Conserving the genetic diversity of Bolivian wild potatoes

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Contents

CHAPTER 1

General Introduction

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Potato

The potato is one of the world's most productive vegetables and provides a major source of nutrition and income to societies across the globe. In terms of global production it is the fourth crop in importance after wheat, rice and maize. Originally, it was restricted to the high mountains in the Andes, but after the conquest of South America by the Spaniards in the 16th century, potato arrived in Europe from where it eventually spread to other continents. Now it is grown in every temperate-climate country and even in many tropical countries throughout the world (Hawkes and Hjerting, 1989; Spooner and Salas, 2006).

In contrast to many other crops, the potato presents the largest amount of genetic diversity in its secondary and tertiary gene pool. The diversity of cultivated potatoes is also exceptionally high including different levels of ploidy and more than 4,000 cultivars (Hawkes, 1990; Estrada Ramos, 2000). The large number of wild potatoes species is widely distributed in the Americas from the southwestern United States to southern Chile, but the thousands of primitive cultivated landrace populations are mainly grown at high altitudes in the Andes (Spooner and Hetterscheid, 2005; de Haan, 2009).

The potato is the most important and best-known member of the plant family Solanaceae, a medium-sized family with 3000–4000 species. It belongs to the genus *Solanum* which has about 1400 species worldwide. Its edible tubers are modified parts of the stolons and so actually swollen underground stems (Huamán, 1986; Spooner, 2001). Potatoes can be propagated both by seed and by tubers.

The potato is of utmost importance as a basic staple food for Andean communities. Its management and conservation is closely related to farmers' livelihood and their survival strategies in the difficult conditions of the high mountains (de Haan, 2009). The wild species related to the potato crop are less important for farmers. Some still persist in nature in isolated and undisturbed areas, others are spread accidentally by human activities (road and other urban constructions and even through child games). However, these wild species form highly valuable genetic resources for crop improvement. They occur in an extremely wide habitat range and certain populations were able to develop resistance to pests and diseases or the ability to withstand environmental extremes of cold, heat or drought.

Potato taxonomy

The potato and its wild relatives, all accommodated in *Solanum* section *Petota*, have been the subject of intensive taxonomic work because of a number of factors:

- the large amount of diversity
- the efforts to elucidate the evolutionary relationships between the wild relatives and the cultivated potatoes
- the necessity to establish the crossability with the cultivated potato and the value of these species for plant breeding programs.

Unfortunately, the taxonomic efforts have not lead to a general consensus. There are many disagreements amongst authors regarding species boundaries and their interrelationships. This poses problems not only for the correct identification of plant material and the application of a uniform system of names, but also for the establishment of the relationships between putative wild ancestors and the cultivated potato. The only fact all taxonomist agree on is that the potato possesses more related wild species than any other major crop species, although several treatments have suggested a re-evaluation of series boundaries within sect. *Petota* and a reduction of the number of species (Spooner and van den Berg, 1992; Spooner *et al.*, 1994; van den Berg *et al.*, 1996; Spooner and Castillo, 1997; Spooner *et al.*, 1997; van den Berg *et al.*, 1998; Spooner *et al.*, 2003; Spooner *et al.*, 2009b).

Within section *Petota*, Hawkes (1990) recognized a total of 232 wild potato species accommodated in 21 series, but the molecular work of Jacobs (2008) provided only partial support for the series classification and a new classification in 10 informal species groups was proposed. Spooner and Hijmans (2001) presented a revised list of 199 wild species arranged in four clades. Hijmans *et al.* (2002) mapped out 196 wild species (accommodated in 20 series) in their Atlas of wild potatoes. Subsequently, Spooner and Salas (2006) lowered the number of wild potato species to 188 and later to only 110 (Spooner *et al.*, 2009b), accommodated into three clades. Finally, the most recent taxonomic treatment, available on the Solanaceae Source website (http://www.solanaceaesource.org), lists around 100 species (plus about 10 hybrid species).

The cultivated potato has also been the subject of many changes in its taxonomic interpretation, from 21 cultivated species recognized by Bukasov and Lechnovich in 1971 (Huamán and Spooner, 2002), to nine (Ochoa, 1990), seven (Hawkes, 1990; Spooner and Hijmans, 2001), four (Spooner *et al.*, 2007; Rodriguez *et al.*, 2010), three (Dodds, 1962) to only one species with eight cultivar-groups: Ajanhuiri, Phureja, Stenotomum, Chilotanum, Chaucha, Juzepczukii, Andigenum, and Curtilobum (Huamán and Spooner, 2002; Spooner *et al.*, 2003; Spooner and Hetterscheid, 2005; Spooner and Salas, 2006).

Regional treatments for Bolivia have been provided by Hawkes and Hjerting (1989) and Ochoa (1990). Spooner and van den Berg (1992) analyzed these treatments and encountered many differences in taxonomic interpretation even for the same material. Still, these treatments were the most widely utilized ones for more than a decade, are still in use in herbaria and gene banks worldwide (Spooner and Hijmans, 2001; Spooner and Salas, 2006) and were also used as the reference for monitoring the status of wild potato populations in Bolivia in the present study. These treatments show that Bolivia is the second most species-rich country (after Peru) for wild and cultivated potatoes.

The Andes, center of origin and domestication of the potato

According to Vavilov (1951), the origin of a cultivated plant is to be found in its region of greatest diversity. The central Andes of Peru and Bolivia, being the area accommodating the highest richness in wild potato species (Hijmans *et al.*, 2002), is generally regarded as the center of both the origin and the diversity of cultivated potato (Brush, 1995).

Hawkes (1990) postulated that the origin of potato domestication is located somewhere in the region from central Peru to central Bolivia, more specifically in the area from Lake Titicaca to Lake Poopó in northwestern Bolivia. The first cultivated material probably originated some 10,000 to 7,000 years ago. This was diploid material, probably derived from *S. leptophyes*-like progenitors and classified as *S. stenotomum*. Hawkes also pointed at the requirements for the establishment of primitive cultivation, like a relatively stable population of hunter-gatherers, settled near rivers or lake margins where a plentiful food supply was available. The southern margins of Lake Titicaca and the northern margins of Lake Poopó seem to fulfill these requirements.

During the early stages of the domestication process, other wild species may also have been involved. Ugent (1970) designated the *S. brevicaule* complex (a taxonomically confusing group of very similar tuber-bearing species distributed from central Peru to northern Argentina) as containing the ancestors of the cultivated potato. Grun (1990) also suggested a wild progenitor from the *S. brevicaule* complex. Hawkes (1990) suggested that crosses between the first diploid cultivated material and a number of wild species (*S. sparsipilum, S. acaule* and *S. megistracrolobum*) led to the polyploid cultigens.

For most crops, it is not clear whether their domestication was a single or multiple event (Zohary, 1999). Blumler (1992) pointed out that in the Near East and Mexico plant species were taken into cultivation and subsequently domesticated only a few times, and perhaps only once, but that in the central Andes multiple domestication events might have taken place. Ugent (1970) postulated that the great variation of the cultivated potato and its sympatry with putative wild relatives render it likely that it is of polyphyletic origin. Ochoa (1990) suggested a polyphyletic origin of *S. stenotomum* (from several different places and at several occasions in time) because of the high polymorphism found in this cultigen. In addition, Hosaka (1995) postulated successive domestication for diploid cultigens, sexual polyploidization for *S. tuberosum* subsp. *andigena,* and selection for the Chilean *S. tuberosum* subsp. *tuberosum.* His findings were supported by a chloroplast NA restriction endonuclease analysis. All these hypotheses suggested that the cultivated potato was the result of successive hybridization events between diploid members of the *S. brevicaule* complex, with chromosome doubling leading to the tetraploid forms.

Recently, Spooner *et al*. (2005) postulated a monophyletic origin of the cultivated potato from the northern component of the *S. brevicaule* complex (species from Peru, plus *S. achacachense* from northern Bolivia). Their conclusions center on a group of around 11 species, which are morphologically very similar (van den Berg *et al*., 1998).

High diversity of wild and cultivated potatoes in Bolivia

The potato has a rich gene pool of more than 100 wild tuber-bearing species and a great wealth of primitive Andean cultivated forms that represent a huge and only partially explored reservoir of germplasm (Hawkes, 1990; Spooner and Salas, 2006). Wild potato species occur in a wide range of habitats from sea level to 4500 m, including humid and dry highlands in the Andes, cool rain forests and dry deciduous forests, in partial shade or in full sun. Some species occur in undisturbed habitats, but others grow in highly anthropogenic places such as fields, roadsides and other ruderal localities (Hijmans *et al*., 2002).

Bolivia harbors a number of cultivated potato groups: Ajanhuiri, Phureja, Stenotomum, Chaucha, Juzepczukii, Andigenum, and Curtilobum (Huamán and Spooner, 2002), ranging from diploid (2n=24), triploid (3n=36), tetraploid (4n=48) to pentaploid (5n=60). Cultigens are grown in the high lands of the Andes in the Titicaca Lake region and on mountain slopes and valleys at altitudes between 2000 to 4500 m (Ochoa, 1990; Hawkes and Hjerting, 1989). They are found in seven out of nine departments of Bolivia. Within this vast area, these native so-called primitive cultivars or landraces are mostly grown in small household gardens and fields as a subsistence or local market crop. There are more than a thousand different landraces grown in the Bolivian Andes (Cadima *et al*., 2010), which vary mainly in the shapes and colors of the tubers.

Taking into account the information available in global databases and the taxonomic classification as applied in the Bolivian germplasm bank, this country harbors all cultivated species and 39 wild taxa: 34 species, two subspecies and two varieties, 24 of which are endemic to Bolivia: 21 species, one subspecies and two varieties (Spooner and Hijmans, 2001; Spooner and Salas, 2006). Three species are considered to be of hybrid origin. The wild species show a broader ecological range than the cultivated groups. They grow at altitudes between 700 to 4500 m in the Cordillera Oriental in the departments of La Paz, Oruro, Potosí, Cochabamba, Chuquisaca, Tarija and Santa Cruz. Some of these species are distributed over large areas while others represent narrow endemics only known from one or a few localities (Patiño, 2009; Spooner *et al*., 1994).

In Bolivia, ten biogeographic provinces have been recognized (Navarro and Ferreira, 2009). The wild potato species are found in four of them, all within the Andean Region: Yungas, Bolivian Tucuman, Puna Mesophytic, and Puna Xerophytic (Table 1).

Table 1. *Wild potato species and their occurrence in biogeographic provinces of Bolivia*

Wild potato species	Biogeographic province			
	Yungas (800-4200 m)	Bolivian Tucuman (500 – 5000 m)	Puna Mesophytic (2300-5200 m)	Puna Xerophytic (3200-5200 m)
Solanum acaule Bitter		X	X	X
S. achacachense Cárdenas			X	
S. alandiae Cárdenas		X		
S. arnezii Cárdenas		X		
S. avilesii Hawkes & Hjrt.		X		
S. berthaultii Hawkes		X		
S. boliviense Dunal		X		
S. bombicynum Ochoa	X			
S. brevicaule Bitter		X	X	X
S. candolleanum Berthault	X		X	
S. chacoense Bitter	X	X		
S. circaeifolium Bitter	X	X		
S. × *doddsii* Correl (aln x chc)		X		
S. flavoviridens Ochoa	X			
S. gandarillasii Cárdenas		X		
S. hoopesii Hawkes & K.A. Okada		X		
S. infundibuliforme Phil.		X		X
S. leptophyes Bitter		X	X	X
S. × *litusinum* Ochoa (ber x tar)		X		
S. megistacrolobum Bitter		X	X	X
S. microdontum Bitter		X		
S. neocardenasii Hawkes & Hjert.		X		
S. neovavilovii Ochoa	X			
S. okadae Hawkes & Hjert.	X			
S. oplocense Hawkes		X		
S. soestii Hawkes & Hjert.	X			
S. sparsipilum (Bitter) Juz. & Bukasov	X	X	X	
S. × *sucrense* Hawkes (adg x opl)		X		X
S. tarijense Hawkes		X		
S. ugentii Hawkes & K.A. Okada		X		

Wild potato species	Biogeographic province			
	Yungas (800-4200 m)	Bolivian Tucuman (500 – 5000 m)	Puna Mesophytic (2300-5200 m)	Puna Xerophytic (3200-5200 m)
S. vidaurrei Cárdenas		X		
S. violaceimarmoratum Bitter	X			
S. virgultorum (Bitter) Cárdenas & Hawkes	X			
S. yungasense Hawkes	X			

Economic and cultural relevance of potato in its center of origin

Primitive cultivars (landraces) and wild species are both important genetic resources for potato breeding. They may possess desirable traits such as resistance to high temperatures, drought, frost, fungi, bacteria, viruses, nematodes, and insects. Extensive literature regarding potato wild relatives and primitive landraces has documented their value in breeding programs for disease resistance, environmental tolerances, and other agronomic traits of interest, but up to now only a very small fraction of the available diversity has been exploited (Spooner *et al.*, 2009a; Jansky *et al*., 2008; Hajjar and Hodgkin, 2007; Hawkes, 1990; Ochoa, 1990). New knowledge and technologies (such as marker-assisted selection) may open possibilities for an intensified use of these genetic resources in future breeding programs and other uses in industry (e.g. starch production) and pharmacy (Bradshaw *et al*., 2006; Bradshaw and Ramsay, 2005).

Plant breeding using wild species generally is a long and difficult process because of the need to backcross to the cultivated material. In potato, the process can take up to 10 or 12 years. According to Estrada Ramos (2000), this is because breeders use modern cultivars (or *Solanum tuberosum* subsp. *tuberosum*) as one of the progenitors and the fertility and genetic diversity of this material is restricted. Potato breeders from the Andes have had good experiences using wild species in crosses with Andean cultivated groups and also crosses among landraces of different groups. For example, Estrada Ramos (1984) obtained an artificial fertile breeding line by crossing *S. acaule* with *S. phureja*, Carrasco (1993) obtained interspecific hybrids with frost tolerance using landraces from the Bolivian potato gene bank, and Gabriel (2010) mentions at least 18 new cultivars obtained in Bolivia as a result of 21 years of work in the potato breeding program using wild species and Andean cultivated groups. However, despite extensive potato breeding programs worldwide, the number of wild species used does not exceed five percent. This could be largely due to the fact that not all wild potato species readily cross with each other or with cultivated material to produce fertile offspring. The degree to which two species are interfertile depends to a great extent on ploidy level and Endosperm Balance Number (EBN) (Gabriel *et al.*, 2001; Hijmans *et al.*, 2003).

The potato also has a prominent role in Andean communities; numerous cultural expressions accompany the cultivation cycle of potato and also determine their use for specific processes, dishes and social relationships (de Haan, 2009). Farmers apply their own folk taxonomy, folk descriptors, and nomenclature to the diversity of potatoes, both cultivated and wild (Brush, 1995; de Haan, 2009). In Peru, de Haan (2009) noted three potato groups according to the indigenous taxonomy: *Atoq Papa* (wild / not consumed), *Araq Papa* (semi-wild / consumed), and *Papa Tarpuy* (cultivated / consumed). Varietal groups within the *Papa Tarpuy* group are abundant. In Bolivia (North Potosí and Oruro), Terrazas *et al.* (2008) distinguished three large groups of cultivated potatoes following the criteria of farmer communities: *Waykus, Phiñus* and *Luk'is*. These groups are distinguished by the type of use and the native cultivars belonging to the groups share some characteristics such as mealy character, cooking time and hardiness against adverse weather conditions. Local knowledge also emphasizes the differentiation of an intermediate group between wild and cultivated, the semi-wild potatoes known popularly as *Leleqo, Tayna Choqe, Chulipako* or *Kkita papa*. The proper taxonomic position of this material is not clear, although Ochoa (1990) recognized them as *S. tuberosum* subsp. *andigena* var. *lelekoya* in his taxonomic treatment of Bolivian potatoes. In Bolivia, wild potatoes are known to indigenous people as *Yuthu Papa, Zorro Papa, Alqo Papa, P'isqo Papa*. These are considered as weeds or simply wild plants with little or no importance, except for children who like to play with the tubers. They are rarely used in the traditional pharmacopoeia (de Haan, 2009).

Ex situ and *in situ* conservation

ue to the high value of biodiversity and genetic resources for present and future generations worldwide, huge conservation efforts have been made using two different and complementary approaches. *Ex situ* conservation, takes place outside the natural habitat or outside the production system, often in facilities specifically created for this purpose (gene banks), but also in botanical gardens. *In situ* conservation, maintaining plant populations in the habitats where they naturally occur, generally involves nature reserves and national parks, and is realized either through species-specific conservative actions or via a more general ecosystem approach (Dulloo *et al*., 2010; Maxted *et al*., 2008; Frankel *et al*., 1995).

Samples of seeds and tubers representing part of the genetic diversity of cultivated and wild potato species are conserved *ex situ* in gene banks worldwide. Most potato germplasm accessions are preserved as seeds. Clonal stocks are kept for cultivated collections where the genotype must be maintained (Spooner and Salas, 2006). The twelve major gene banks conserving genetic resources of potato are cited in Table 2.

There is a concern about the loss of valuable genetic diversity, especially in gene banks. This can be caused by genetic drift over serial increase cycles (involving genetic bottlenecks due to small sample sizes), unintentional selection, differential fertility and accumulation of deleterious mutations (Del Rio *et al*., 1997; Spooner, 1999). To a large extent these threats depend on the quality of gene bank management procedures. van Soest (2006) reported potato genetic resources management practices to differ considerably between gene banks. Even some of the more critical gene bank functions, such as regeneration, documentation, storage, health control and safety duplication, which are essential for optimal conservation, are not adequately performed in a number of gene banks due to different reasons. In Latin America, proper resource management activities are threatened because of a lack of funding.

Targeted collecting expeditions (either to centers of diversity or to trace rare taxa) could be a way to regenerate and refresh the diversity in *ex situ* collections. These should follow strict procedures to ensure the capture of maximum diversity (Spooner, 1999). The challenge is to find funds and sponsors to support such activities.

Strategies for *in situ* conservation differ between cultivated potatoes and their wild relatives. *In situ* conservation of cultivated material is also called on-farm management, because the maintenance of a crop involves human interactions with the crop (de Haan, 2009). Several thousand potato landraces are grown by Andean farmers, who maintain the diversity of potatoes in fields that are deliberately planted as a mixed collection of local landraces (Brush, 1995; Terrazas *et al*., 2008; de Haan, 2009).

Table 2. *Composition and size of the potato collections in the twelve major gene banks*

Gene bank	Total accessions	Type of accession (%)		
		Wild species	Landraces	Other *
Institut national de la recherche agronomique/Station d'Amélioration Pomme de Terre et Plantes à Bulbes (France)	10461	6	84	92
N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry (Russian Federation)	8889	-	46	54
International Potato Center (Peru)	7450	2	69	29
Institüt fur Pflanzenbau und Pflanzenzüchtung (Germany)	5392	18	37	45
Potato Germplasm Introduction Station, United States Department of Agriculture, Agricultural Research Services	5277	65	21	14
National Institute of Agrobiological Sciences (Japan)	3408	3	1	96
Centro de Investigación La Selva, Corporación Colombiana de Investigación Agropecuaria (Colombia)	3043	-	-	100
Centre for Genetic Resources (the Netherlands)	2716	72	27	1
Central Potato Research Institute (India)	2710	15	-	85
Instituto Nacional de Innovación Agrícola y Forestal INIAF (Bolivia)	2428	22	78	0
Potato Research Institute Havlickuv Brod Ltd. (Czech Republic)	2207	5	1	94
Banco Activo de Germoplasma de Papa, Forrajeras y Girasol Silvestre (Argentina)	1739	85	15	-

* research materials/breeding lines, advanced cultivars and a mixture of two or more types
Source: FAO (2010) and personal communication with gene bank managers

In situ conservation of wild species is commonly associated with the maintenance of biodiversity in nature. The *in situ* conservation of crop wild relatives (CWR) deserves special attention, because in the mid-1970s and 1980s the scientific community realized that CWRs were no safer than other wild plants in natural environments (Meilleur and Hodgkin, 2004). uring the last decade scientists have developed theoretical and methodological frameworks for *in situ* CWR conservation (Meilleur and Hodgkin, 2004; ulloo *et al.*, 2010). espite the valuable recommendations, these are still theoretical frameworks that need to be put into practice. Hopkins and Maxted (2010) mention that there are several *in situ* conservation mechanisms in place to conserve wild plants (legal species protection, site designation or protected areas, agri-environment programs) and each is likely to already contribute to *in situ* conservation of CWRs. However, at present we have an incomplete understanding of which species benefit from these conservation mechanisms and we do not know whether there are significant omissions. A number of published papers deal with initiatives specifically aimed at the conservation of CWRs (Heywood and ulloo, 2005, Maxted *et al.*, 2008, Maxted and Kell, 2009, VMA-BIOVERSITY, 2010, ulloo *et al*., 2010, FAO, 2010, Hunter and Heywood, 2011). Some of these aimed at specific crops, small groups of species or limited areas, but most of the *in situ* conservation efforts are still strategies and plans waiting for or that are in the process of implementation. If national parks and other conservation areas cannot guarantee the *in situ* conservation of all CWRs, localities outside protected areas must be selected to complement and offer a higher degree of protection for the threatened CWRs. A range of actions to complement a protected area system is recommended by Hunter and Heywood (2011) in their Manual for *in situ* conservation, but such actions may depend on engaging private landowners in the conservation process and the policies and particular context of each country.

