe</i>)	● Otero-Arnaiz et al. (2003) ● Ordóñez-Salanueva (2008)
Seed ecophysiology of <i>Polaskia chichipe</i> (Cactaceae) in a soil seed bank	● In preparation
Chemotype assessment of <i>Lippia graveolens</i>	● Hernández et al. (2009a) ● Hernández et al. (2009b)
Evaluation of the conservation status of 10 Cactaceae species of Mexico	● Casas et al. (1999a) ● Casas et al. (1999b) ● Téllez-Valdés and Dávila (2003) ● Godínez-Álvarez et al. (2004) ● Rodríguez-Arévalo et al. (2006) ● Solórzano et al. (2009)
Genetic and ecologic evaluation of some globose Cactaceae endemic to the Tehuacán-Cuicatlán valley	● Dávila et al. (forthcoming)
Global change effects on the distribution range of some Cactaceae of the Tehuacán-Cuicatlán valley	● Dávila et al. (forthcoming)
Seed catalogue of the columnar Cactaceae of Mexico	● Guillen et al. (2009) ● Flores et al. (2011)

Application of seed banks as part of the global Millennium Seed Bank partnership

The effectiveness and impact of seed banking worldwide have often been limited by constraints of infrastructure, or of trained personnel with access to research support for solving problems. For example, as recently as 2006, 60 gene bank staff from 38 sub-Saharan African countries reported difficulty in germinating over half of the useful plant species prioritised by farmers and technicians (RBG Kew, unpublished data). One compelling strategy to improve and increase the impact of seed banking activities is to share best practice through networks and to transfer technology through international cooperation. The CBD agreed at the 1992 Earth Summit set the framework for such partnerships in conservation of biodiversity worldwide. Although the national variations in implementation of Access and Benefit Sharing regulations by signatory nations have presented genuine obstacles to the exchange of research and conservation material between many states, botanical institutions such as the Royal Botanic Gardens Kew, UK, have been able to establish effective seed banking collaborations through development of comprehensive Access and Benefit-Sharing Agreements (Cheyne 2003) with partners.

In 1992 the Trustees of RBG Kew recognised that the seed banking of wild species could be expanded dramatically through providing extensive technical and research support to partners worldwide. Duplicates of each seed collection would be stored in the UK for long-term conservation and to help gain a deeper understanding of the biology of seeds. Botanic gardens are particularly suited to host such projects as they can provide extensive technical support and can showcase the achievements to their visitors. A £30 million grant from the UK Millennium Commission, a distributor of proceeds from the National

Lottery, provided the impetus for an ambitious programme to collect and bank seeds from the entire UK native flora by the year 2000 and in the following decade to safeguard seeds from 10% of the world's plant species through international collaboration. The selection of species included endemic, endangered, and useful species as prioritised at national, local and institutional levels.

In October 2009 the collection of seed from *Musa itinerans* Cheesman, a Chinese wild banana, marked the achievement of the 24,200 species seed-banking target to which 100 Millennium Seed Bank (MSB) Partner organisations from 50 countries had contributed. The collections are accompanied by comprehensive field data and a set of pressed herbarium specimens which facilitate the correct naming of the collection. After processing, seeds are dried, banked and germination tested to ensure that it meets quality standards. Seed banks respond to the needs of bona fide users for samples of seed, for basic research and conservation only. For example, since 2000, RBG Kew alone has provided over 11,000 seed samples to non-commercial users, largely in the university, botanic garden, and education sectors.

The MSB Partnership achievements extend beyond the seed collections: over 1150 personnel from 43 countries received technical training in seed conservation techniques, and Kew's active seed science programme has developed research capacity through over 50 postgraduate students, resulting in publication of over 150 papers. In addition to direct technical support from RBG Kew, opportunities have been taken to strengthen direct contact between partners to share expertise and spread best practice within the network. For example, reciprocal exchanges took place between the seed bank officer and technician (FESI-UNAM, Mexico) and the propagation research officer (INIA, Chile) to participate in seed conservation

activities. Wider research collaboration across the region was achieved through the compilation of seed biology data for 350 species of Cactaceae studied by cooperating researchers in Argentina, Chile, Mexico, Peru, the UK and USA (Seal et al. 2009). Technical cooperation has also developed within the Caribbean centred on the Dominican Republic, where leadership has been shown by the National Botanical Garden (JBN, Santo Domingo) and the Ministry of Environment and Natural Resources to develop seed collections respectively of threatened wild species and forestry species. Support from RBG Kew and the Lombardy Seed Bank (University of Pavia, Italy) has helped the JBN make 97 seed collections of 68 species and to improve seed processing and storage protocols.