Recently, across Europe, important practical conservation actions are being studied and implemented under the EU FP7-funded PGR Secure project (www.pgrsecure.org). These include the development of CWR conservation strategies for individual nations (Taylor *et al.*, 2013). These initiatives complement the CWR conservation plans developed under the Global Environmental Facility (GEF) project "*In situ* conservation of crop wild relatives through enhanced information management and its field application", which include five countries (Armenia, Bolivia, Madagascar, Sri Lanka and Uzbekistan). This project developed national information systems and a web-based portal to access international CWR information, with the purpose to show that information management is an essential part of CWR conservation (www.cropwildrelatives.org). In Bolivia, in the period 2006 – 2008, this project allowed initiating activities involving wild species of several genera

(*Annona*, *Vasconcellea*, *Cyphomandra*, *Rubus*, *Annacardium*, *Theobroma*, *Bactris*, *Euterpe)* and wild relatives of Andean crops such as potatoes, quinoa, beans, groundnuts, cassava and peppers.

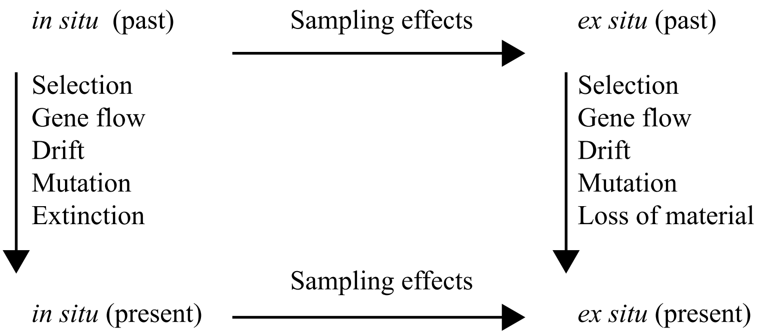
Information on the wild potato species occurring in Bolivia was collected from international and national sources as well as directly from field observations, in order to provide data to both the national and international information system of the project. Distribution maps were designed and a proposal for a methodology to assess the status of the *in situ* conservation of crop wild relatives in Bolivia was developed. In addition, *ex situ* conservation of wild potato germplasm in Bolivia was boosted by the acquisition of about 600 accessions belonging to 35 species, which were obtained mostly from the Centre for Genetic Resources, the Netherlands, but also from collections made during collecting trips as part of the project (Patiño and Cadima, 2009). Furthermore, it was suggested that the conservation of potato genetic diversity should be addressed through a coordinated effort involving proper gene bank collection management, periodic recollecting to maintain genetically variable germplasm, habitat preservation for *in situ* conservation, and, as many CWRs occur in agricultural environments, also efforts to conserve specific elements of the agricultural production systems (ulloo *et al*., 2010; Meilleur and Hodgkin, 2004; Spooner, 1999; Altieri *et al*., 1987; Altieri and Merrick, 1987).

Aims and scope of this thesis

This thesis will focus upon the Bolivian wild potato diversity. As mentioned in the previous paragraphs, vast efforts have been made to conserve these genetic resources, particularly *ex situ* in gene banks. On the other hand, many populations of wild potatoes are frequently found in disturbed areas such as roadsides, field margins, etc., and often occur in traditionally managed agro-ecosystems or in natural environments. *In situ* conservation of natural populations in such areas is incidental and not the result of planned policy. Regarding the *in situ* and *ex situ* conservation of Bolivian wild potato species, a number of key issues can be identified (Fig. 1):

1. <u>Potential changes in distribution patterns of Bolivian potato species *in situ*.</u> ifferences in past and current distribution of individual species may have occurred due to different factors, such as threats driven by human activities and natural causes. Depending on the severity of these factors, the probability of losing genetic diversity or even extinction may

Figure 1. *Potential influences on levels of genetic diversity* in situ *and* ex situ



vary among wild potato species in their natural habitats. etection of changes in distribution patterns over time as well as in the threats to wild potato populations are fundamental for conservation decisions.

2. Inadequate representativeness of the existing diversity in *ex situ* potato germplasm originating from Bolivia. The risk of a poor coverage of genetic diversity is more likely in rare and/or narrowly endemic species, which are more difficult to find in natural environments. In Bolivia, at least 21 endemic species have been reported, many of them rare, occurring in very restricted areas. In some cases only herbarium samples were obtained but no seeds were collected for *ex situ* conservation. Consequently, there is a need to inventory the *ex situ* potato germplasm originating from Bolivia, to identify collection gaps and to organize additional collecting activities for wild potato species under-represented in the Bolivian gene bank.

3. Potential changes in the genetic diversity during *ex situ* maintenance of accessions. uring the regeneration of *ex situ* conserved germplasm, the genetic integrity of accessions may be compromised by factors such as unintentional selection, gene flow, genetic drift and mutation. Insight in the magnitude of the effects of these factors is essential to evaluate the effectiveness of regeneration procedures that are currently practiced.

4. Genetic differences between *ex situ* conserved accessions and their original *in situ* populations. Ideally, the genetic diversity of accessions maintained in gene banks is representative for that of the original populations occurring in their natural habitats. Factors determining this include sampling effects during collecting expeditions and developments

in genetic diversity in both *in situ* and *ex situ* circumstances. The extent to which the diversity occurring *in situ* is effectively maintained *ex situ* is an important issue in developing a conservation strategy.

In this thesis these four issues are addressed in order to evaluate whether the current management efforts adequately conserve the genetic diversity of Bolivian wild potato species, and if not, what recommendations can be provided for improvement. More specifically, the following questions are addressed in the research presented in chapters 2 to 6 of this thesis:

1) To what extent have the wild potato species documented decades ago, including endemic and threatened rare species, persisted in the natural ecosystems in Bolivia?
2) What is the distribution pattern of endemic wild potato species in Bolivia, and which potential risk factors affecting their survival can be identified?
3) What are the main gaps in current *ex situ* collections of Bolivian wild potato species?
4) Has the genetic integrity of accessions of Bolivian wild potato species been maintained during *ex situ* conservation?
5) To what extent do *ex situ* conserved accessions of Bolivian wild potato species represent the genetic diversity occurring in their ancestral *in situ* population?
6) What recommendations can be provided to develop a sound conservation strategy for Bolivian wild potato species?

Chapter 2 "**The state of natural populations of Bolivian wild potato species**" addresses question 1. To answer this question extensive field monitoring and collecting trips took place from 2006 to 2010 based on information about previous collecting efforts obtained from databases and related literature. Additionally, a review of the ecological geography was conducted to understand the distribution of the vegetation and ecosystems where wild potato species occur.

In Chapter 3 "**Biodiversity status of endemic wild potato (*Solanum* spp.) in Bolivia: reasons for conservation concern**" questions 2 and 3 are addressed. The *in situ* and *ex situ* conservation status of wild potato species was evaluated based on spatial analysis, while hotspots of endemic wild potato diversity were identified. Based on these results, recommendations were provided to improve the conservation status of several endemic species.

Chapter 4 “**Comparative assessment of the conservation status of wild potato species using two methodologies**” is related to question 2. It compares two methodologies to evaluate the threat status of Bolivian endemic wild potato species: the standard methodology applying the categories and criteria of the IUCN Red List and a second approach using the criteria developed by the CWR Project “*In situ* conservation of crop wild relatives through enhanced information management and field application”.

In Chapter 5 “G**enetic change during *ex situ* conservation of wild potato species from Bolivia and a comparison with recollected *in situ* populations**” questions 4 and 5 are addressed. Microsatellite markers were used to detect potential genetic changes over time in *ex situ* conserved wild potato species, while these accessions were also compared to recently recollected material from the original *in situ* populations. Implications of the results for the future conservation management of Bolivian wild potato species are discussed.

Chapter 6 “**General Discussion”** addresses question 6, summarizing the main findings of this thesis and their implications for improving the conservation of Bolivian wild potatoes.

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CHAPTER 2

The state of natural populations of Bolivian wild potato species

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Abstract

Within Bolivia, thirty nine wild taxa of potato are found. Here, they occur in four biogeographic provinces (Yungas, Puna Mesophytic, Puna Xerophytic and Bolivian-Tucuman) of the Andes Region at altitudes from 700 up to 4500 m, and in a wide range of habitats with pluviseasonal and xeric bioclimates, thermotropical to criotropical thermotypes and semiarid to humid ombrotypes. From 2006 to 2010, a number of exploration trips was scheduled in all four Bolivian biogeographic provinces in order to monitor the occurrence of wild potato populations in their natural habitats as a basis for designing *in situ* conservation plans. Based on the results of earlier expeditions, 19 species (one of which is considered to be of hybrid origin) and 40 collecting sites were selected to assess their *in situ* state. Populations of wild potatoes have been most affected by human activities in the Puna Mesophytic and Bolivian Tucumán biogeographic provinces. Here, human settlements have been intensified and further expanded, reducing the natural habitat for wild potatoes and displacing them into marginal areas around fields and human constructions. The smallest effect of human activities on the natural potato habitat was detected in the Yungas biogeographical province. Here, notably the difficult access to areas where wild potatoes grow has prevented habitat loss. The highest number of wild potato species (at least 20) has been reported from the Bolivian Tucumán biogeographic province, most likely as a result of its diverse climate and marked altitudinal gradient (500 – >5000 m). Here, in large areas with a pronounced dry climate and xeric plant ecosystems, several wild potato species have developed drought tolerance. They represent valuable genetic material for breeding purposes, especially since drought is becoming one of the most severe problems due to the effect of climate change on potato producing areas in Bolivia. The species reported as being widespread in Bolivia were confirmed as such, indicating their strong capacity for resilience and adaptation even to environments disturbed by human activities. This feature also indicates a widespread resistance to biotic and abiotic factors.

Introduction

Crop Wild Relatives (CWRs) are the wild ancestors of crop plants. CWRs are likely to play a significant role in securing 21st century food security by improving agricultural production and sustaining productivity. This is due to their potential use in plant breeding to produce crops which can withstand the adverse impacts of climate change, of the growing scarcity of nutrients, water and other inputs, and of new pests and diseases (Hopkins and Maxted, 2010, Hunter and Heywood, 2011).

Potato is one of the most important crops in Bolivia. This country harbors a huge genetic diversity of both cultivated and wild potatoes. The wild relatives of the cultivated potato (species belonging to *Solanum* sect. *Petota*) occur in a wide range of habitats. They have developed resistance to a range of pests and diseases as a consequence of their evolution under natural conditions, which makes them of great importance for the genetic improvement of this crop (Hawkes, 1990; Guarino *et al.*, 2002).

Wild potato populations are susceptible to a number of factors that threaten their habitats. To ensure the conservation of such populations, specific action needs to be taken. In the past, large areas of Bolivia have been explored searching for wild potatoes, and the collected germplasm has been deposited in gene banks for *ex situ* conservation. This material also served extensive taxonomic studies as well as breeding and evaluation experiments (Hawkes and Hjerting, 1989; Ochoa, 1990; Spooner *et al.*, 1994; Spooner *et al.*, 1997; Jackson and Hanneman Jr, 1999; Coleman, 2008).

The conservation of wild relatives of an important crop, such as the potato, requires long-term strategies. The efforts of gene banks are undoubtedly valuable, but the design of appropriate *in situ* conservation plans is necessary as a complementary strategy. For this, we need to collect the largest possible amount of information to make effective decisions, e.g. we need to monitor the current status of the species, to determine the habitat availability and ecosystem health, and to assess the effects of threats on the current occurrence (Heywood and Dulloo, 2005, Hunter and Heywood, 2011).

The purpose of this study was to investigate whether certain populations of wild potato species detected decades ago persisted in the natural ecosystems in Bolivia, and if so, to examine their present state. In this study, special attention was given to endemic and threatened rare species. The resulting data form the basis for designing appropriate conservation strategies.

Brief summary of previous collecting missions in Bolivia

The importance for food security at the global level and the economic value of potatoes have stimulated research into various aspects of the crop, such as genetics and systematics, as well as the collecting of germplasm of cultivated and wild material. Over the past 40 years, more than 40 potato collecting expeditions have been undertaken by various international explorers and botanists (Correll, 1962; Hawkes and Hjerting, 1989; Ochoa, 1990; Spooner and Hijmans, 2001).

Bolivia has been the target country of many of such collecting expeditions for both wild and cultivated material, which have contributed to our present-day understanding of the potato genetic resources (Ochoa, 1990; Spooner *et al.*, 1994; Hawkes, 2003). The first species of *Solanum* were collected and described for Bolivia during the first decades of the 19th century, but the most intensive explorations were performed during the 20th century (Table 1). The germplasm collections from these expeditions have been deposited at various gene banks worldwide. More recently, in 2006, 2007, 2008 and 2009, collecting expeditions were conducted with funding provided by the Crop Wild Relatives (CWR) Global Project – Bolivia component. The trips were led by PROINPA technicians from Bolivia assisted by A. Salas from the International Potato Center (CIP), Peru. In 2010, a collecting trip was organized by PROINPA technicians in cooperation with R.G. van den Berg from Wageningen University (The Netherlands). The main purpose of these recent expeditions was not so much to collect new germplasm material, but to monitor wild potato species in their natural environment, although new material and herbarium vouchers were collected when possible and deposited at the Bolivian potato gene bank.

Bolivian ecosystems and habitats for natural populations of potato

Bolivia has been identified as a mega-diversity country (Ibisch and Mérida, 2003) as it harbors a huge diversity of habitats and climates. Climate is the main determinant of vegetation and ecosystems in general. Climatic conditions are characterized by a marked seasonality of rainfall. The seasonality is particularly marked in the Andean Region, where there are areas with 5 to 8 dry months (Navarro and Maldonado, 2002). Herbaceous plants like wild potatoes can generally only be found in the rainy season, while their tubers survive the dry season in the soil, sometimes with additional protection from stones, shrubs or trees.

Table 1. *Overview of all expeditions in Bolivia that focused on collecting wild and cultivated potatoes*

Year / Period	Collector(s)
1830 – 1833	A.D’Orbigny (France)
1855 – 1857	H. Algernon Weddell (England)
1855 – 1861	G. Mandon (France)
1885 – 1891	M. Bang (Denmark)
1903 – 1904	K. Fiebrig (Germany)
1906 – 1036	O. Buchtien (Germany)
1913 – 1920	W.F. White (USA) & E. Asplund (Sweden)
1921	E. Asplund (Sweden), H.H. Rugby (USA) & M. Cárdenas (Bolivia)
1927 – 1928	S.V. Juzepczuk (Russia)
1931	E. Baur & R. Schick (Germany)
1932	H.G. MacMillan & C.O. Erlanson (USA)
1933 – 1934	K. Hammarlund (Sweden), O. Braun (Germany) & M. Alvarez (Bolivia)
1939	E.K. Balls, W.B. Gourlay, J.G. Hawkes (England) & M. Cárdenas (Bolivia)
1944 – 1968	M. Cárdenas (Bolivia)
1950	W. Brooke (England)
1953	E. Petersen & J.P. Hjerting (Denmark)
1955	C. Ochoa (Peru)
1959	H. Ross, R. Rimpau & L. Diers (Germany)
1960	D.S. Correll, K. Dodds, G. Paxman & H. Brucher (Germany)
1963	D. Ugent (USA), M. Cárdenas & A. Vidaurre (Bolivia)
1970’s – 80’s	H. Gandarillas, S. Alandia & M. Zavaleta (Bolivia)
1971	J.G. Hawkes, P. Cribb (England), J.P. Hjerting (Denmark) & Z. Huamán (Peru)
1974	J.G. Hawkes (England), A. van Harten (The Netherlands) & J. Landeo (Peru)
1978 – 1979	C. Ochoa (Peru)
1979 – 1981	J.G. Hawkes, D. Astley (England), W. Hondelmann, J.P. Hjerting (Denmark), A. van Harten, L. van Soest (The Netherlands), Z. Huamán, J. Landeo (Peru), I. Avilés, C. Alarcón, A. Moreira, G. Caero (Bolivia) & K.A. Okada (Argentina)
1983 – 1984	A. Salas (Peru)
1986	R. Hoopes (USA), I. Avilés (Bolivia) & K.A. Okada (Argentina)
1993 – 1994	D. Spooner (USA), R. van den Berg (The Netherlands), W. García & M. Ugarte (Bolivia)
2006 – 2009	X. Cadima, F. Patiño, F. Terrazas, A. Mamani (Bolivia), A. Salas (Peru)
2010	X. Cadima, F. Patiño (Bolivia) & R. van den Berg (The Netherlands)

In mountainous areas of strong relief, like the Andes, the key environmental factors that control vegetation are altitude, local climate, and erosion. In flat areas or areas of moderate relief like the eastern lowlands of Bolivia, the environmental factors that control vegetation are the geomorphology (soil texture, degree of drainage and soil susceptibility to flooding) and the regional climate zone (Navarro and Maldonado, 2002). Populations of wild potatoes are only located in mountainous areas in well drained soils.

Discontinuities in the distribution and composition of the natural vegetation of an area is generally related to domestic fauna like cows, goats, and sheep (Navarro and Maldonado, 2002). Potatoes are highly susceptible to grazing by such animals, although these, as well as other elements of the fauna (like birds and reptiles), might also contribute to the distribution of seeds as is the case with different herbaceous Solanaceae (Moreno Casasola, 1996).

In large parts of Bolivia, human impact is the main force acting on the current landscape and vegetation. In some areas the interaction of cultures with the environment has led to landscapes with remarkable heterogeneity, diversity, balance and persistence, like in parts of the lowlands of Beni and the Yungas of La Paz, but in other situations the interaction has resulted in depleted and eroded landscapes. This is the case in large areas of the Andean and lowlands of northern Chaco in Santa Cruz (Navarro and Maldonado, 2002). Some wild potato species, like *S. sparsipilum, S. microdontum* and others seem to be well adapted to human activities that might even contribute to the spread of their tubers and seeds These species are widely distributed and behave like weedy species in open places and disturbed areas, although they may or may not persist under constant disturbance (Hijmans *et al.*, 2002).

Bioclimates

In general, Bolivia presents three bioclimates (pluvial, pluviseasonal and xeric); six thermotypes or bioclimatic zones (infratropical, thermotropical, mesotropical, supratropical, orotropical and criotropical), and five ombrotypes (semiarid, dry, subhumid, humid and hyperhumid) (Navarro, 2002), defined by bioclimatic indices. etails about these bioclimatic zones, types and indices are provided in Table 2.

There is a correlation between the range of bioclimatic indices and the limits to the distribution of plants and ecosystems. For instance, wild potatoes are restricted to the pluviseasonal and xeric bioclimate, with thermotropical to criotropical thermotypes and semiarid to humid ombrotypes.

Table 2. *Ranges of bioclimates, thermotypes and ombrotypes for Bolivia*
Io is annual ombrothermic index = P/12T, where P is average annual total precipitation, and T is average annual temperature; Iod2 is ombrothermic index of dry season = P2/T2 and shows the ombrothermic index of the two driest consecutive months of year; It is index of thermicity = (T+M+m)10, where T is average annual temperature, M is average maximum temperatures of the coldest month of the year, m is average minimum temperature of the coldest month of the year; Tp is positive annual temperature, the sum of the average temperature of every month of the year in which that average is above 0ºC.

Bioclimates of Bolivia	Io*	Iod2*	Presence of potatoes
9GOPF>G	U)%,	U(%+	7J
9GOPFMB>MJI>G	U)%,	T(%+	=BM
<BLF@	'%& S)%,	$	=BM

Thermotypes of Bolivia (bioclimatic zones)		It*	Tp*	Presence of potatoes
4ICL>NLJKF@>G	5JQBL :OKBLFJL	.'&$./& -)&$.'&	U))+&)'&&$))+&	7J
;EBLHJNLJKF@>G	5JQBL :OKBLFJL	,'&$-)& */&$,'&	(/&&$)'&& (-&&$(/&&	=BM
6BMJNLJKF@>G	5JQBL :OKBLFJL	)/+$*/&)(&$)/+	(*&&$(-&& ('&&$(*&&	=BM
:OKL>NLJKF@>G	5JQBL :OKBLFJL	(*&$)(& ',&$(*&	'+-+$('&& '&+&$'+-+	=BM
8LJNLJKF@>G	5JQBL :OKBLFJL	'&+$',& +&$'&+	-+&$'&+& *+&$-+&	=BM
1LFJLJNLJKF@>G	5JQBL :OKBLFJL	$ $	'+&$*+& '$'+&	=BM
0NEBLH>NLJKF@>G		$	&	

Ombrotypes of Bolivia		Io	Presence of potatoes
:BHF>LFA	5JQBL :OKBLFJL	'%&$'%+ '%+$(%&	7J
2LR	5JQBL :OKBLFJL	(%&$(%. (%.$)%,	=BM
:O?EOHFA	5JQBL :OKBLFJL	)%,$*%. *%.$-%&	=BM
3OHFA	5JQBL :OKBLFJL	-%&$'&%+ '&%+$'*%&	=BM
3FKBLEOHFA	5JQBL :OKBLFJL	'*%&$('%& ('%&$(.%&	7J

?FJ@GFH>NF@ FIAF@BM >@@JLAFID NJ 7>P>LLJ !(&&("

Biogeography of Bolivia

South America belongs to the Neotropical-Austroamericano biogeographic kingdom; Bolivia is located in the Neotropical sub-kingdom, which is divided into four biogeographic regions: the Amazonic, Brasilian-Paranense, Chaco and Andean Region (Navarro, 2002). These are in their turn subdivided into smaller biogeographic provinces (Fig. 1) (Navarro, 2002; Navarro and Ferreira, 2009).

Potatoes are present only in the four biogeographic provinces of the Andean Region. It is very unlikely that any potato species could be found in other provinces, because those are too warm and humid (Amazonic and Brazilian-Paranense regions), or too warm and dry (Chaco Boreal province).

A short description of each biogeographic province where wild potato species occur is given below.

- **Yungas province**

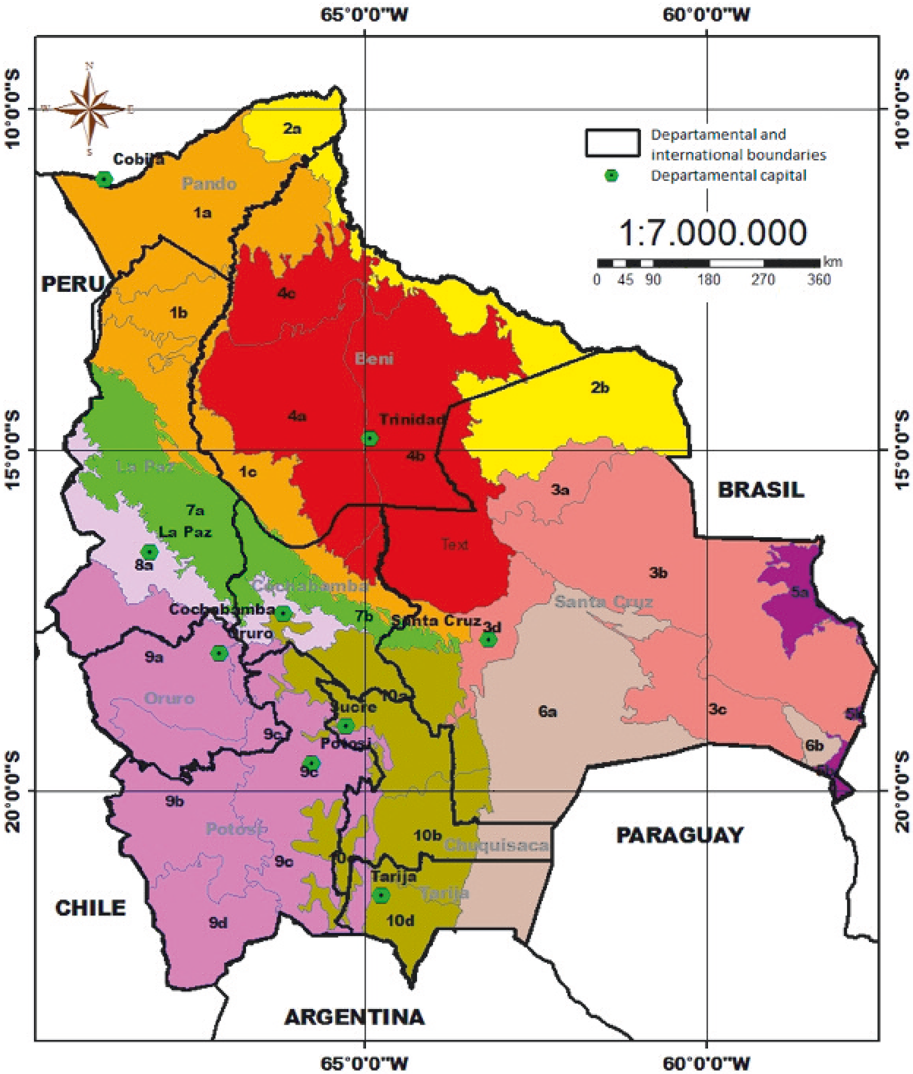
The Yungas province encompasses the valleys, hills and mountains of the Andean Cordillera Oriental, from the border with Peru to central Bolivia. It shows the greatest diversity of bioclimates (pluvial, pluviseasonal and xeric) due to the strong altitudinal gradient (from 800 to 4200 m.a.s.l) and the diversity of landscape and topography (Navarro and Maldonado, 2002, Navarro and Ferreira, 2009).

Many wild potato species were reported from the Yungas province, such as *S. bombicynum, S. brevicaule, S. circaeifolium, S. chacoense, S. flavoviridens, S. neovavilovii, S. okadae, S. soestii, S. sparsipilum*, *S. violaceimarmoratum, S. virgultorum, S. yungasense,* and on the border with the Puna Mesophytic Province also *S. candolleanum*.

- **Puna Mesophytic province**

This province occupies the north of the Altiplano (high plateau) and the Andean Cordillera Oriental, specifically the western La Paz department, except for the Pacajes province and the southwestern provinces Pando and Aroma. It also occupies the western department Cochabamba, except west of the provinces Tapacarí, Arque and Bolívar. The predominant bioclimate is pluviseasonal, while the xeric bioclimate is limited to the mesotropical and

Figure 1. *Map of biogeographic provinces of Bolivia.* Amazonic Region is 1= Southwestern Amazonian Province (where 1a= Sector of Acre and Madre de Dios, 1b= Sector of Heath and bajo Madidi, 1c= Pre-Andean Sector of northern Bolivia and southern Peru), and 2= Central-southern Amazonian Province (where 2a= Sector of Alto Madeira, 2b= Sector of Guapore). Brazilian-Paranense Region is 3= Cerrado Province (where 3a= Transitional chiquitano Sector to Amazon, 3b=Central chiquitano Sector, 3c=Transitional chiquitano Sector to Chaco, 3d= Cruceño chiquitano Sector), 4= Beni Province (where 4a= Beni Western Sector, 4b= Beni Eastern Sector, 4c= Beni Northern Sector) and 5= Pantanal Province (where 5a= Sector of Northwestern Pantanal, 5b= Sector of the Southern Pantanal. Chaco Region is 6= Chaco Boreal Province (where 6a=Sector of Northwestern Chaco, 6b=Sector of Northeastern Chaco). Andean Region is 7=Yungas Province (where 7a=Sector of the upper basin of Beni, 7b= Sector of the upper basin of Ichilo), 8= Puna Mesophytic Province (where 8a= Sector southern mesophytic Puna), 9= Puna Xerophytic Province (where 9a=Sector of Sajama-Desaguadero, 9b= Sector of Salar de Uyuni, 9c= Potosí Sector, 9d=Sector of Southwestern Lípez), and 10= Bolivian-Tucuman Province (where 10a=Sector of Piray-Rio Grande, 10b= Sector of Pilcomayo Alto Parapetí, 10c= Pre-puna sector of San Juan del Oro, 10d= Sector of Bermejo)



Source: Gonzalo Navarro and Wanderley Ferreira, 2009 lower supratropical zones of the high watershed of the rivers La Paz and Luribay (Navarro and Maldonado, 2002, Navarro and Ferreira, 2009).

In the Puna Mesophytic province, four ecosystem levels are found and mountains with peaks over 6000 m altitude. The pre-puna level, represented by high valleys in the upper area of the watershed of the La Paz river (2300–3200 m altitude), presents ecosystems generally very degraded by livestock grazing and wood extraction (Navarro and Maldonado, 2002). In this area two wild potato species, *S. leptophyes* and *S. sparsipilum,* were reported.

The puna level (3100–4000 m altitude) presents forests of low canopy composed mainly of species of the genus *Polylepis*, although today many of these forests have been replaced by grasses and weeds (Navarro and Maldonado, 2002). In this level, at least three wild potato species, *S. candolleanum, S. acaule,* and *S. megistracrolobum*, have been reported and at the lower altitudinal limit also *S. circaeifolium*.

The high-Andean level (3900–4700 m altitude) presents vegetation of wet grasslands, peat bogs and aquatic vegetation. From this level, *S. achacachense* and frost-resistant populations of *S. acaule* and *S. megistracrolobum* have been reported.

The subnival level (4600–5200 m altitude) is the lower limit of the snow line in Bolivia. The typical vegetation of this level is low grassland, frost desert vegetation, and subnival peat bogs. In Bolivia, no wild potato species are reported from this level.

- **Puna Xerophytic province**

This province extends from the southwest of La Paz (Pacajes and southern Pando provinces), Oruro (all except the eastern department) and Potosi (entire department except the east) from the western Pie de monte of the Andean Cordillera Oriental in the east to the border with Chile and Argentina in the west.

It is determined by its predominant xeric bioclimate, an altitude range between 3200 and 5200 m and the presence of lakes (Poopó, Uru Uru and Chungará) and salt flats such as Coipasa and Uyuni.

The wild potato populations reported from this province mostly belong to the frost-resistant *S. acaule*, and in lower frequencies to *S. megistacrolobum, S. infundibuliforme, S. brevicaule, S. leptophyes* and *S.* ×*sucrense*.

- **Bolivian-Tucuman province**

This province includes part of the departments of Cochabamba, Chuquisaca, Potosí and Tarija, and the west of the Santa Cruz department. It includes both the puna and high-Andean levels of the Cordillera Oriental, and also the valleys and sub-Andean levels belonging to the inter-Andean parts of the watersheds of the rivers Grande, Pilcomayo and Bermejo.

There is a climatic diversity caused by the mountain ranges and marked altitudinal gradient from less than 500 m to over 5000 m, generally with a pluviseasonal and xeric bioclimate.

In this province, the highest number of Bolivian wild potato species occurs: *S. acaule, S. alandiae, S. arnezii, S. avilesii, S. berthaultii, S. boliviense, S. chacoense, S. circaeifolium, S. gandarillasii, S. hoopesii, S. infundibuliforme, S. leptophyes, S. megistracrolobum, S. microdontum, S. neocardenasii, S. oplocense, S. sparsipilum, S. ×sucrense, S. tarijense,* and *S. ugentii*.

Methods

To monitor the occurrence of wild potato populations in natural habitats in Bolivia, a number of field trips was conducted between 2006 and 2010 during which new collections were made and evident stress factors affecting the observed species were registered. For this, the following steps were taken:

1) To identify potential collecting sites we used databases from different sources, namely The Centre for Genetic Resources, the Netherlands (CGN), the United States Potato Genebank (USPG), the Institute of Plant Genetics and Crop Plant Research of Germany (IPK), the Intergenebank Potato atabase (IP), the International Potato Center of Peru (CIP), and the Genebank of Andean tubers and roots of Bolivia (BGTRAs). We also used the data published by Spooner *et al*. (1994). In total, 1100 geo-referenced wild potato collections were used to select sites to be visited for an assessment of the *in situ* state of wild potato populations reported since 1955. Priority was given to sites where species endemic to Bolivia had been collected, but also non-endemic species with wide or limited distribution were considered. The aim was to select species representative for all four biogeographic provinces. The resulting 40 prioritized collecting sites, where previously 19 wild potato

species were recorded (including one considered to be of hybdrid origin), are presented in Table 3.

2) The 40 sites were plotted on the road map of Bolivia to generate travel routes (Fig. 2). The six collecting trips took place in March and ecember 2006, February 2007, March 2008, January 2009 and February 2010. In trying to re-locate the populations of wild potatoes at the selected sites, we sometimes used the help of local people, often children, who directed us to the target locations. Using a Global Positioning System (GPS), latitude, longitude and altitude were recorded at each collecting site.

3) At each site, additional information was registered related to the status of the wild potato populations in their natural habitats. The abundance (number of individuals observed), area of coverage (limited, localized or widely distributed) and presence of actual or potential stress factors (e.g. the presence of human settlements, execution of civil works as construction of roads, bridges, etc.), the change of land use and the grazing of cattle, sheep or goats were recorded.

4) Herbarium specimens were taken, which were later on used to identify the material with the help of Alberto Salas, curator of wild potatoes from CIP, Peru, following the nomenclature proposed by Spooner and Salas (2006). Herbarium vouchers were deposited at the Herbaria Martin Cardenas of Cochabamba. Where possible, berries (true seed), tubers and living plants were also collected to obtain germplasm for the Bolivian National Genebank.

Results

The data of the 19 monitored species are presented per biogeographic province in Table 4-7.

- **Yungas biogeographic province** (Table 4)

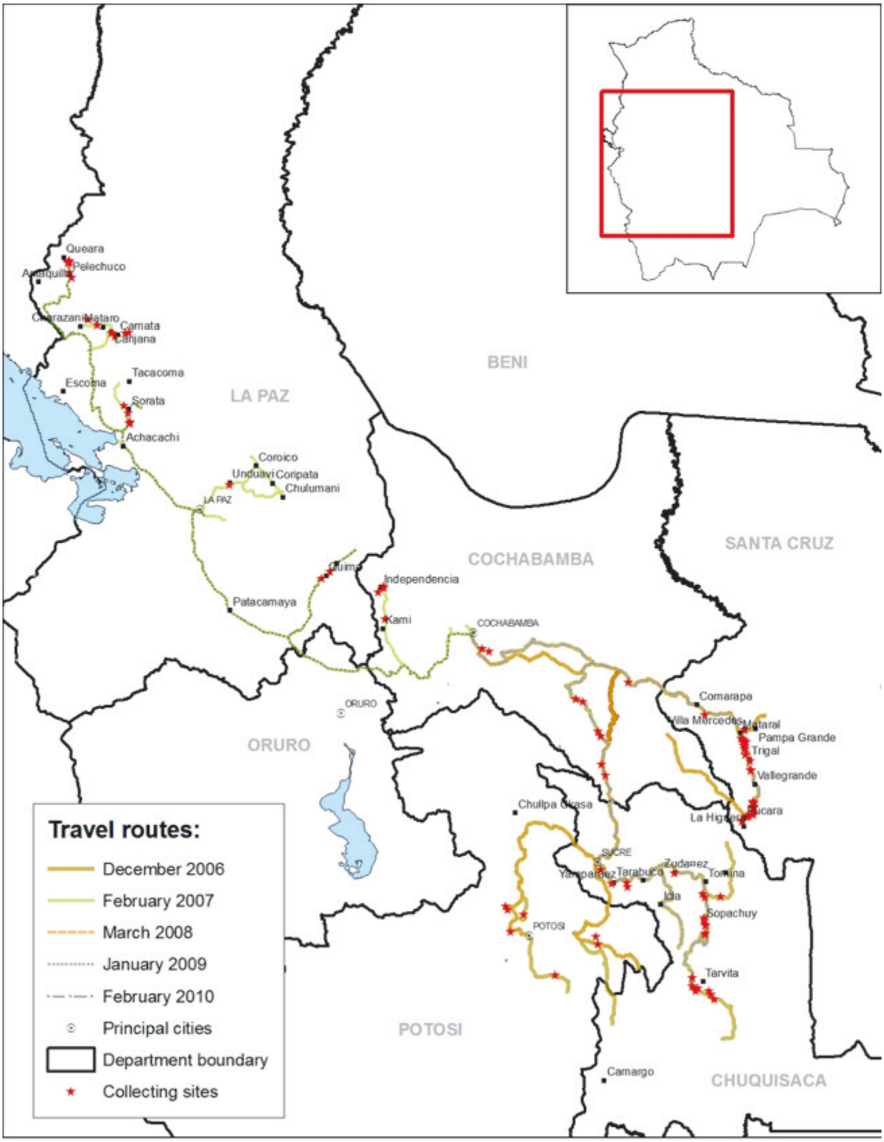
In the Yungas province, six species were monitored, including four endemics that have different ranges of distribution (from very limited distribution to widespread). Monitoring results generally show stability of the of the wild potato populations found in this province.

Table 3. *Details of collecting sites of wild potato species prioritized for assessment of their conservation status*

Species	Collection number	Department	Province	Latitude	Longitude	Altitude (m asl)	Collecting date
S. achacachense*	VSU 649	La Paz	Omasuyos	15º53’	68º39’	4120	12/02/1994
S. acaule	SFVU 6742	La Paz	Camacho	15º32’	69º04’	3792	24/03/1993
	EBS 1863	La Paz	Ingavi	16º 33’	68º 42’	3600	March 1959
	VSH 222	Potosi	Frias	19º34’	65º45’	3800	31/03/1980
S. arnezii*	VSUG 634	Chuquisaca	Tomina	19º18’	64º21’	2170	21/01/1994
	HAM 126	Chuquisaca	Tomina	19º18’	64º27’	2140	09/03/1980
	SVG 6692	Chuquisaca	Zudañez	19º07’	64º38’	2295	08/03/1993
S. avilesii*	HHA 6519	Santa Cruz	Vallegrande	18º38’	64º09’	2850	01/03/1980
	HHA 6521	Santa Cruz	Vallegrande	18º38’	64º09’	2950	01/03/1980
	HHA 6522	Santa Cruz	Vallegrande	18º38’	64º09’	2850	01/03/1980
S. candolleanum	SVU 6743	La Paz	Camacho	15º32’	69º05’	4080	24/03/1993
	ZAV 1597	La Paz	Larecaja	15º32’	68º39’	3550	20/04/1971
S. flavoviridens*	OCHS 11900	La Paz	Saavedra	15º00’	68º50’	1800	01/03/1978
S. gandarillasii*	CAR 5068	Santa Cruz	Florida	18º07’	64º12’	2000	01/03/1955
	OCH 15588	Santa Cruz	Vallegrande	18º55’	64º12’	1900	01/04/1984
S. hoopesii*	VSU 622	Chuquisaca	Azurduy	19º60’	64º26’	2690	16/01/1994
	SVG 6685	Chuquisaca	Azurduy	20º01’	64º25’	2613	07/03/1993
S. infundibuliforme	ROR 181	Potosi	Linares	19º53’	65º41’	3550	01/03/1959
S. leptophyes	VSOA 088	La Paz	Manco Kapac	16º14’	68º68’	3860	08/03/1980
S, megistacrolobum var. toralapanum	ROR 203	Cochabamba	Tiraque	17º25’	65º42’	3500	23/02/1971
	VSAL 136	Cochabamba	Tiraque	17º26'	65º32’	3800	17/03/1980
S. microdontum	SG 6599	Tarija	Oconnor	21º27’	64º64’	2100	21/02/1993
S. neocardenasii*	HHA 6496	Santa Cruz	Florida	18º07’	64º12’	1400	29/02/1980
	OCHS 15555	Santa Cruz	Caballero	18º30’	64º10’	1600	28/02/1984
S. neovavilovii*	OCHS 14961	La Paz	Franz Tamayo	14º42’	69º04’	3000	06/02/1983
	VSU 654	La Paz	Franz Tamayo	14º41’	79º01’	3300	18/02/1994
S. okadae	HHA 6585	Cochabamba	Ayopaya	17º06’	66º55’	2450	11/03/1980
	HPS 002	La Paz	Inquisivi	16º58’	67º13’		n/d
	OCHS 15481	La Paz	Inquisivi	16º57’	67º10’	2800	23/02/1984
S. soestii*	SFVU 6719	La Paz	Inquisivi	16º 57’	67º 12’	2816	15/03/1993
	SVG 6722	La Paz	Inquisivi	16º 57’	67º 11’	2819	15/03/1993
S. ×sucrense*	HAO 064	Chuquisaca	Yamparez	19º11’	65º10’	2820	09/03/1986
	HAM 087	Potosí	Tomás Frías	19º27’	65º48’	3100	02/03/1980
	HAM 088	Potosí	Tomás Frías	19º27’	65º48’	3120	02/03/1980
	HAM 089	Potosi	Frias	19º27’	65º48’	3120	02/03/1980
S. violaceimar-moratum*	HHCH 5040	La Paz	Sud Yungas	16º19’	67º54’	-	03/04/1971
	HHCH 5042	La Paz	Sud Yungas	16º19’	67º54’	3225	03/04/1971
S. yungasense	VSU 602	La Paz	Nor Yungas	16º12’	67º44’	1680	05/01/1994
	SVG 6732	La Paz	Nor Yungas	16º12’	67º38’	1300	19/03/1993
	SVG 6739	La Paz	Nor Yungas	16º12’	67º38’	1243	20/03/1993

* Species endemic to Bolivia

Figure 2. *Travel routes of the six collecting expeditions*



Populations of *S. flavoviridens* were found in the same limited area (between Carijana and Camata) 29 years after being reported there for the first time. We found at least seven populations (plants in senescence, some flowering but no berries) growing in natural habitats and in orchards near the villages and co-occurring with *S. yungasense*.

S. neovavilovii was still present in its type locality 24 years after its discovery there. The habitat of this species was virtually unchanged, with only rare human settlements. However, in 2007 a project started to improve access to those remote communities by building roads in the area of occurrence of this species, which may cause a future threat to the populations.

S. okadae is not an endemic species, but in Bolivia it has a limited distribution. During the site visit, two other wild potato species turned out to coexist with *S. okadae*, namely *S. soestii* (a rare and endemic species) and *S. sparsipilum* (non-endemic).

S. violaceimarmoratum is an endemic species, but widely distributed in two departments of Bolivia. After 35 years, populations of this species were still present in the same site and in good condition. Close to this population an industrial waste dump from an industry of aggregates for construction (sand, gravel, crushed stone) was present, which could be a threat to these populations.

S. yungasense was previously reported only from the La Paz department, provinces Nor Yungas and Sur Yungas. During the last expedition in February 2007, a new occurrence was registered in the northern province Muñecas of the La Paz department, very far from its type locality (Nor Yungas province). In the new site, the species coexisted with *S. flavoviridens*.

- **Puna Mesophytic biogeographic province** (Table 5)

In the Puna Mesophytic province, three species were monitored with different ranges of distribution (from very limited distribution to widespread). Only *S. achacachense* was endemic and found to be severely endangered.

S. achacachense is endemic to a small area located on the border of two provinces (Omasuyos and Larecaja) of the La Paz department. In 1994, small plants were identified near steep cliffs. In 2007, very few plants were located on steep slopes but in the surrounding area no additional individuals were encountered. The area showed signs of intensive grazing (scarce pasture and abundant presence of manure).

S. candolleanum is non-endemic but it has been reported from a limited area in La Paz. In this biogeographic province it is found at the puna level but it is also found at the border with the Yungas biogeographic province (high rainfall region “Ceja de Monte”). One of the monitoring sites selected for this species was a cemetery where it was first encountered in 1971, 36 years later a large population of vigorous plants was still present.

- **Puna Xerophytic biogeographic province** (Table 6)

In this province, only *S. acaule* was selected for monitoring purposes. It is non-endemic and widely distributed in three countries: Perú, Bolivia and Argentina. It is a frost-resistant species that is found in natural and disturbed areas.

- **Bolivian Tucuman biogeographic province** (Table 7)

In this province, the endemic species *S. gandarillasii* and *S. neocardenasii* from the valley zone were selected for monitoring purposes. From the montane zone the endemic species *S. arnezii, S. avilesii, S. hoopesii,* and the non-endemic species *S. microdontum* were selected. From the pre-puna zone the endemic species *S.* ×*sucrense,* and from the puna zone the non-endemic species *S. infundibuliforme* and *S. leptophyes* were studied. Apart from the two widespread species *S. leptophyes* and *S. microdontum*, all showed a tendency of population decline.