Increasingly, RBG Kew has been supporting local propagation initiatives in order to assist communities recover their useful native plants. For example, Millennium Seed Bank expertise has assisted the initiative to restore the relict Huarango *Prosopis pallida* (Humb. & Bonpl. ex Willd.) Kunth forest (Whaley et al. 2011) in the Atacama-Sechura Desert of Peru. The Project MGU 'Useful Plants Project' has pioneered the collection and propagation of 132 useful wild species in local communities in Mexico, South Africa, Mali, Kenya and Botswana (RBG Kew 2010b). These projects have demonstrated that cooperation between academia, regional government, and local communities provides an effective platform for ecological restoration, and the materials and knowledge from seed banks underpin technical restoration activities.

As the MSB Partnership enters its second decade, the achievements of individual seed banking initiatives in Latin America have been locally and nationally significant, but wider cooperation across the continent will be needed to fully safeguard plant diversity of priority habitats and threatened species. A new model of cooperation emphasising harmonisation of technical standards and data exchange between participating seed banks will enable additional regions to benefit from the technology transfer and academic support offered within the network. Improved understanding of desiccation sensitivity and storage biology across the plant kingdom will enable seed conservation projects to expand into more challenging biomes such as sub-humid forests and cerrado savannahs. This will require better integration with other conservation measures to ensure that threats to livelihoods, as a result of depletion of plant diversity, can be countered and reversed. Seed banks will provide essential founder collections from which restoration material and associated technical support can be provided.

General discussion and conclusions

Seed banks have a vital role to play in supporting integrated strategies for conservation and sustainable use of plants. The cost effectiveness of seed banking technology clearly depends on the seed biology of the target species, and the role that the collection will play in long-term conservation and ecological restoration strategies. Experience

demonstrates that the frequency of recalcitrant seed storage behaviour currently limits use of the technique in biomes such as Amazonian humid forest (Hong et al. 1998), but even in that challenging ecosystem, seed banking of orthodox species enables users to have access to that seed material for many decades. The large majority of species within biomes such as the Caatinga, Mediterranean Chile and dry Mesoamerica have orthodox seeds and can be efficiently collected and preserved for study and future use on restoration.

As seed bank managers recognise the opportunity to provide ecological restoration materials to users, they will need to pay attention to testing and documenting the viability and germination of the species on media comparable with the eventual field soil situation. This will ensure that seed banks support users across the range of biological sciences by providing high-quality and genetically representative samples to users. The diversity and quantity of plant material needed for restoration will often demand a scaling-up of seed conservation operations. For example, UFLA (2008) projected that 27 collecting teams would be needed for 18 years to provide seed for storage, propagation and use in the proposed sustainable reforestation of the São Francisco river basin spanning four Brazilian states.

FAO (2010) noted that an estimated 80,000 species of plant are cultivated in living collections in 2500 Botanic Gardens worldwide. Of these gardens, 800 focus on conservation but only 160 have seed banks. As examples, significant seed bank collections are held within Europe and China: the European Native Seed Conservation Network has conserved 37,780 accessions representing 7903 species of native plant (ENSCONET 2011), and the China Germplasm Bank of Wild Species manages over 50,000 collections (CAS 2011) representing over 7000 species. Although seed bank facilities are present in the majority of Latin American countries, most of them concentrate on plant genetic resources for food and agriculture and wild relatives, and just a few have active programmes on wild plant conservation. The diversity of storage conditions (see Table 2) between the region's recognised seed banks demonstrates that seed bank standards are not universal and suggests that there are obstacles to accessing all required technology.

There are therefore benefits to enhancing cooperation between seed banks across sectors. The need to strengthen ex situ conservation practice through improved international cooperation, and closer links with in situ conservation and to germplasm users is a key conclusion of FAO (2010). For example, in the case of crops, Mexico is host to the International Maize and Wheat Improvement Centre, CIMMYT, supported by the Consultative Group on International Agricultural Research. The seed collections include 27,000 samples of maize held to international gene bank standards (Tabata et al. 2004) and duplicated for safety at the Fort Collins seed bank in the USA and recently also in the Svalbard Global Seed Vault, Norway. The Mexican government also completed the construction in 2011 of a National Centre of Genetic Resources to provide long-term

Table 2. Principal seed banks for Plant Genetic Resources for Food and Agriculture (PGRFA) summarised across Latin America. Data are primarily from FAO (2010) and from *WISM-GPA* (2012), the world information sharing mechanism on the implementation of the Global Plan of Action (GPA) for PGRFA. Additional material was collected from literature and personal communication. Although the majority of facilities primarily store seed of crops and are classified as 'active' banks, the majority of the countries have an established base bank with dry cold conditions allowing long-term seed storage. Non-domesticated species, including crop wild relatives, are also stored in the majority of banks. An extensive survey of the forestry sector would be likely to indicate additional wild species collections under long-term storage conditions. Following FAO/IPGRI (1994), a base bank is a facility holding germplasm accessions preserved for the long term, not to be distributed directly to users; an active bank preserves germplasm accessions which are immediately available for multiplication and distribution for use.