S. arnezii was monitored in the years 2006 and 2010, under very different circumstances. The year 2006 was very dry, and only poorly developed plants were present in places protected by thorny bushes. In the year 2010, rainfall was more intense which had significantly favored their growth, and plants were found under the partial shade of trees, shrubs and bushes, and also among rocks protected from the cattle.

All specimens of *S. avilesii* have been collected in a limited area of around 25 km long in the Vallegrande province of the Santa Cruz department. This species was monitored in three years. In March 2006, no populations were found in natural habitats (hills and slopes). In March 2008 and 2010, the type locality of this species was revisited, and several populations were found in full bloom. They occurred in natural areas and along the road between the villages of Vallegrande and Pucara.

S. gandarillasii is an endemic species but with a wide distribution in three departments of Bolivia. Trips were made in December 2006, March 2008 and March 2010 to verify the occurrence of this species in previously known sites. Although 2006 was a dry year, making it difficult to locate wild potatoes, it was possible to find a few plants of this species growing under trees and bushes with some organic soil. In 2008, at least four other populations of *S. gandarillasii* were found in the same locality, but again each consisting of a few plants in a small area of no more than 10 m^2. In 2010, two additional populations were found, each comprising more than 300 plants in an area of around 50 m^2.

S. infundibuliforme is widely distributed but it was monitored in only one collecting site, where a single population was found consisting of less than 10 small plants with flower buds, in very dry and rocky soil. These plants were the only green herbs around, and hence this particular population might well possess a particular tolerance to drought.

S. microdontum was monitored in a number of selected sites, but many other populations of this species were encountered in different places around the routes to the Tarija, Chuquisaca and Santa Cruz departments, mostly along roads, but also under bushes and trees.

S. neocardenasii coexists with *S. gandarillasii* in the Florida and Vallegrande provinces of the Santa Cruz department. Collecting sites were revisited four times. In March of the dry year 2006, this species was not encountered. In ecember of the same year, only one small population of *S. neocardenasii* was found under spiny trees and shrubs, consisting of less than five small plants. In March of the wet year 2008, few plants of this species were found at several sites. In March 2010, additional populations of this species, consisting of more than 200 large and vigorous plants, were found along the same route followed previously in 2006 and 2008.

S. ×sucrense is a widely distributed endemic species of hybrid origin (Hawkes, 1990, Spooner and Hijmans, 2001). Small populations found in the middle of wheat fields showed tubers damaged by weevils and the fungus *Rhizoctonia solani*. The type locality of *S. ×sucrense* was greatly altered by newly constructed houses, including dumps of debris. Only a small population of this species was found with less than five dried out plants, in poor rocky and dry soil.

Table 4. *Wild potato species monitored in the Yungas Province*

Species	Occurrence	Area of distribution	Characteristics	Altitudinal range (m)	Monitoring year (s)	Trends of occurrence in the monitoring year	Main factors influencing occurrence
flavoviridens	Endemic, narrow distribution	Northern La Paz Department (Provinces Saavedra, Muñecas and Larecaja)	Subandean zone, thermotropical pluviseasonal bioclimate	1148 - 2790	2007	Stable, various populations growing on stony soils, along forest margins, in hill slopes under and in the middle of crop fruits	Agriculture at low extent
neovavilovii	Endemic, very limited distribution	Northern La Paz Department (Prov. F. Tamayo)	Ceja de Monte, supratropical bioclimate	2800 – 3500	2007	Stable, various populations growing under evergreen forests, in mountain streams, stone walls or over large rocks covered with moss and succulent plants	Human settlements at low extent
okadae	Non-endemic, limited distribution	Departments of La Paz (Prov. Inquisivi) and Cochabamba (Prov. Ayopaya and Tiraque)	Yungas montane forest	2400 – 3000	2009	Stable, large populations growing in the middle of shrubs, under spiny trees on steep slopes	Deforestation and soil erosion (landslides)
soestii	Endemic, restricted distribution	La Paz Departament (Province Inquisive)	Yungas montane forest	2800 – 2900	2009	Tendency to decline, large population of small plants growing among herbaceous plants, protected by spiny shrubs and rocks under partial shade of trees	Deforestation and soil erosion (landslides)
violaceimarmoratum	Endemic, widespread	Departments of La Paz (Prov. Nor Yungas, Sur Yungas, Saavedra and Murillo) and Cochabamba (Prov. Chapare and Ayopaya)	Ceja de Monte and Yungas montane	1800 – 3700	2007	Stable, large population of big plants growing on hill slopes, between stones and soil with high organic matter content	Industry of aggregates for construction close to wild populations

Species	Occurrence	Area of distribution	Characteristics	Altitudinal range (m)	Monitoring year (s)	Trends of occurrence in the monitoring year	Main factors influencing occurrence
S. yungasense	Non-endemic, limited distribution	La Paz Departament (Prov. Nor Yungas, Sur Yungas and Muñecas)	Yungas montane	1200 – 1800	2007	Stable, large populations in the middle of tropical fruit trees	Agriculture at low extend

Table 5. *Wild potato species monitored in the Puna Mesophytic Province*

Species	Occurrence	Area of distribution	Characteristics	Altitudinal range (m)	Monitoring year (s)	Trends of occurrence in the monitoring year	Main factors influencing occurrence
S. achacachense	Endemic, very limited distribution	La Paz Department (Prov. Omasuyos and Larecaja)	High.Andean zone, humid pluviseasonal bioclimate	3800-4000	2007	Drastic declining, small population growing near steep cliffs	Overgrazing
S. candolleanum	Non-endemic, limited distribution	La Paz Department (Prov. Larecaja, F. Tamayo, Camacho and Muñecas)	Puna and Ceja de Monte	3100-4000	2007	Stable, large populations of vigorous plants growing on a rugged surface with organic soil in the middle of abundant vegetation	Human settlements at low extend
S. megistacrolobum	Non-endemic, widespred	From the Lake Titicaca region of south Peru and north Bolivia, to northwestern Argentina	High, cold and moist Puna and high-Andean levels of the Puna Mesophytic and Bolivian-Tucumán Biogeographic Provinces, and in the North Altiplano	till 4450	2010	Stable, large population growing under trees, on open spaces and near cultivated crops	Agriculture and livestock

Table 6. *Wild potato species monitored in the Puna Xerophytic Province*

Species	Occurrence	Area of distribution	Characteristics	Altitudinal range (m)	Monitoring year (s)	Trends of occurrence in the monitoring year	Main factors influencing occurrence
acaule	Non-endemic, widespread	Large areas from Peru, Bolivia and northern Argentina	Puna and high-Andean zones	till 4500	2006	Stable, large populations growing in natural areas, in the middle of potato crops and at road borders	Human settlements, agriculture

Table 7. *Wild potato species monitored in the Bolivian Tucuman Province*

Species	Occurrence	Area of distribution	Characteristics	Altitudinal range (m)	Monitoring year (s)	Trends of occurrence in the monitoring year	Main factors influencing occurrence
arnezii	Endemic, limited distribution	Chuquisaca Department (Prov. Tomina)	Montane zone, mesotropical pluviseasonal bioclimate	1900-3200	2006 and 2010	Declining, small populations growing in partial shade of trees, shrubs and bushes between rocks	Strong presence of cattle and goat
avilesii	Endemic, very limited distribution	Santa Cruz Department (Prov. Vallegrande)	Montane zone, mesotropical pluviseasonal bioclimate	2500 - 2800	2006, 2008 and 2010	Declining, populations growing in hills and slopes, and along road borders	Presence of cattle
gandarillasii	Endemic, wide distribution	Departments of Chuquisaca (Prov. Oropeza and Zudañez), Cochabamba (Prov. Carrasco and Campero) and Santa Cruz (Prov. Vallegrande, Florida and M.Caballero)	Sub-andean and valley zone with a thermotropical bioclimate in interandean semiarid deciduous woodlands	1500 – 2500	2006, 2008 and 2010	Declining, populations of plants in small patches growing under woody and thorny shrubs with some organic soil	Strong presence of cattle and goat

Species	Occurrence	Area of distribution	Characteristics	Altitudinal range (m)	Monitoring year (s)	Trends of occurrence in the monitoring year	Main factors influencing occurrence
S. hoopesii	Endemic, very limited distribution	Chuquisaca Department (Prov. Azurduy)	Montane zone, mesotropical pluviseasonal bioclimate	2500 - 3400	2006	Declining, populations growing on steep and rocky slopes, and at the edge of field crops	Expansion of agriculture
S. infundibuliforme	Non-endemic, widespread	From northen Argentina to central Bolivia (Departments of Chuquisaca, Potosí, Tarija and Cochabamba)	Puna, supratropical zone, xeric and pluviseasonal bioclimate	3000 - 4000	2006	Declining, small population growing in dry and rocky soil	Presence of sheep, goats and Andean camels
S. leptophyes	Non-endemic, widespread	From southern Perú, through Bolivia to northern Argentina	Puna, supratropical zone, xeric and pluviseasonal bioclimate	3000 - 4000	2007	Stable, large populations growing in different environments, under shrubs, grasses, in rocky, sandy and organic soils	Urban growth and human settlements
S. microdontum	Non-endemic, widespread	Northern Argentina to Bolivia (Departments of Tarija, Chuquisaca and Santa Cruz)	Montane zone, mesotropical pluviseasonal bioclimate	1900-3000	2006 and 2010	Stable, large populations growing at the edge of roads, field crops, in the middle of bushes and under trees	Human settlements and agriculture
S. neocardenasii	Endemic, very limited distribution	Santa Cruz Department (Prov. Florida and Vallegrande)	Valley zone with a thermotropical bioclimate, and in interandean semiarid deciduous woodlands	1400 – 1700	2006, 2008 and 2010	Declining, populations of plants in small patches growing under woody and thorny shrubs with some organic soil	Strong presence of cattle and goat
S. ×sucrense	Endemic, widespread	Departments of Chuquisaca (Prov. Nor Cinti, Oropeza, Yamparaez, Azurduy), Potosí (Prov. Bilbao, Chayanta, T. Frías, Saavedra, Linares, Nor Chichas and Sur Chichas)	Pre-punean level with xeric, mesotropical and lower supratropical bioclimate, and puna, supratropical, xeric and pluviseasonal bioclimate	2100- 4000	2006	Declining, populations growing in the middle of crops (wheat, corn), and on rocky hillsides, poor and dry soils	Agriculture and excessive use of pesticides, human settlements

Discussion

In the following paragraphs the implications of the monitoring results for the persistence of the examined wild potato species are discussed per biogeographic province. We try to analyze how the current and potential factors perceived in the five years of field exploration could influence the specific characteristics and occurrence of each species.

Yungas biogeographical province

S. neovavilovii and *S. flavoviridens* occur in the northwest corner of this province. No obvious factors that might negatively affect the occurrence of these species were detected. Local children are aware of these wild potato populations because they play with their tubers and this might even contribute to the dispersion of those populations. The area is difficult to access (Spooner *et al*., 1994) although there are some roads. There are scattered human settlements that do not expand due to the migration of the local population to the main cities and only underdeveloped agriculture, while large extensions of evergreen forest ecosystems have remained almost unchanged. This has favored the occurrence of wild potato species. Recently, municipalities are developing plans to improve roads and open new ones, particularly near the type locality of *S. neovavilovii*. Such activities might lead to fragmentation of the populations or other negative effects on the occurrence of this species and of *S. bombicynum* which is also present in the vicinity of this locality (Spooner *et al*., 1994; Ochoa, 1990), although there is no information on how sensitive these species are to changes in their environment.

The type localities of *S. okadae* and *S. soestii* are located in the southeast of the department of La Paz (Inquisivi province). Here, a large number of plants of these species was found co-existing in the same area of approximately 500 m^2. Apparently the decrease of the primary forest caused by intensive wood extraction and road improvements has not significantly affected their population size, although *S. okadae* was far more common than *S. soestii*. This capacity of *S. okadae* to survive in semi-natural habitats was already mentioned by Hawkes and Hjerting (1989).

Within the Yungas biogeographic province, *S. violaceimarmoratum* and *S. yungasense* are the species with the highest tolerance to human activities. Populations are located in roadsides, around buildings or in the midst of human settlements. These disturbed circumstances may even have contributed to the dispersal of the plants, but both species are also found in less disturbed areas.

Puna Mesophytic biogeographic province

Of the three wild potato species studied in this area, only *S. achacachense,* occurring in the northwest of the Puna province in the Andean high-level (above 3800 m altitude), appears to be at serious risk of extinction due to excessive animal grazing. For this species only a very small number of plants was found in the vicinity of the type locality. The vegetation of the area consists mainly of peat bogs and grasslands (Navarro, 2002), leaving cattle with only limited options for food.

For *S. candolleanum,* two collecting sites were visited that were more than 200 km apart. At both sites, the climate is cold and humid. The encountered plants were always large and vigorous, with tubers up to 7 cm long. Only some plants showed symptoms of late blight, suggesting that this species may have partial resistance to *Phytophthora infestans*, although there are no references confirming this (Jacobs *et al.*, 2010). We could not detect obvious factors negatively affecting the occurrence of this species in its natural habitat.

Solanum megistracolobum has been described as a widely distributed species (Hawkes, 1990; Ochoa, 1990) and this was confirmed during the recent field trips. Also, its var. *toralapanum* was abundantly present in the surroundings of Toralapa Station. Apparently, the distribution of this species is even positively affected by human influences such as livestock and agriculture practices.

Bolivian Tucuman biogeographic province

The number of plants and populations of *S. gandarillasii* and *S. neocardenasii* varied considerably among the three different years of observation. The ecosystem is strongly affected by burning, cutting of firewood and grazing by cows and goats (Navarro, 2002). Grazing has an even stronger negative effect during dry years. In wet years the animals prefer other herbs. Even in dry years it was still possible to find a few populations of *S. gandarillasii*, confirming its tolerance to drought (Coleman, 2008).

In the montane zone of this biogeographic province four species were studied: *S. arnezii, S. avilesii, S. hoopesii* and *S. microdontum*. During the first visit in the dry year 2006, plants of *S. arnezii, S. hoopesii* and *S. microdontum* were found, but none of *S. avilesii*.

The monitored sites where *S. arnezii* and *S. hoopesii* occur were generally dry and rocky (not suitable for agriculture), and these species grew in protected places under shrubs, where they were also protected from the voracity of livestock. Even in those harsh conditions these species, in particular *S. hoopesii*, produced big and vigorous plants. It seems that this species has at least partial tolerance to drought.

As observed during the three site visits, the occurrence of *S. avilesii* is greatly affected by the climate. Apparently, the species has a large ability to tolerate dry years by developing new plants from tubers and/or seeds in years with more favorable conditions.

The large numbers of individuals and the wide dispersal of *S. microdontum,* even in dry years, suggest the potential presence of a range of resistances to diseases as well as adaptations to environmental stresses (Hawkes and Hjerting, 1989).

The type locality of *S. ×sucrense* has been greatly altered by human activities and this species is expected to rapidly disappear from this location. However, there are still many other populations of this species.

The observation of a very small population of less than five plants of *S. infundibuliforme* growing in a very dry and rocky soil is in line with its reported drought tolerance (Hawkes and Hjerting, 1989).

Puna Xerophytic biogeographic province

Solanum acaule has a wide geographical distribution and is usually found at high elevation and in cold environments, in places heavily disturbed by human settlements as well as in natural habitats (Hawkes and Hjerting, 1989; Ochoa, 1990). Because two of the three sites where *S. acaule* was observed (surroundings of Potosí city and near Cariquina, La Paz) were heavily influenced by human activities, we conclude that this species has a high resilience to highly disturbed environments. For this species, the influence of human activities even seems to be positive for its dispersion, but it is important to realize that populations of this wild species were always found in rural environments. A complete urbanization certainly would lead to large scale loss of populations.

Taxonomic issues

Monitoring wild potato populations in the field heavily relies on a correct identification of the species. We used the taxonomic keys of Ochoa (1990) and Hawkes and Hjerting (1989), and the experience of the curator of the wild potato germplasm of the CIP, A. Salas. For several species such as *S. acaule, S. megistracolobum, S. neocardenasii, S. gandarillasii, S. infundibuliforme, S. soestii,* and *S. microdontum*, taxonomic identification is not problematic. But in other species, even with the help of taxonomic experts and publications, it was not always possible to conclusively settle the identity of the plants encountered due to characters shared by different species and the variability within the species exceeding that indicated in the taxonomic keys and descriptions.

The taxonomic keys currently in use sometimes have imprecise terminology. Plants are keyed out with terms like ‘delicate’, ‘of medium vigor’ or ‘vigorous’, ‘of medium height’, stem wings ‘broad’ or ‘narrow’, and pubescence ‘sparse to very sparse’, ‘medium to very dense’, ‘medium-length’ or ‘variable’. We observed that plants growing in sunny places or in the shade regularly show differences that are not considered in the taxonomic keys. Thus, precise measurements and the use of standard terms are needed (Spooner and van den Berg, 1992; Spooner *et al.*, 1994) and further taxonomic research is necessary to assess the range of variability within species.

An example of such problematic identifications formed the plants found in central Chuquisaca department, Azurduy province. They could not be identified directly in the field and small plants were collected and grown in a greenhouse to complete their development. Here A. Salas was able to identify them as *S. tarijense*. However, the presence of glandular hairs which is the distinguishing feature of *S. tarijense* was hardly evident in the greenhouse material. These plants resembled *S. arnezii*, and were collected in the distribution range of that species, but the white (not creamy yellow) color of the corolla suggested that the name of species was correctly assigned, which would mean a new locality of occurrence for *S. tarijense* in Chuquisaca.

In general, wild potatoes considered as “weedy species” were difficult to identify. These behave as weeds growing along the edges or even within crop fields and closely resemble the cultivated potato. One example is *S.* ×*sucrense*. The few plants we found were almost dry, were very hard to recognize, and may have formed hybrid populations with the cultivated material complicating the interpretation of this ‘species’ (Spooner *et al*., 1994). The morphological similarity of *S.* ×*sucrense* to the cultivars is remarkable, leaving only characters like small tubers and long stolons to identify the “wildness” of the species.

epending on whether the year was dry or wet, the plants found at the collecting sites showed different stages of development. Where possible, berries were collected, but mostly only small plants were found, especially in dry years. In those cases, living plants were collected to try and complete their development in the greenhouse at PROINPA. Regularly, the characters used to define species proved impractical when germplasm collections were planted together and identifications were attempted without knowledge of the place of origin. These difficulties were also faced by Salas *et al.* (2001) with new collections of wild potato species in Peru. Hence, in order to ascertain the correct identity of such material, preliminary species identifications need to be checked with an assessment of the ploidy level and additional morphological or molecular evaluations.

ue to the problematic taxonomy of some of the Bolivian wild potato species, especially those accommodated in series *Tuberosa* (Spooner and van den Berg, 1992) and the so-called brevicaule-complex (van den Berg *et al*. 1998), caution is necessary in the interpretation of our findings at the species level.

Quality of collection data

For the selection of collecting sites we used information from databases of different gene banks. In the past, these gene banks made efforts to standardize information, solve missing and conflicting data and produce the Intergenebank Potato atabase (IP) (Huamán *et al*., 2000), which was also used as a source of information. Nevertheless, some collections still do not have precise geographical coordinates, making it necessary to take into account additional locality information from field reports. Notes taken in the field during collecting trips are often scanty or imprecise, which made verification of the exact location of some sites difficult. Hence, we may erroneously have scored some populations as being absent.

The wild potato expeditions in Bolivia have been mainly conducted by foreign researchers but significant contributions were made by Bolivian researchers at different periods. M. Cardenas contributed substantially during four decades (1920´s to 1960´s), subsequently other researchers contributed in the 80's and the 90´s (Table 1). The present work has been an effort to retrieve the information and lay the foundations for local field work with the support of experts to enable future initiatives in the exploration, monitoring and collecting of wild potatoes. Some methodological steps should be improved. The information in databases should be updated thoroughly to get more precise coordinates and a proper designation of political/administrative units. A larger number of collecting sites should be monitored, more time should be invested in field visits and locations should be visited more than once given that large fluctuations in population size may occur between years. The morphological variability found within species during the monitoring process should also be documented.

Conclusions

After explorations during five years in the four biogeographic provinces of Bolivia where wild potatoes occur naturally, we conclude that the annual variation in rainfall has a profound influence on the occurrence of wild potato populations.

In the Yungas biogeographic province, populations of the six wild potato species monitored are maintained, despite the progressive tendency of human activities that are altering natural ecosystems.

In the Puna Mesophytic biogeographic province, one of the three species monitored, the narrow endemic *S. achacachense,* seems to be at serious risk of disappearing because excessive animal grazing endangers the occurrence of this species in its natural habitat.

In the Bolivian Tucuman biogeographic province, the nine species monitored were found in a wide area with a markedly dry climate and xeric plant ecosystems. uring the explorations carried out in a period of five years, significant populations of most species (except *S. infundibuliforme* and *S.*

×*sucrense*) were still encountered, showing a great capacity of survival and resilience under hard conditions. Still, these species face increasing stress factors caused by the progressive human settlements, agriculture and husbandry in this region.

Solanum acaule was found abundantly in three biogeographic provinces (Puna Xerophytic, Puna Mesophytic and Bolivian-Tucuman) confirming its wide geographical distribution which reduces the risks that affect the occurrence of natural populations.

The unexpected low frequency of the wide-spread *S. infundibuliforme* and *S. ×sucrense* in this study suggests the need of additional expeditions for these and similar cases when monitoring results show low occurrences of populations, in order to assess whether this is due to stochastic variation or to an actual decline in the number of their populations.

Wild potato species were found to occur in natural and semi-natural environments, with different grades of disturbance, often in the same areas where they were first encountered decades ago. Their survival until now is not the result of any intervention or management action. The pressure of human activities on the habitats where wild potato occurs, although not known with any precision, is likely to increase substantially over the coming years, to an extent that could exceed the capacity of survival of wild potato species. Endemic species with a limited distribution run the greatest risk. Therefore, it is necessary to change the current passive to an active conservation, promoting positive actions to ensure the existence of viable populations of these taxa, like habitat monitoring and the maintenance of the natural, semi-natural or artificial ecosystems that contain them.

The results obtained from the examined populations provide a good representation of species growing in the four biogeographical provinces of Bolivia were wild potatoes are known to occur. Nevertheless, this study should be considered as a starting point, and further research on population structure, and genetic and morphological variability should be conducted to determine the necessary spatial scale for conservation measures.

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CHAPTER 3
Biodiversity status of endemic wild potato (*Solanum* spp.) in Bolivia: reasons for conservation concerns[1]

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Abstract

Crop wild relatives possess important traits for crop improvement and therefore *ex situ* and *in situ* conservation efforts are essential. Bolivia is a centre of wild relative diversity for several crops, among them potato, which is an important staple worldwide and the principal food crop in this country. espite their relevance for plant breeding, limited knowledge exists about their *in situ* conservation status. We used Geographic Information Systems (GIS) and distribution modelling to better understand geographic patterns of endemic wild potato diversity in Bolivia. In combination with threat layers, we assessed the conservation status of all 21 endemic species. We prioritized areas for *in situ* conservation by using complementary reserve selection and excluded 25% of the most-threatened occurrence sites because costs to implement conservation measures at those locations may be too high compared to other areas. Following the IUCN Red List guidelines, we assessed a vulnerable status or even more severe category of threat for 15 out of the 21 species. Our results show that four of these species require special conservation attention because they were only observed in 15 or less locations and are highly threatened by human accessibility, fires and livestock pressure. Although the highest species richness occurs in south-central Bolivia, in the departments Santa Cruz and Chuquisaca, the first priority area for *in situ* conservation according to our reserve selection exercise is Cochabamba in central Bolivia. This area is less threatened than the potato wild relatives' hotspot in south-central Bolivia. Only seven of the 21 species were observed in protected areas. To improve coverage of potato wild relatives' distribution by protected areas, we recommend starting inventories in parks and reserves with a high modelled diversity. Finally, to improve *ex situ* conservation, we targeted areas for germplasm collecting of species with less than 5 accessions conserved in genebanks.

Key words: Crop wild relatives; *Ex situ* conservation; *In situ* conservation; IUCN red listing; Potato breeding material; Reserve selection; Species distribution modelling; Threat assessment.

Introduction

Crop wild relatives (CWRs) include crop progenitors and species that are closely related to crops. Many of the latter species possess traits of interest for crop improvement, providing plant breeders with genes coding for biotic and abiotic stress resistance (e.g. resistance against pests and diseases, temperature, drought or salinity stress) or higher values for nutritional traits to name but a few (Tanksley and McCouch, 1997). Besides their role in providing genes for crop breeding, many CWRs are already exploited by local communities as they directly contribute to food security through provision of fruits, leaves, tubers and/or seeds.

Populations of some CWRs occur in existing protected areas but many others grow in unprotected natural environments. ue to the absence of active *in situ* management, the conservation status of most CWRs is still largely unknown. Many CWRs are increasingly menaced by habitat loss due to agricultural intensification, the impact of invasive species, deforestation, overgrazing and overexploitation (Maxted *et al.,* 2008; VMABCC-BIOVERSITY, 2009). In addition to these direct threats, global climate change is expected to become a long-term threat (Jarvis *et al.,,* 2008). The Convention on Biological Diversity (CBD, 2010), the Status of Plant Genetic Resources for Food and Agriculture (FAO, 2010) and the Global Network for *In Situ* Conservation of Crop Wild Relatives (Maxted and Kell, 2009), all highlight that active *in situ* (in wild populations and on farm) and *ex situ* conservation of CWRs is essential for future crop improvement. Several global initiatives are currently being implemented to improve both *in situ* (VMABCC-BIOVERSITY, 2009) and *ex situ* conservation (GCDT, 2010) of CWRs.

Bolivia is located in one of the main centres of origin of domesticated plants in the world (Vavilov, 1951), and its high diversity of climatic conditions, soils and habitats, combined with the high cultural wealth of indigenous peoples, played a key role in the process of domestication (Ibisch and Mérida, 2003). Bolivia is an important centre of diversity of several globally important staple crops such as potatoes (*Solanum tuberosum* L.), peanuts (*Arachis hypogaea* L.) and chili peppers (*Capsicum* spp.), but also crops of local importance such as the Andean grains quinoa (*Chenopodium quinoa* Willd.) and amaranth (*Amaranthus* spp.), and Andean roots and tubers. Bolivia is also an important secondary centre of diversity of several other species such as maize (*Zea mays* L.), cassava (*Manihot esculenta* Crantz) and pineapple (*Ananas comosus* (L.) Merr.), and home to many wild relatives of all of these crops.

In terms of net production, potato is the fourth most important crop in the world, after rice, wheat and maize. The crop and its wild relatives are therefore included in Annex I of the International Treaty on Plant Genetic Resources for Food and Agriculture, which facilitates the access to these genetic resources (http://www.planttreaty.org/texts_en.htm). In Bolivia, potato is the most important food crop for the local population with over 1000 native potato cultivars being cultivated by over 200,000 families (Cadima and Gandarillas, 2009; Zeballos *et al.,* 2009).

espite their potential for breeding programmes, CWRs are still underutilized in the development of new cultivars, albeit that new technologies are available to better target their use (i.e. molecular maps, QTL analysis) (Hajjar and Hodgkin, 2007). In the case of potato wild relatives, several endemic Bolivian species have been studied, revealing traits important for future potato breeding (see Table 1). Ten species were found to show resistance against late blight (*Phytophthora infestans*), while twelve species proved to be resistant to nematodes (*Globodera* spp.), the main diseases affecting potato production worldwide. Seven species show tolerance to abiotic stress, such as high temperature, drought or frost (Hawkes and Hjerting, 1989; Ochoa, 1990; Coleman, 2008).

Wild potato relatives (*Solanum* spp. section *Petota*) occur in the Americas from the south-western United States to central Argentina and Chile. Some species, such as *Solanum acaule* Bitter, have a wide distribution range but most of them are confined to limited areas and ecological zones (Spooner and Salas, 2006; Hijmans *et al.,* 2002; Hawkes, 1990). The highest number of wild potato relatives is found in the Andes area from north-central Peru to central Bolivia.

In Bolivia, 34 wild potato species have been recorded following the classification of Spooner and Salas (2006), of which 21 species are endemic to the country. Wild potato species grow at altitudes between 700 to 4500 m (Ochoa, 1990) and occupy many different ecological niches in mesothermic and inter-Andean valleys, and in the subtropical Andean rainforest (Yungas). They are only absent from the Bolivian tropical lowland forests (Spooner *et al.,* 1994).

There have been many efforts to collect germplasm of wild potato species in Bolivia. Nevertheless, a significant amount of the diversity remains unrepresented in gene bank collections (Hijmans *et al.,* 2000). Several species have only a small number of known *in situ* populations whereas they are not conserved *ex situ*. At the same time, there is limited knowledge about the *in situ* conservation status of these potato relatives (VMABCC-BIOVERSITY, 2009).

Table 1. *Documented properties of interest for crop improvement in wild potato relatives endemic to Bolivia*

Species	Uses (Resistances)*
S. achacachense	Cyst nematode (*Globodera pallida*)
S. alandiae	Wart (*Synchytrium endobioticum*), Blackkeg (*Erwinia carotovora*), Cyst nematode (*Globodera pallida*), Flea beetle (*Epitrix cucumeris*), Potato aphid (*Macrosiphum euphorbiae*) Heat tolerance
S. arnezii	Late blight (*Phytophthora infestans*), Blackkeg (*Erwinia carotovora*) Root-knot nematode (*Meloidogyne* spp.), Cyst nematode (*Globodera pallida*)
S. avilesii	Late blight (*Phytophthora infestans*), Wart (*Synchytrium endobioticum*), Blackkeg (*Erwinia carotovora*), Root-knot nematode (*Meloidogyne* spp.), Cyst nematode (*Globodera pallida*), Flea beetle (*Epitrix cucumeris*), Potato aphid (*Macrosiphum euphorbiae*).
S. berthaultii	Late blight (*Phytophthora infestans*), Wart (*Synchytrium endobioticum*), Black scurf (*Rhizoctonia solani*), Verticillium wilt (*Verticillium* spp.), Blackkeg (*Erwinia carotovora*), Common scab (*Streptomyces scabies*), Bacterial wilt (*Ralstonia solanacearum*), Root-knot nematode (*Meloidogyne* spp.), Cyst nematode (*Globodera* spp.), Virus resistance PVX, PVY, PSTV Colorado beetle (*Leptinotarsa* spp.), Peach-potato aphid (*Myzus persicae*), Leaf hopper (*Empoasca fabae*), Flea beetle (*Epitrix* sp.), Leaf miner (*Liriomyza* spp.), Chinche (*Lygus* sp.), Spider mite (*Tetranychus* spp.)
S. circaeifolium	Late blight (*Phytophthora infestans*), Blackkeg (*Erwinia carotovora*), Cyst nematode (*Globodera pallida*). Heat and Drought tolerance
S. ×doddsii	Wart (*Synchytrium endobioticum*).
S. flavoviridens	Peach-potato aphid (*Myzus persicae*), Colorado beetle (*Leptinotarsa* sp.), Spider mite (*Tetranichus* spp.), Leaf hopper (*Empoasca* sp.), Leaf miner (*Lyriomiza* spp.)
S. ×litusinum	Late blight (*Phytophthora infestans*), Wart (*Synchytrium endobioticum*), Black scurf (*Rhizoctonia solani*), Cyst nematode (*Globodera* spp.) Colorado beetle (*Leptinotarsa* spp.), Chinche (*Lygus lineolaris*)
S. neocardenasii	Peach-potato aphid (*Myzus persicae, Macrosiphum euphorbiae*), Leaf hopper (*Empoasca fabae*), Flea beetle (*Epitrix cucumeris*), Spider mite (*Tetranichus urticae*), Drought tolerance
S. soestii	Late blight (*Phytophthora infestans*), Blackkeg (*Erwinia carotovora*), Cyst nematode (*Globodera* spp.), Heat tolerance.
S. ugentii	Late blight (*Phytophthora infestans*), Cyst nematode (*Globodera* spp.)

Species	Uses (Resistances)*
S. virgultorum	Late blight (*Phytophthora infestans*), Wart (*Synchytrium endobioticum*) Blackkeg (*Erwinia carotora*) Cyst nematode (*Globodera* spp.)
S. gandarillasii	Drought tolerance Cyst nematode (*Globodera* spp.)
S. ×sucrense	Verticillium resistance Late blight (*Phytophthora infestans*), Wart (*Synchytrium endobioticum*). Cyst nematode (*Globodera* spp.), Blackkeg (*Erwinia carotovora*) Virus resistance PVX, PVA Potato tuber moth (*Phthorimaea operculella*) Frost resistance
S. violaceimarmoratum	Colorado beetle (*Leptinotarsa* spp.) White mold (some) Late blight (*Phytophthora infestans*) Frost resistance

*Uses found for only 16 out of the 21 endemic Bolivian species. Data obtained from Hawkes and Hjerting, (1989), Ochoa, (1990), Coleman (2008), Spooner and Bamberg (1994), Jansky *et al.,* (2008) and from databases of Centre for Genetic Resources (CGR), the Netherlands, Intergenebank Potato Database (USDA), International Potato Center (CIP), Peru, Institute of Plant Genetic Resources and Crop Plant Research (IPK), Germany.

Geographic information systems (GIS) are an effective tool that can contribute to generate new knowledge on the conservation status of plant species (Brummitt *et al.,* 2008). GIS is widely applied in different areas of environmental sciences and biodiversity, and has become an important tool in the development of strategies for the conservation and sustainable use of plant genetic resources (Jarvis *et al.,* 2003). GIS, and notably environmental niche modelling, is increasingly used to evaluate the geographic distribution and *in situ* conservation status of plant species, including CWRs (Scheldeman *et al.,* 2007; Penn *et al.,* 2009; Hauptvogel *et al.,* 2010; Gonzáles_Orozco *et al.*, 2012), as well as to guide targeted germplasm collecting trips (Jarvis *et al.,* 2005; Scheldeman *et al.,* 2007). Since species with a narrow distribution range are more prone to become extinct (Baillie *et al.,* 2004; Işik, 2011), spatial analysis has been widely used to assess species conservation status by identifying the potential extent of species distribution ranges (Willis *et al.,* 2003). Spatial layers that contain information about human intervention (e.g. roads, agricultural conversion) can be overlaid in GIS over maps of species distribution and provide further information about the threats and conservation status of cultivated plant species and their relatives (Willemen *et al.,* 2007; Maxted *et al.,* 2008) or of entire ecosystems (Jarvis *et al.,* 2010).

Recent collecting missions by the Fundación para la Promoción e Investigación de Productos Andinos (PROINPA) have increased the number of wild potato accessions for *ex situ* conservation (Patiño and Cadima 2009). This new occurrence data combined with existing information about wild potato relatives' distribution and with new spatial information about threats allows a comprehensive survey of the conservation status of endemic potato wild relatives in Bolivia. In this study, we will (1) evaluate the *in situ* and *ex situ* conservation status of endemic wild potato relatives in Bolivia, based on a spatial analysis; and (2) identify hotspots of endemic wild potato diversity, including areas that are threatened by human activities causing disturbance to the habitat of the potato wild relatives. The newly obtained results will all add to improve the conservation status efforts of endemic potato wild relatives and contribute to the maintenance of a future base for potato breeding. We use the term biodiversity hotspot as referring to an area of exceptional species-richness following Reid (1998), and not in the sense of Myers *et al.,* (2000) who use the presence of a specific threat as an additional criterion.

Methods

Data sources

Georeferenced passport data from existing genebank databases (Centre for Genetic Resources, the Netherlands, United States Potato Genebank, Institute of Plant Genetics and Crop Plant Research of Germany, Intergenebank Potato atabase and International Potato Center of Peru) were used to map de geographic occurrence of the 21 Bolivian endemic wild potato species. These data were supplemented by georeferenced herbarium records on wild potato species developed by Hawkes and Hjerting (1989), Ochoa (1990) and Hijmans and Spooner (2001). uplicates were removed after merging the different data sets, and 331 georeferenced observation points remained. Subsequently, 101 new presence points, obtained through PROINPA's germplasm collecting missions during 2006 to 2010 were added to this dataset. Finally, we added 52 georeferenced herbarium and genebank records (presence points) obtained from the Global Biodiversity Information Facility (GBIF), twelve of which were without coordinates and were georeferenced based on locality descriptions with the use of Google Earth® and www.geonames.org. Presence point datasets were checked for inconsistencies between coordinates and department information in the passport data after Scheldeman and van Zonneveld (2010) and removed accordingly.

Species identification followed the taxonomy of Spooner and Salas (2006) which is commonly used in global databases and also in the Bolivian germplasm bank. We are aware that our results depend on the chosen taxonomy, and might be different if we would take into account the last taxonomic treatment of wild potatoes reported on the Solanaceae source website (http://www.solanaceaesource.org) where for example the delimitation between various species of the "brevicaule complex" as defined by van den Berg *et al.,* (1998) is questioned (see Appendix A).

The reliability of the taxonomic identity of collection data obtained from third parties such as through GBIF is often unknown (Chapman, 2005). As an additional quality control, we therefore identified for each species, observation points in a-typical environments, which may represent taxonomic misidentifications. For each species, we calculated a-typical values using the 1.5 interquartile ranges as threshold for four bioclimatic variables representing different facets of intra-annual climatic conditions: mean annual temperature, annual precipitation, temperature seasonality and precipitation seasonality. These calculations were done in R version 2.15.2 (R evelopment Core Team, 2010). Climate values were obtained through the raster package (Hijmans, 2012) from the 2-5 minutes resolution Worldclim dataset (www.worldclim.org). We considered observation points as outliers when they scored a-typical values for two or more of the four climate variables. Following this method, only three observation points were identified as outliers of whom one came from a database herbarium record and two from the recently collected herbarium and gene bank records by PROINPA. No outlier points were observed from the herbaria that made their data accessible through GBIF. The three 'suspicious' points all had a confident taxonomic identification (verified by the expert on wild potatoes from CIP) and we decided to maintain them in our dataset.

Species richness

A layer of the observed species richness based on presence points was created in IVA-GIS using a five-minute resolution grid and applying a circular neighbourhood of 30-minute diameter (about 50 km at the equator) (see Scheldeman and van Zonneveld, 2010).

To create a potential species richness map, we estimated potential natural distribution ranges using a species distribution modelling approach. This technique defines the ecological niche, based on the combination of different environmental layers at the sites of the records, and identifies areas with similar environmental conditions as zones where the species could potentially occur and discriminates it from areas with an environment outside the ecological niche. Maxent is a distribution modelling tool (Phillips *et al.,* 2006; Elith *et al.,* 2011) for which the applied algorithm has been evaluated as performing very well, in comparison to other distribution modelling software (Aguirre-Gutiérrez *et al.,* 2013; Elith *et al.,* 2006; Hernandez *et al.,* 2006). Therefore, Maxent was selected to model the potential natural distributions of the 21 species.

We used both climatic and soil data as environmental parameters to produce our environmental niche models. Nineteen bioclimatic variables were obtained from the WorldClim database at 30 arc-sec spatial resolution (http://www.worldclim.org/current) (accessed 03-10-2012) (Hijmans *et al.,* 2005). These were tested for multicollinearity using Pearson's correlation test (Raes *et al.,* 2009) and only those with Pearson's |r| < 0.80 were retained. Soil data were collected from the Harmonised World Soil atabase (http://webarchive.iiasa.ac.at/Research/LUC/External-World-soil-database/HTML/index.html?sb=1) (accessed 03-10-2012) (FAO/IIASA/ISRIC/ISSCAS/JRC, 2009), of which we used data of top soil only. Soil data were tested for multicollinearity using Spearman's rank correlation test and soil variables with Spearman's |rho| < 0.7 were retained. For groups of correlated variables we used a PCA analysis to select the one with the highest information content (highest factor loading on axis one or two) combined with ecological interpretation. Additionally, the standard deviation of altitude (DEM_sd) within the 30 arc-sec grid cell based on the 90 m SRTM altitude data, obtained through (<srtm.csi.cgiar.org>) (accessed 07-10-2011), was calculated and serves as a proxy for ruggedness of the landscape (Gamache, 2004). In total, fifteen environmental layers were selected to generate the models: six climatic, the standard deviation of altitude and eight soil layers (Table 2). All 15 layers contain continuous data.

Distribution modelling with Maxent and the 15 environmental variables cited in Table 2 was used to predict the occurrence of wild potatoes species in Bolivia. To train the model for each species, we used background points within a 50 km radius around the presence points. Model outcomes were generated with logit probability values. All other Maxent settings were kept default. As a threshold probability value to distinguish potential areas of occurrence from areas where a species would be absent, we chose the probability value where the value of sensitivity (true positive rate) plus specificity (true negative rate) is maximum, as recommended (see Liu *et al.,* 2005).

Table 2. *Abiotic variables used for environmental niche modelling*

Code	Description
Bio3	Isothermality (BIO1/BIO7) * 100
Bio4	Temperature seasonality
Bio6	Minimal temperature of coldest month
Bio12	Annual Precipitation
Bio14	Precipitation of driest month
Bio15	Precipitation seasonality
DEM_sd	Standard deviation of altitude
REF_DEPTH	Reference Soil Depth
T_CEC_SOIL	Topsoil CEC (soil)
T_ESP	Topsoil Sodicity (ESP)
T_GRAVEL	Topsoil Gravel Content
T_OC	Topsoil Organic Carbon
T_PH_H2O	Topsoil pH (H_2O)
T_SILT	Topsoil Silt Fraction
T_TEXTURE	Topsoil Texture

Then, to develop a potential richness map that is comparable to the observed richness map, we aggregated for each species its presence-absence map to the same resolution as the observed richness map, i.e. five minutes. The aggregated cells received a value for species presence (grid cell value=1) when species presence was modelled in one or more of its composing cells. Our final potential richness map consisted of the sum of all aggregated presence-absence maps.

Species with only few occurrence data may be sensitive to over-prediction in Maxent, although Maxent may even produce useful models with only 5-10 observations if these species have a rare and narrow distribution (Hernandez *et al.,* 2006). This is likely true for several of our potato species that have a narrow distribution restricted to Bolivia: five of the 21 species had less than 15 unique locations (Table 3). Therefore, we restricted all generated potential distribution layers with a buffer zone around each register to avoid overestimation of the modelled distribution ranges. A circular radius of 50 km was chosen for this buffer zone after the potato distribution maps developed by Hijmans and Spooner (2001). By restricting the model outcomes with the buffer zones, our predictions of modelled species richness remain relatively conservative.