Country/ International Centre	Seed banks	Number of seed banks	Storage temperature (°C), Typical measured seed moisture content (%)	Volume (m ³) of storage room	Room available	Type of material other than PGRFA
Argentina	Base	3	-20°, <5%	3 and 123	Yes	Wild relatives; some ornamental forest and native forage seeds
	Active	14	0 to 7°, 5-7%	3-190	Yes	
Bolivia	Base	2	-20°, 7%	1-70	Yes	Wild relatives; some native PGRFA
	Active	5	0-12°, 5-10%	22-190	Yes	
Brazil	Base	2	-10 and -20°	570	Yes	Wild relatives; some native genetic resources and native forest seeds
	Active	9*				
Chile	Base	2	-18°, 5-7%	350	Yes	Wild relatives and forage species; native and threatened species
	Active	5	-5-5°		Yes	
Costa Rica	Base	1	-18°, 5-7%	96	Yes	Some wild relatives and native forest seeds
	Active	1	-4°, 6-12%	36	Yes	
Colombia	Base	1	-20°, 5-7%	48	No	Wild relatives and native plant genetic resources
	Active	2	-10° and 5°, 5-7%	50, 74	No	
Cuba	Base	0	-	-	-	Wild relatives and native plant genetic resources
	Active	5	5-16°, 2-6%	1-17	No	
Ecuador	Base	2	-15°	22-24	Yes	Wild relatives
	Active	3	6-8°	22-24	Yes and No	
El Salvador	Base	1	-20°, 11%	80	Yes	Wild relatives
	Active	1	5°, 11%	85	Yes	
Guatemala	Base	0	-	-	-	
	Active	1	4-6°, 4-7%		Yes	

(Continued)

Table 2. (Continued).

Country/ International Centre	Seed banks	Number of seed banks	Storage temperature (°C), Typical measured seed moisture content (%)	Volume (m ³) of storage room	Room available	Type of material other than PGRFA
Mexico	Base Active	2 22	-18°, 3-7% -5-14°, 2-15%	10-15 2354	Yes	Native, endemic and threatened species, wild relatives
Nicaragua	Base Active	0 0	-	-	-	Mostly in vitro and field collections of tropical fruit trees
Panama	Base Active	0 0	-	-	-	In vitro unit and short-term seed storage facilities
Paraguay	Base Active	0 0	-	-	-	Short-term seed storage facility for PGRFA
Peru	Base Active	7 1	2-7° -	20-201 -	Yes and No -	Wild relatives and some native PGR Some wild relatives; native and endangered species; forestry species
Dominican Republic	Active Base	2 1	10 and 15°, 12% -	48 and 1200 41	Yes Yes	Mainly forage and other native species
Uruguay	Base Active	2 2	-18°, 5% 5°, 5%	20 and 100 63 and 196	Yes Yes	Wild relatives and some forestry species
Venezuela	Active Base	2 2	-18°, 5% 5°, ~5%	240 and 459 400	No / Yes No	Teosinte seeds, < 1% of total accessions
CIMMYT	Base Active	1 1	-18°, 6-8% -3°; 6-8%	400 400	No No	Wild bean and forage collections, ca. 5% of total collections
CIAT	Base/Active	1	-18°, 5%	614	No	

* Nine active germplasm banks recorded within the Brazil National Genetic Resource Network lead of EMBRAPA. <http://plataformag.cenargen.embrapa.br/rede-vegetal/projetos-componentes> (accessed 17 April 2012).

storage for germplasm samples for agricultural, forestry and livestock development. The challenge is to develop the science and technology support from other sectors to ensure that seed collections benefit from an active and relevant programme of research, in order to solve seed problems and to respond to the needs of users. In particular, through enhanced cooperation the capacity of seed banks needs to be strengthened to respond to the increasing demand for seed and information to support habitat restoration and also reintroduction of threatened species. This potential is demonstrated by the rapid development of the Ibero-American Network of ecological restoration (RIACRE) since its founding in Cuba in 2007 and the active Ecological Restoration Network for Latin America (REDLAN 2012) – see González-Espinosa et al. (2008) and Williams-Linera et al. (2011) for various recent forest restoration ecology experiences in Latin America.

Acknowledgement

PL has received support from INIA, specifically the Project Centro de Recursos Biológicos Públicos funded by the Ministry of Agriculture of Chile; Rio Tinto PLC and the Arcadia Foundation (UK). ML acknowledges support from CNPq 27/2005, PCE/MMA 2010 and the Amazonian State Institute of Agricultural and Forestry Development; PD acknowledges the support received from CONABIO for the FESI Seed Bank. MW acknowledges support from the UK Millennium Commission, Kew, Latin American Research Fellowships, and private donor MGU. The authors would also like to acknowledge the help from correspondents who provided updated seed bank information for Latin America. We thank T. Ulian and H. Pritchard from MSBP Kew for comments on the draft manuscript.

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