Table 3. *Total number of presence points of each endemic wild potatoes species in Bolivia, number of points in protected areas, preliminary IUCN conservation status, average threat value and identification of most important threats per species*

Species	Nr. of locations	Nr. of locations in protected areas	AOO (km^2)	EOO (km^2)	Tentative IUCN threat status*	Mean threat value	Largest threat **	Second largest threat**
S. achacachense	10	0	29	129	EN B2ab(iii)	0.35	fires (0.86)	access (0.75)
S. alandiae	34	0	6874	20586	NT	0.30	access (0.60)	fires (0.53)
S. arnezii	23	0	5124	5488	VU B1ab(iii)	0.36	access (0.78)	livestock (0.71)
S. avilesii	19	0	38	59	CR B1ab(iii)	0.30	access (0.74)	Convers (0.61)
S. berthaultii	71	0	25085	36307	NT	0.30	access (0.84)	livestock (0.61)
S. boliviense	33	0	5205	10076	VU B1ab(iii, iv)	0.29	access (0.95)	livestock (0.45)
S. bombycinum	3	3	5	0.3	CR B2ab(iii)	0.16	fires (0.39)	access (0.37)
S. brevicaule	47	13	111659	105673	LC	0.36	fires (1.05)	access (0.70)
S. circaeifolium	42	4	42095	46386	NT	0.27	fires (0.68)	access (0.56)
S. flavoviridens	7	4	39	67	CR B1ab(iii)	0.34	fires (0.95)	convers (0.46)
S. gandarillasii	21	0	2913	12308	VU B1ab(iii)	0.27	access (0.68)	livestock (0.49)
S. hoopesii	11	0	264	430	EN B2 ab(iii)	0.34	fires (1.00)	livestock (0.57)
S. neocardenasii	14	0	37	507	CR B1 ab(iii)	0.28	access (0.75)	fires (0.56)
S. neovavilovii	17	17	61	180	EN B2 ab(iii)	0.17	fires (0.52)	access (0.35)
S. soestii	6	0	1	3	CR B2 ab(iii)	0.16	access (0.57)	livestock (0.29)
S. ugentii	12	0	324	401.4	EN B2 ab(iv)	0.42	fires (1.28)	livestock (0.60)
S. violaceimarmoratum	22	9	8830	13703	NT	0.28	fires (0.73)	access (0.65)
S. virgultorum	9	2	18792	25035	NT	0.18	access (0.63)	livestock (0.22)
S. ×doddsii	18	0	3268	11985	VU?***	0.20	access (0.65)	livestock (0.55)
S. ×litusinum	9	0	1663	10161	VU?***	0.29	access (0.80)	livestock (0.58)
S. ×sucrense	66	0	25436	48284	NT	0.37	fires (0.99)	access (0.86)

*CR: Critically Endangered; EN: Endangered; VU: Vulnerable; LC: Lower Concern; NT: Not threatened.
** access: accessibility to humans; livestock: livestock activities pressure; convers: conversion to agriculture.
*** Based on EOO (<20.000 km^2) calculated for *S. ×doddsii* and AOO (<2.000 km^2) calculated for *S. ×litusinum* both species could have a Vulnerable (VU) status, but they still need a verification of population size and/or quality of habitat.

Ecogeographic analysis

For each endemic wild potato species we identified the different climatic zones in its distribution range according to the climate classification of Köppen (see Kottek *et al.,* 2006). This allows us to identify for each species, putative ecotypes adapted to different environmental conditions, including rare and unusual ones. Such an analysis helps to determine potentially interesting germplasm for potato breeding that would use adaptive traits to unusual and interesting environmental conditions.

We used 30 seconds resolution monthly precipitation and mean temperature layers from the Worldclim dataset to define the different climate zones according to the criteria provided by Kottek *et al.,* (2006). We calculated these zones using the R statistical environment (R Development Core Team 2010; for the final map please refer to Appendix B). In addition, we provided for each endemic wild potato species the altitudinal range in which it occurs. Elevational data was derived at 30 seconds resolution from the Worldclim set.

In situ *conservation status*

On the basis of the presence points, we calculated for each species the Extent of Occurrence (EOO), the Area of Occupancy (AOO) (both in km^2). These were used to assess the preliminary IUCN red list category of threat, based on criterion B (IUCN 2010). The EOO and AOO values were calculated on the basis of observed species distribution in ArcView 3.2 with the CATS tool (Willis *et al.,* 2003; Moat, 2007). The CATS tool calculates the areas using the Equal Area Cylindrical Projection.

EOO is a measure of the distribution range in which a taxon occurs and is defined as the area within the shortest boundary that encompasses all occurrence sites, also called its convex hull (IUCN, 2010). Taxa with a higher EOO have a broader distribution range and are therefore less vulnerable to extinction compared to narrow distributed taxa. AOO is a parameter that represents the area of suitable habitat for species occurrence within its EOO (IUCN, 2010). AOO is calculated as the area of all grid cells in which one or more species records are located (IUCN, 2010). For each species, we defined the grid cell size by taking the 10% of the maximum geographic distance between two collection sites, following Willis *et al.,* (2003). The more suitable habitat a taxon has within its EOO, the less likely it is going to extinct within a short term.

The taxon must then meet at least two of three other options listed for criterion B to qualify for any of the IUCN threat categories Vulnerable, Endangered or Critically Endangered (IUCN, 2010). The options are provided in Appendix C.

We calculated in ArcGIS 10 (ESRI, Redlands, California, USA), the number and percentages of records per species within protected areas. The protected area layer was derived from the World Database on Protected Areas (WDPA) (UNEP-WCMC, 2010). All classes of protected areas were considered, i.e. national, international and private protected areas, in assigning the different IUCN categories. The ecosystem map followed the Nature Serve classification that was developed by Josse *et al.,* (2003).

As an estimation of potential population decline, we used threat maps for natural ecosystems developed by Jarvis *et al.,* (2010) to understand the major factors affecting distributions and species richness of endemic wild potato species. They calculated the threat levels for specific locations by (1) mapping the geographic distributions of recorded threat occurrences; (2) developing threat specific decay functions after expert consultation (these were used to calculate the relation between threat exposure and geographic distance); (3) the magnitude of each threat impact on 608 ecosystems according to experts; and (4) the response of these ecosystems to specific threats according to experts. The latter indicated whether the threats' impact to specific ecosystems were linear, exponential (low levels of threat would have a minimal impact), logarithmic (any level of threat has large impacts), or polynomial (low impact in mid-threat levels). Final threat values for locations were between 0 (low) and 3 (high).

Jarvis *et al.,* (2010) describe the following datasets to determine the geographic distribution of each threat in South America:

- Accessibility to humans: road, river and rail access per capita using data from the Digital Chart of the World (DCW), Vector Map (VMAP), and the Center for International Earth Science Information Network (CIESIN) (1:250,000–1:1,000,000 scale);
- Conversion to agriculture: number of major crops per 10 km resolution grids as indicated by distribution maps for the 22 principal crops developed by You and Wood (2006);
- Fire: 250 m resolution MODIS satellite-based fire occurrence;

- Livestock pressure: 8 km resolution maps of cattle, goat and sheep density from FAO' Livestock Atlas of the World (FAO, 2004);
- Infrastructure: airport or dam presence according to DCW and King's College London database of dams (1:1,000,000 scale);
- Oil and gas: recorded oil and gas drill sites according to the World Petroleum Assessment 2000 Digital Data Series (DDS) 60 (1:5,000,000 scale).

The spatial resolution of these maps was defined to 30 s (□1 km) considering the accuracy of the various data sources and ease of applicability for practitioners in the field (Jarvis *et al.,* 2010).

Because the sensitivity has been determined on ecosystem level, the threat values at species level should be interpreted with caution because some taxa may be more sensitive to a specific threat than others. In our threat analysis, we assume that all wild potato species occurring in a specific ecosystem have a similar level of sensitivity to the different.

For each species, we determined the average threat value at each occurrence by overlaying the six threat maps in DIVA-GIS. We also identified, for each species, the major threats on the basis of the mean value across the occurrences.

Prioritization of areas for in situ *conservation*

We carried out a complementary analysis (Rebelo and Siegfried 1992) in DIVA-GIS (www.diva-gis.org), using a 30 minutes-resolution grid (~50 km^2) to prioritize areas for *in situ* conservation. This analysis identifies the minimum number of grid cells required to conserve all taxa of interest. The grid cell with the highest number of species records is determined as the first priority area for *in situ* conservation. Second priority is given to the grid cell that covers the highest number of additional species that did not occur in the first priority cell. This prioritization exercise goes on until all species are covered in one or more cells.

We considered 30 minutes (~50 km^2) an appropriate scale to detect spatial patterns at the country level. It is also a representative size for a protected area. The median size of the protected areas that are listed for Bolivia in the WDPA database is 36 km^2, while their mean size is 61 km^2.

ifferent approaches to define priority conservation areas were tested. In a first analysis, a complementary analysis was carried out without taking into account whether the locations of presence points are threatened or not. In a second analysis we included only presence points at locations below the 75% percentile of average threat value. The areas that are most susceptible to threats like human accessibility, livestock pressure and agricultural production can be very costly to conserve compared to more isolated and less-threatened areas (Carwardine *et al.,* 2008). Limited budgets for conservation planning can thus be used more efficient in these isolated and less-threatened areas. The reserve selection exercise was then repeated with only occurrence sites from protected areas. We carried out this analysis to evaluate how well the current protected area network in Bolivia conserves endemic potato wild relatives. Finally, we carried out the reserve selection considering different putative ecotypes within each species that occur in the different climatic zones.

Ex situ *conservation status*

To examine the *ex situ* conservation status of the endemic wild potato relatives of Bolivia, we consulted the Global Strategy for the *Ex Situ* Conservation of Potato (GCDT, 2006) which provides an overview of material from Bolivia in gene banks. We identified species not yet conserved in any gene bank or with less than five accessions conserved *ex situ*. We then identified, using Maxent, the areas these species are likely to occur (gap analysis, Scheldeman and Zonneveld, 2010). Targeted future collecting efforts in these areas is likely to improve the Bolivian wild potato species *ex situ* conservation status.

Results

Species richness

Wild potato relatives can be found from the northern high Andean part of Bolivia across the Andean-Amazon transition zone towards dry subtropical south-central Bolivia (Fig. 1). Observed species richness is highest in south-central Bolivia (Fig. 2), in Santa Cruz (mesothermal valleys of Florida and Vallegrande provinces), and in Chuquisaca (provinces Zudañez, Azurduy, Tomina and Oropeza). According to the potential species richness map (Fig.

3), most species are expected to occur in northern Chuquisaca and to a lower degree in Cochabamba. This area is situated more towards the centre of Bolivia than towards the mesothermal valleys of Santa Cruz where currently most species are known to occur. Most areas of observed high diversity are situated outside protected areas. The only protected area where the highest number of species is predicted to occur is 'El Palmar', but so far this area has not been visited to collect wild potato species (Figs. 2 and 3). To a lower degree, the national park 'Carrasco' and "Tunari" in Cochabamba and "Apolobamba" in La Paz harbour several endemic wild potato species (Figs. 2 and 3).

Figure. 1. *Distribution of the 21 endemic wild potato relatives on the basis of herbarium and genebank records*

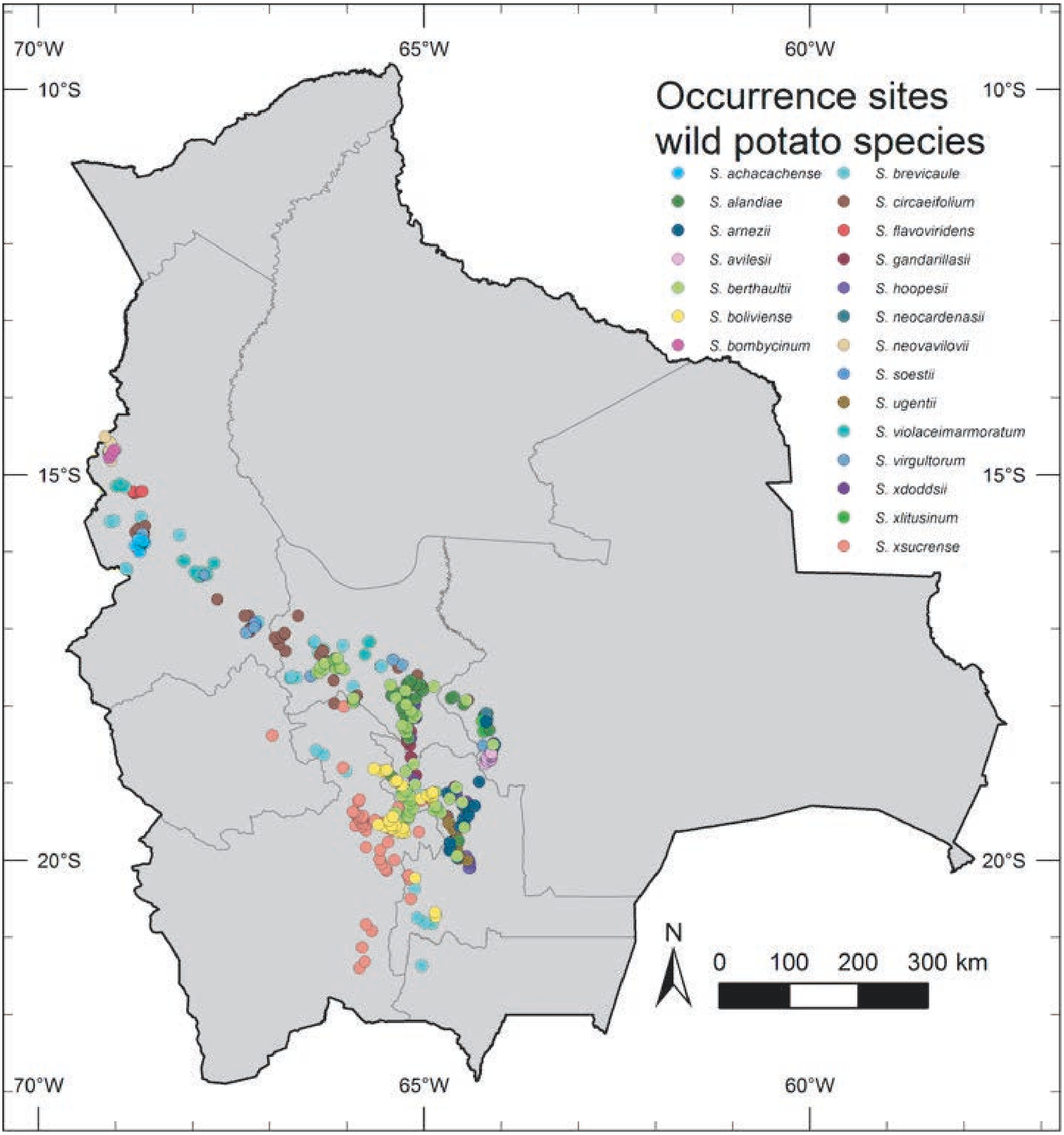


Figure 2. *Observed wild potato species richness with a 5-min resolution grid-cell and 30-min circular neighborhood based on herbarium and genebank records of the 21 endemic wild potato relatives*

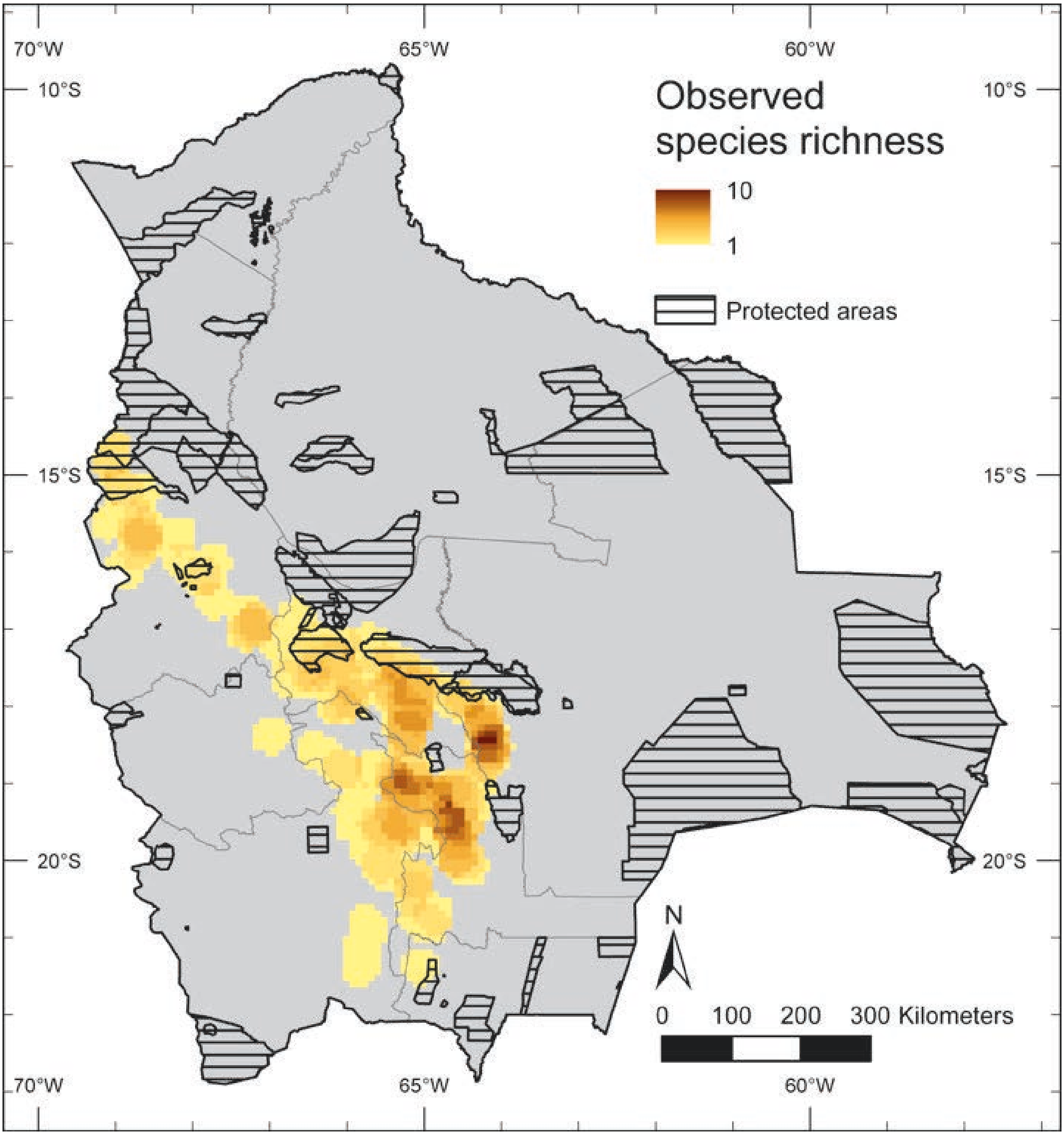
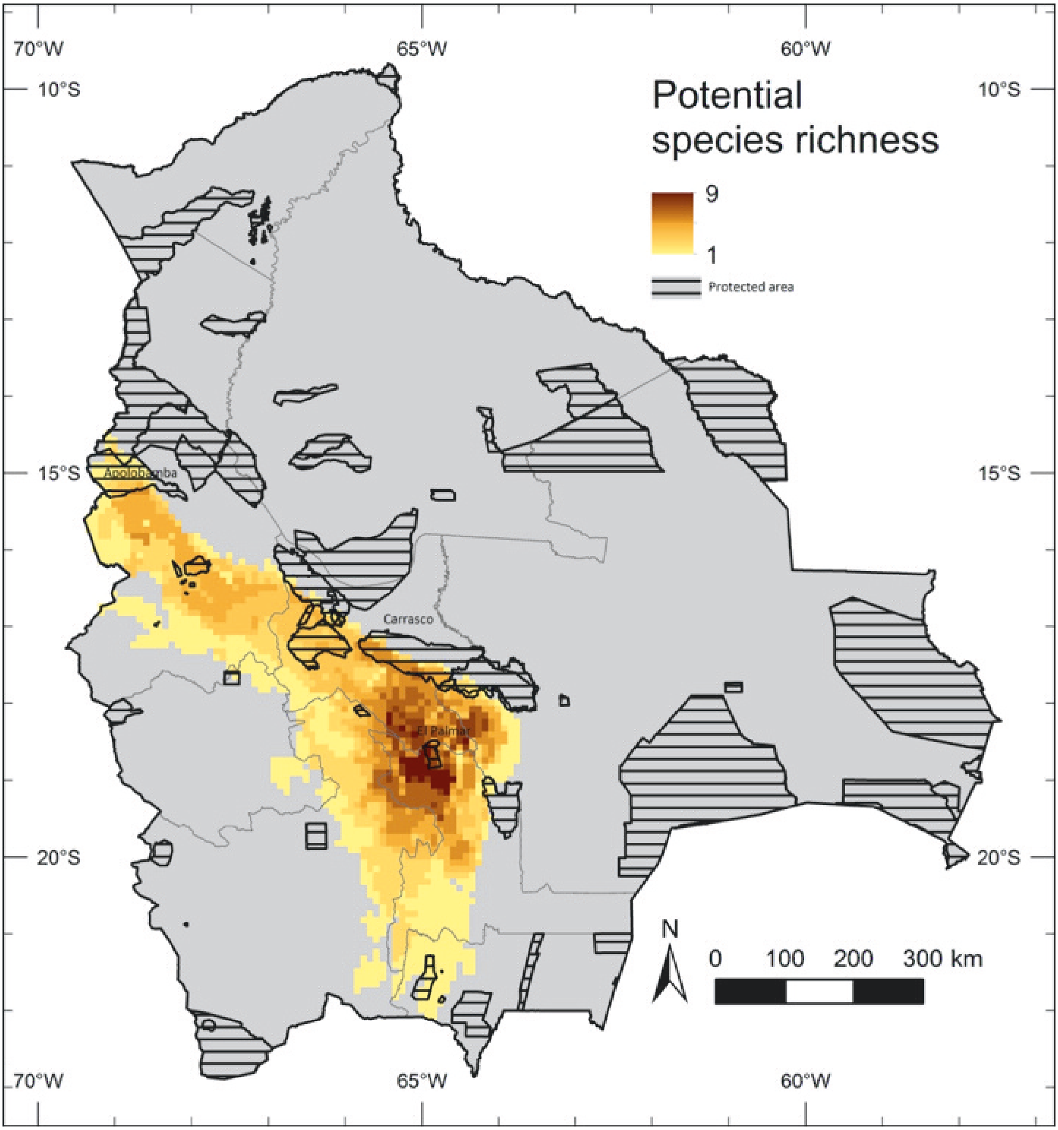


Figure 3. *Potential wild potato species richness with a 5-min resolution grid-cell based on the 21 endemic wild potato relatives using species distribution modelling in Maxent*



Ecogeographic analysis

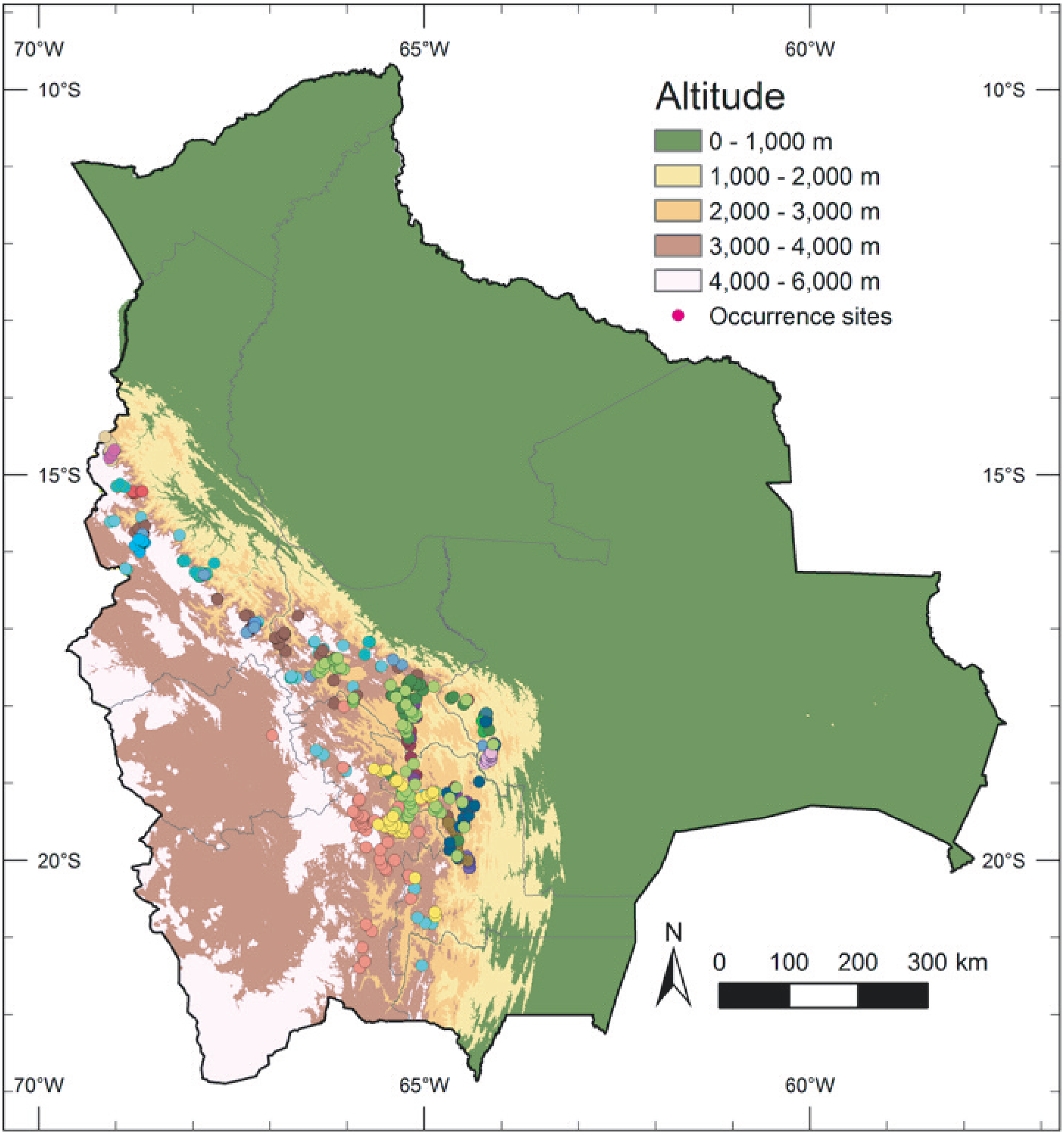
Almost all species have been observed in warm temperature climates with dry winters and warm summers according to our Köppen climate map (Table 4, Cwb). In this climate zone half of all observations have been registered. In general, these areas correspond to inter-Andean valleys and mid-elevation subtropical forests. The second most diverse climate zone is the cold arid steppe climate (Table 4, Bsk). This corresponds to highland grass and shrub vegetation. Material from the hot arid steppe climate zone (Table 4, Bsh) may have interesting breeding traits for climate change adaptation like drought and heat tolerance. This zone is the third-most rich and abundant in endemic wild potato species (Table 4). All species occur above 1,200 m.a.s.l. and it is common to find species above 3,000 m.a.s.l. (Table 4 and Fig. 4). Some species occur even up to elevations above 4000 m.a.s.l. Almost all species occurred in two or more climate zones.

Table 4. *Distribution of occurrence sites of endemic Bolivian wild potato species across Köppen climate zones and the altitudinal range in which they occur*

Species	As[1]	ET[2]	BWk[3]	BSh[4]	BSk[5]	Cfb[6]	Cwb[7]	Cwc[8]	min. alt. (m.a.s.l.)	max. alt. (m.a.s.l.)
S. achacachense		9					1		3745	4165
S. alandiae				9	3	1	21		1633	3377
S. arnezii				5	6		12	23	1738	2771
S. avilesii							19	19	2145	2841
S. berthaultii				16	20		35		1692	3219
S. boliviense					16		17		2869	3732
S. bombycinum		2					1		2610	4643
S. brevicaule		11		1	13		18	4	2152	4315
S. circaeifolium		3			2		36	1	1933	4753
S. × doddsii				5	8		5		1977	2762
S. flavoviridens	2						5	7	1336	2850
S. gandarillasii				19	1		1		1411	2740
S. hoopesii							11		2360	3950
S. × litusinum				2	5		2		1925	3090
S. neocardenasii				13	1				1392	1867
S. neovavilovii		1					16		2444	4155
S. soestii							6	6	2862	3595
S. × sucrense		2	3	1	47		13		2117	4550
S. ugentii							12	12	2700	3950
S. violaceimarmoratum	1					2	18	22	1226	4002
S. virgultorum		2		1			6		1441	4714
Total species richness	2	7	1	10	11	2	20	3		
Total observations	3	30	3	72	122	3	255	6		

[1]As = equatorial savannah with dry summer; [2]ET = tundra climate; [3]BWk = cold desert climate; [4]BSh = hot steppe climate; [5]BSk = cold steppe climate; [6]Cfb = warm temperature climate, fully humid and with warm summer; [7]Cwb = warm temperature climate with dry winter and warm summer; [8]Cwc = warm temperature with dry summer and cool summer.

***Figure 4.** Distribution of endemic wild potato species across altitude ranges*



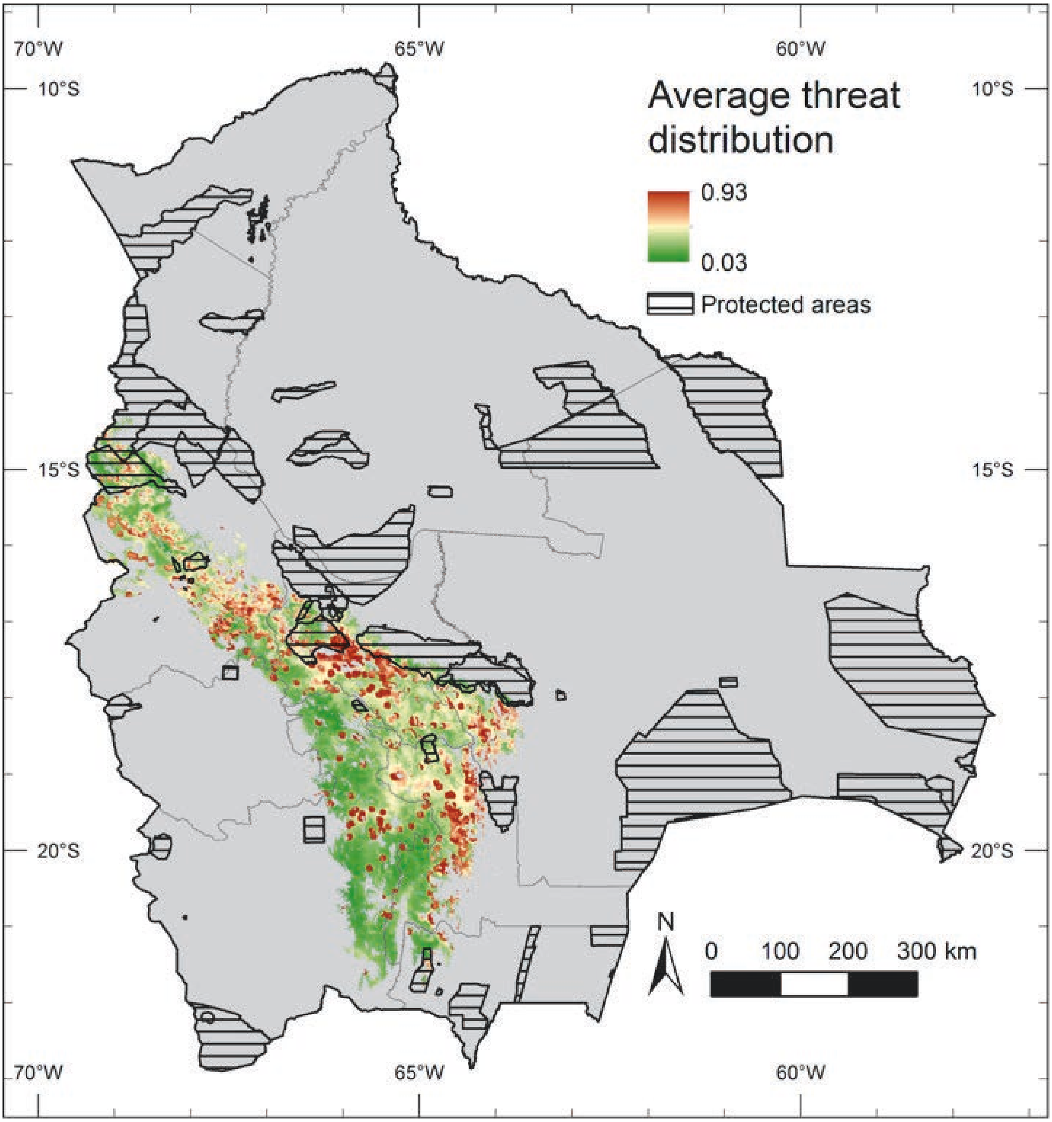
In situ *conservation status and threat assessment*

Our preliminary assessment in IUCN Red List categories shows that 24% (five of the 21 species) of the endemic wild potato relatives is critically endangered (CR), which is due to their restricted observed distribution areas (Table 3). Another 19 % (four of the 21 species) is endangered (EN) according to these parameters, whereas 24 % (five of the 21 species) has a vulnerable status (VU). The remaining seven species are placed in the categories Not Threatened (NT) or Least Concern (LC).

According to our combined threat map (Fig. 5), the areas with highest average threat levels can be found in the western part of Cochabamba, and to a lower degree in northern Chuquisaca and western Santa Cruz where currently the highest numbers of species are observed. The most significant threats for all species considered in this study are accessibility to humans, fire and to a lower degree livestock pressure (Table 3). A substantial part of the protected area "Tunari", where potentially several potato species occur, shows a high level of threat (Fig. 5). The seven most threatened species are *S. achacachense* (EN), *S. arnezii* (VU), *S. brevicaule* (LC), *S. flavoviridens* (CR), *S. hoopesii* (EN), *S. ugentii* (EN) and *S.* ×*sucrence* (NT).

Of these seven species, four have a vulnerable conservation status or worse while another four have only been observed in a low number of locations. *Solanum flavoviridens* has been observed in less than 10 locations and *S. achacachense, S. hoopesii* and *S. ugentii* in less than 15 locations (Table 3).

Figure 5. *Mean threat values (average of human accessibility, conversion to agriculture, fires, livestock pressure, infrastructure, and oil and gas) in a 30-second resolution map across the modelled distribution range of endemic wild potato species in Bolivia*



Prioritization of areas for in situ *conservation*

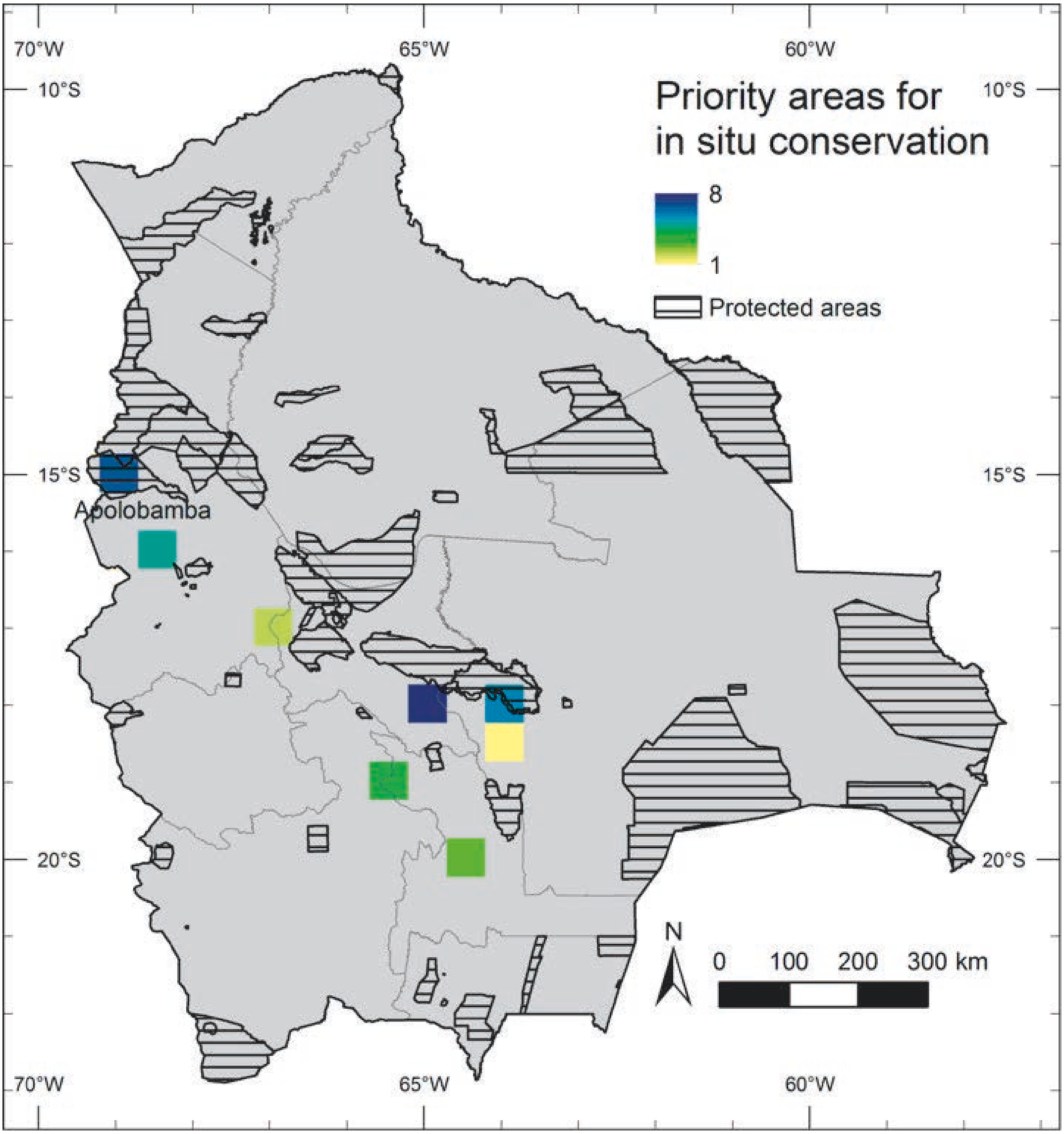
All 21 species can be conserved *in situ* in eight areas of ~50 km² when 25% of the most threatened occurrence sites are not taken in account (Table 5). This is only one area of ~50 km² less than when all occurrence sites are considered, including the most threatened ones.

Table 5. *Results of the reserve selection analysis to prioritize areas for* in situ *conservation*

Methodology	Nr. of cells included	Nr. of species included
All occurrence sites are included in the reserve selection (threats not taken into account)	7	21
25% of the occurrence sites with the highest average overall threat not included in the reserve selection	8	21
Only occurrence sites coinciding with protected areas are included in the reserve selection	3	7

By excluding 25% of the most-threatened occurrence sites, the areas of highest species richness, i.e. northern Chuquisaca and western Santa Cruz, were less pronounced in the reserve selection because large parts of the natural vegetation in those areas are threatened by human accessibility, fires and livestock pressure (Fig. 6). Instead, the area of highest priority is situated in south-eastern Cochabamba, where six species can be conserved *in situ* in an area of ~50 km². The second priority are the northern highlands in western La Paz, where three additional species can be conserved in an area of ~50 km² and which, moreover, is within a protected area (Area Natural de Manejo Integrado de Apolobamba (Fig. 6)). The third priority area for conservation is western Santa Cruz were two additional species could be conserved. The fourth priority area is again located in La Paz. The latter also comprises the only known location of the endangered species *S. achacachense* (Fig. 6 and Appendix D). The endangered and highly threatened species *S. hoopesii* and *S. ugentii* are both located in Chuquisaca (Fig. 6 and Appendix D). When we restricted the reserve selection to only the protected areas, only seven (33%) of the 21 species could be conserved while of the four most endangered species only *S. flavoviridens* was covered (see Appendix D).

Figure 6. *Prioritized areas for* in situ *conservation of 21 endemic wild potato species with the use of the complementary reserve selection and excluding 25 % of the most threatened locations*

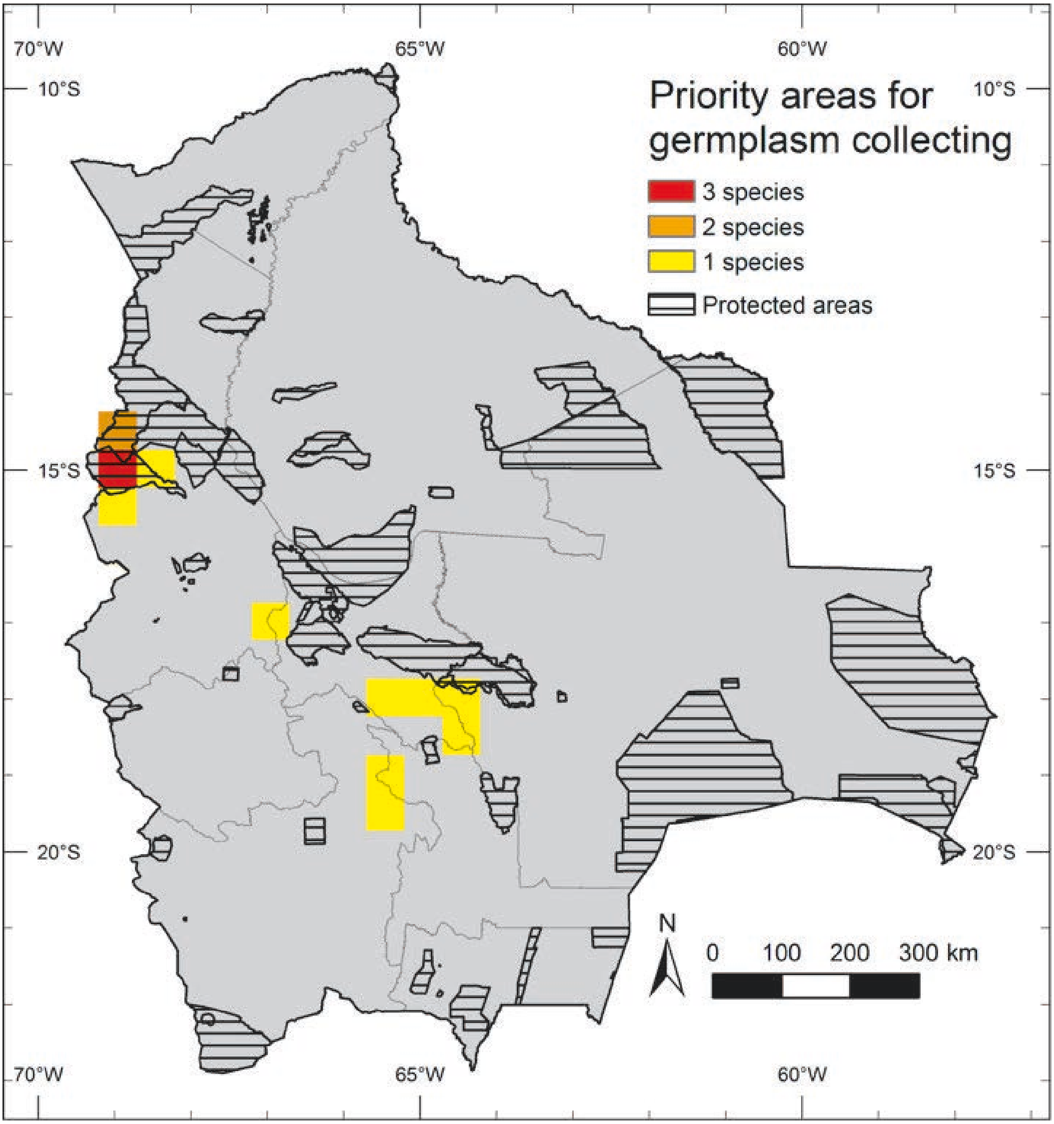


Ex situ *conservation status*

According to data reported in the Global Strategy for the *Ex Situ* Conservation of Potato (GCDT, 2006) updated with data from PROINPA, there are 10 gene banks in the world collectively holding 1062 accessions of 19 out of the 21 wild potato species endemic to Bolivia (Appendix E). The *ex situ* collection of wild potato in Bolivia maintained in the National Genebank of Andean tubers and roots is the result of repatriated materials from the Centre of Genetic Resources the Netherlands (CGN) and new collection trips in recent years. This national collection has currently 235 accessions of 18 endemic species (the total wild potato collection has 618 accessions), 65 of these concern new material collected in the 2006–2010 period.

Some species are well-represented in the gene bank collections, such as *S. berthaultii* which has the largest number of accessions (228), followed by *S. ×sucrence* (195) and *S. boliviense* (141). On the other hand, no germplasm of *S. bombycinum* and *S. ×litusinum* is conserved in any *ex situ* collection. Other species poorly conserved are *S. neovavilovii* (two accessions), *S. soestii* (two) and *S. flavoviridens* (four), and these are present only in the Bolivian collection. The small number of samples for these species in gene banks also coincides with a restricted distribution in the field and limited accessibility of the natural habitats of these species. Prioritized areas for collecting trips are La Paz (Provinces Tamayo and Saavedra) where populations of *S. flavoviridens, S. neovavilovii* and *S. bombycinum* have been observed (Fig. 7). *Solanum soestii* could be explored in La Paz (Province Inquisivi) and Cochabamba (Province Ayopaya). *Solanum ×litusinum* is most likely to occur in the Cochabamba-Santa Cruz border area and at the frontier between Potosi and Chuquisaca (Fig. 7).

Figure 7. *Map with prioritized cells to target germplasm collections of the five potato wild relatives for which no or less than five accessions are currently conserved (*S. bombycinum, S. ×litusinum S. neovavilovii, S. soestii *and* S. flavoviridens*)*



Comparison of conservation priorities of species and putative ecotype diversity

In addition to a reserve selection exercise at the species level, we also carried out a prioritization of areas for conservation considering climate zones. Recorded individuals from a species present in different climate zones possibly represent distinct ecotypes that can be useful in breeding programs for different adaptive traits. In total we identified 56 putative ecotypes, of which 49 can be conserved even when we exclude 25% of the most threatened collection sites (Table 5). These are scattered across the wild potato distribution range in Bolivia and can be conserved in 19 grid cells (Fig. 8).

When we excluded 25% of the most-threatened collection sites, the area with most ecotype diversity coincides with the one of highest species diversity. Other areas of unique putative ecotype diversity also coincide with areas of high species diversity such as the northern highlands in western La Paz. In addition to the previously defined areas for *in situ* conservation, a new prioritized area of unique high putative ecotype diversity is located in eastern Potosí.

Seven of the 56 putative ecotypes occur exclusively in the most-threatened collection sites. These are *S. circaeifolium, S. gandarillasii* and *S. necocardenasii* populations in cold arid steppe climate; *S. virgultorum* and *S.×sucrense* populations in hot arid steppe climate; a *S. neovavilovii* population in tundra climate; and a *S. violaceimarmoratum* population in equatorial savannah environments with dry summers. These seven putative ecotypes were represented by only one occurrence site. In addition to the prioritized species for targeted germplasm collecting, these putative ecotypes should be targeted as well as they are susceptible to *in situ* extinction (Fig. 9).

Figure 8. *Prioritized areas to conserve* in situ *the 56 putative ecotype of the 21 endemic wild potato species with the use of the complementary reserve selection and excluding 25 % of the most threatened locations*

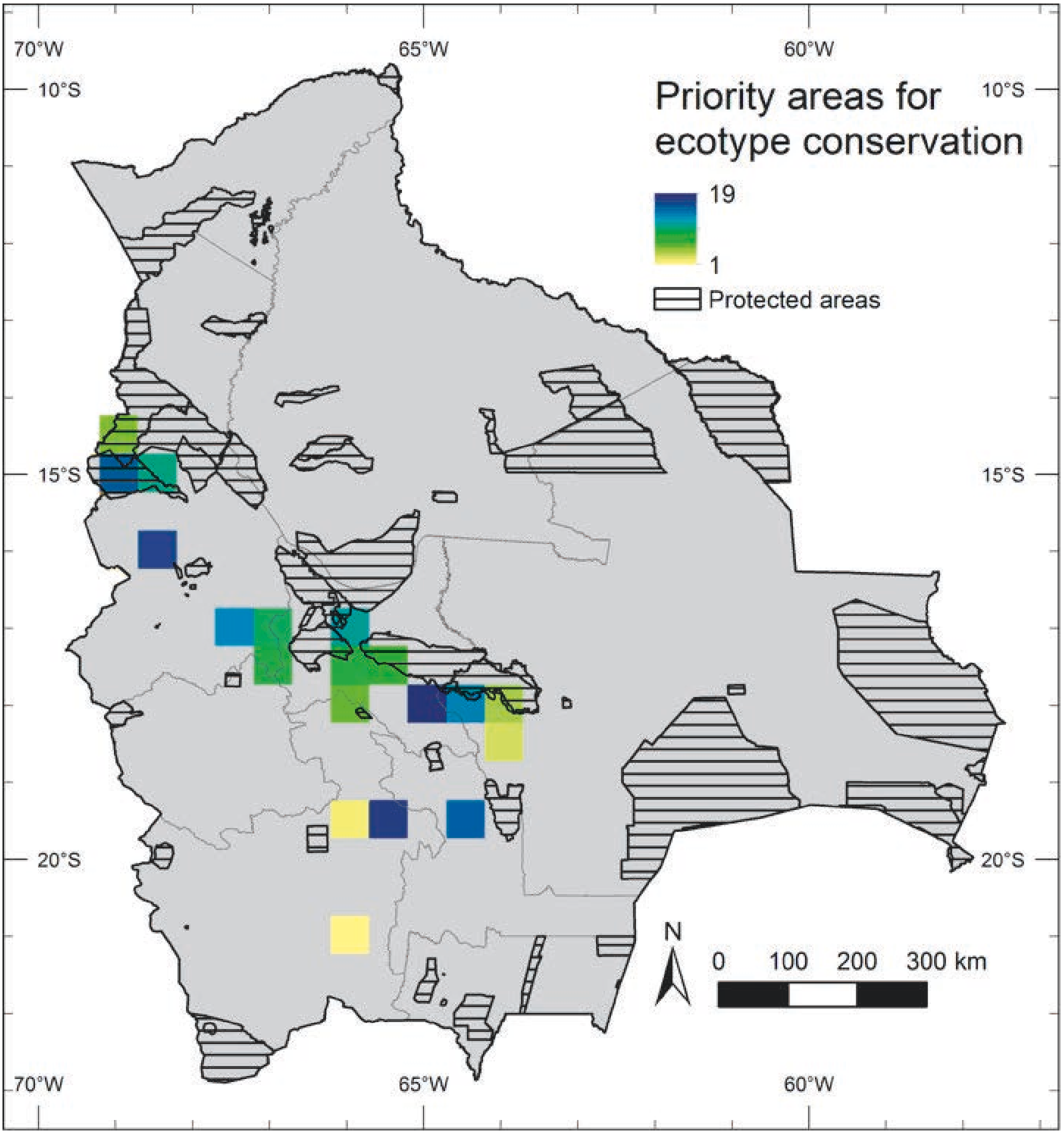
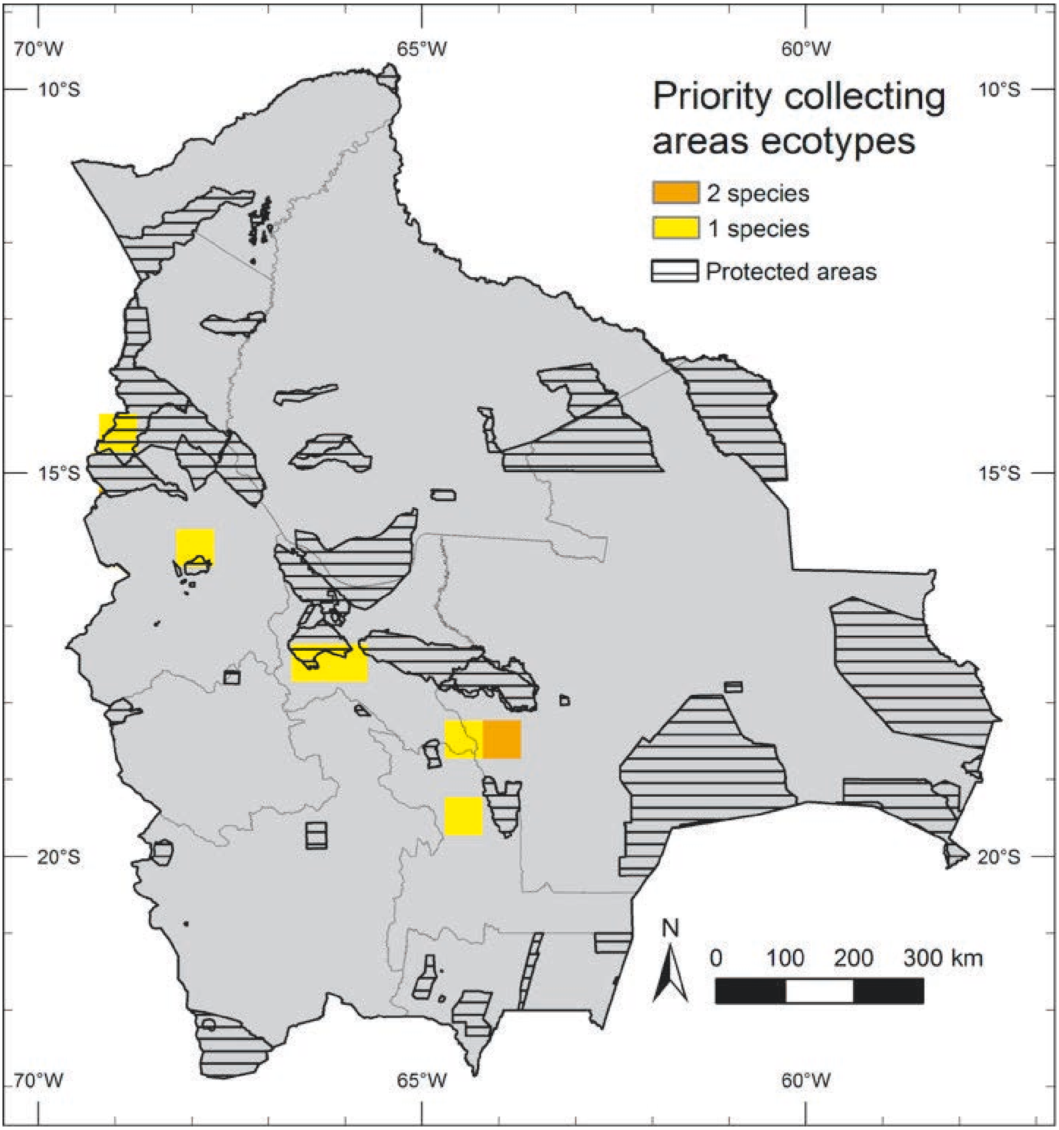


***Figure 9.** Map with prioritized cells to target germplasm collecting trips of the seven putative ecotypes that occur exclusively in the 25 % most threathened collection sites*



Discussion

In this study we followed the classification of Spooner and Salas (2006), which is widely accepted and used in genebanks. Yet new taxonomic studies suggest that several accepted species should in fact be synonymized with other species and that the number of species in the group of wild potatoes should be reduced (http://www.solanaceaesource.org). We expect the results of our study to differ substantially if this new taxonomy was followed. Fewer areas would be required to conserve all species and several of the prioritized rare species would turn out to be co-specific to a well-protected species. However, as long as the taxonomy of wild potato species is not clarified in a satisfactory way, we are obliged to follow the wild potato taxonomy of Spooner and Salas (2006). Molecular characterization studies can help to delineate species and estimate their phylogenetic relationships (González-Orozco *et al.,* 2012).

Fourteen out of the 21 endemic wild potato relatives that we studied have a preliminary vulnerable or worse status of threat according to the IUCN criterion B. Of these, five species are of particular concern for protection because they are facing significant threats, particularly by fire (*S. achacachense, S. arnezii, S. flavoviridens, S. hoopesii* and *S. ugentii*) (Table 3), while they are important as a source of resistance genes to important pests and diseases in cultivated potato (Table 1). *Solanum achacachense* (EN)*, S. flavoviridens* (CR)*, S. hoopesii* (EN) and *S. ugentii* (EN) were only observed in a restricted number of locations (< 15) and therefore be prioritized for conservation.

Among these species, *S. flavoviridens* is underrepresented in genebanks. Fortunately, a considerable number of accessions of the other species is conserved *ex situ*. Occurrence sites of two species that have a broader distribution than the five species mentioned above, i.e. *S. brevicaule* and *S.* ×*sucrense*, are also highly threatened. Although these threats may have a substantial impact on the genetic diversity of the populations of these species, new occurrence sites in less-threatened parts of their distribution range may be identified for their *in situ* conservation. Species distribution modelling will help in identifying those areas.

In addition to *S. flavoviridens*, four other species should be prioritized for targeted collecting trips because they are either not yet conserved in any gene bank (*S. bombycinum, S.* ×*litusinum*) or are underrepresented (*S. neovavilovii, S. soestii*) (Appendix E). The department having the highest priority for such collecting is La Paz (Provinces F. Tamayo and B. Saavedra) within the protected area “Area Natural de Manejo Integrado de Apolobamba” where three of the five species were documented to occur. The areas in the northwest of La Paz are locations of difficult access which may explain the few samples collected in these areas.

The scenario in which we excluded 25% of the most threatened occurrence sites had our preference to prioritize areas for *in situ* conservation. The reduced cost of *in situ* conservation in less-threatened areas may outweigh the cost of implementing conservation measures in an additional area. However, all priority areas identified for conservation are areas where farming is important, except one that lies within the protected area of Apolobamba in northern La Paz. In the case of Santa Cruz, livestock is also important. These areas are not related to any system of conservation or protection, so even while we excluded 25% of the most-threatened occurrence sites, the other locations may still be vulnerable to threats as a result of human activities. For example, although *S. virgultorum* occurrence sites do not have particular high threat values, known populations of these species reported in the past (Ochoa 1990) were not found any more during recent field visits (between 2006 and 2010). Similar indications of decline may even be more pronounced in populations of species that are highly threatened according to our analysis.

Studies on the effectiveness of conservation efforts of vertebrates to reduce their threat level demonstrate a significant contribution of protected areas (Hoffmann *et al.,* 2010). This could be similarly true for higher plants and more specifically for CWRs. In Bolivia, there are 22 protected areas established to protect wild populations of flora and fauna, but none explicitly consider CWRs in their inventories (SERNAP, 2011). According to our study, only one third of the wild potato species endemic to Bolivia (seven species) have been observed to occur within the protected areas. This clearly demonstrates the poor coverage of the actual protected area network in Bolivia in protecting populations of potato wild relatives. The remaining species occur in natural vegetation habitats, sometimes even as weeds in agricultural fields or in road banks, dispersed by human activities. As a consequence, an inventory should be made of protected areas that we modelled as potentially having high species richness but which have not yet been visited for collecting, particularly “El Palmar” at the border of Chuquisaca and Cochabamba (Fig. 3), to get a full understanding as to what extent the existing protected area network in Bolivia can contribute to *in situ* conservation of endemic wild potato diversity. The poor representativeness of wild potatoes in protected areas may also be due to sampling bias. Maximum coverage of such areas precludes roaming far from main roads. Hijmans *et al.,* (2000) mentioned that the main bias in the Bolivian wild potato collection was the infrastructure bias meaning a disproportionate sampling near roads and towns, perhaps due to time limitations, particularly for expeditions in vast, rugged areas such as the Bolivian Andes.

Assisted migration to less-threatened areas, e.g. by translocating plants to existing close-by protected areas, may be an option for *S. achacachense* and *S. flavoviridens*. The concept is still under debate as an adaptive management option particularly for forestry (Ste-Marie *et al.,* 2011; Beardmore and Winder, 2011; Williams and umroese, 2013), but it may be worthwhile to further explore this option with the national government body responsible for the protected areas.

We also observed high threat levels in a few protected areas (Fig. 5). So even within these conservation areas, species may be threatened by human disturbance. On the other hand, national networks of protected areas are the principal measure for *in situ* conservation of biodiversity. This is of great conservation concern. Although the observed *S. flavoviridens* populations occur in a protected area (see Appendix), they are threatened by fire according to our analysis, while also several parts of the protected area 'Tunari' are severely threatened. This protected area is close to some urban populations with people exploiting the natural resources in this area (Valenzuela and Padilla Suarez, 2002).

On-farm conservation may offer an alternative to conserve these species, especially those that grow in disturbed areas. Recently, the UNEP/GEF-supported project "*In situ* conservation of wild crop relatives through enhanced information management and field application" (VMABCC-BIOVERSITY, 2009) worked on raising awareness of indigenous communities and farmers on the importance of building a participatory conservation strategy for CWRs. Guidelines or protocols help raise consciousness and guide farmers in the conservation of CWRs (Dulloo *et al.,* 2010). However, there is an on-going discussion about the feasibility to protect CWRs on-farm, questioning especially how farmers can benefit when these wild relatives may not have direct use (except as genitors in breeding programs), or may even have negative effects on the productivity of their crops through cross-pollination.

Threat assessment is an important step in setting conservation priorities. In this study, we performed such an analysis based on threat maps developed by Jarvis *et al.,* (2010). These maps, however, were made on a continental scale and so may well lose their precision at a local scale. Therefore, our threat analyses should be seen as being exploratory and where relevant, such as in the area of highest threat levels, a local, more-detailed threat analysis should in fact be carried out. In addition to the observed immediate threats, i.e. accessibility and fire, field observations denote livestock pressure as an important threat. This threat has been identified in our analysis as a third threat after accessibility and fire.

Since fire seems to be the most important threat for half of the endemic wild potato species, it would be interesting to investigate the tolerance of these species to fire events. Many plant species have adapted to such conditions (Pekin *et al.,* 2009; Ansley *et al.,* 2010; Segarra-Moragues and Ojeda, 2010) and for some of them fire even favours colonization and regeneration. Hijmans *et al.,* (2002) state that wild potatoes are fire-tolerant, but no further details are provided. It could be that these species can survive fire events as underground tubers and resprout when environmental conditions are more favourable. On the other hand, human-induce fire events can lead to a higher frequency and intensity of fires, causing the degradation of ecosystems adapted to natural fire events (Kessler, 2000). Further ecological research is required to understand the impact of fire on natural wild potato species.

Another serious threat to CWRs is climate change. This study did not include scenarios of climate change in order to obtain expectations about the future distribution of wild potato species in Bolivia, because we aimed at short-term urgent threats. However, earlier studies by Jarvis *et al.,* (2008) estimated potential future changes in the distribution of 108 species of potato as a result of climate change. They used current and projected future climate data for ~2055, and a climate envelope species distribution model. They conclude that climate change will strongly affected all taxa. No less than 7 to 13 species of wild potato were predicted to go extinct (at least one endemic to Bolivia), and their range sizes were reduced by approximately 38–69%. It was also shown that not all species had a predicted reduction in their climatically suitable area under climate change: 21 species of wild relatives of potato gained area assuming unlimited migration, and nine species under a limited migration scenario. These predictions show that the impact of climate change on CWRs can be heterogeneous in space, and may also depend on habitat and migrational capacities of the species. Future climate models need to be applied to local conditions, e.g. specific to Bolivia, ideally coupling those with other parameters such as land-use projection models to predicted future patterns of distribution and habitat fragmentation (Hannah *et al.,* 2002). Such projected impacts may very well have implications on the target areas for *in situ* conservation identified by the present study.

Most collection sites are located in areas of natural vegetation. A possible reason could be that many species do not thrive well in areas disturbed by agriculture. However, Hijmans *et al.,* (2002) state that wild potatoes can grow well in disturbed areas even though they do not explain this in further detail. Another possibility is that there has been a sampling bias towards collecting wild potato species in natural vegetation. It is therefore worthwhile to monitor or set up experiments to determine how well these species may survive disturbance, for example following conversion to agriculture.

This study has identified eight areas where the 21 endemic species could be conserved *in situ*, although our analyses did not take in account the conservation of genetic diversity within species. In general, endemic species have low levels of within-species genetic diversity, whereas comparatively high levels of genetic differentiation can be observed between their populations (Hamrick and Goft, 1996), and such populations are therefore susceptible to inbreeding effects. Consequently, the viability of endemic and narrowly distributed species populations may be more sensitive to fragmentation and habitat reduction compared to more widespread species. We therefore recommend that population genetic studies be carried out on these wild potato species.

On the other hand, species with a larger distribution area may consist of several ecotypes that are adapted to different environmental conditions across the species distribution range. In that case, different ecotypes should be conserved to capture as much of the within-species genetic diversity as possible. In our study, we found that most wild endemic potato species occur in different climate zones. We hypothesize that their populations have developed different adaptive traits to be able to survive in each specific environment. Related studies of other wild potato species have shown a wide variation in resistance between accessions of the same species collected in different localities (Ronning *et al.,* 2000; Del Rio *et al.,* 2001).

In general, the areas with high putative ecotype diversity coincided with the eight prioritized areas for species conservation. An additional area with high putative ecotype richness was identified in Potosí, which may be relevant to consider in an *in situ* conservation strategy.

We identified seven putative ecotypes represented by only one occurrence site and which are therefore likely to represent populations in extreme environments with potentially interesting traits. Because this rare material is most susceptible to extinction, we recommend *ex situ* conservation.

To maximize the conservation of wild potato genetic resources one must consider *in situ* and *ex situ* conservation as complementary strategies. Gene banks can facilitate the use of these species in genetic improvement programmes. On the other hand, the management of *ex situ* collections requires appropriate monitoring efforts in order to maintain the genetic integrity of the conserved material, which means the addition of new accessions implies extra costs for storage, regeneration, etc. *Ex situ* conservation is considered a static form of conservation while under *in situ* conditions plant populations can evolve in interaction with their environment, thus ensuring new variation critical for future crop improvement. Therefore, it is widely recognized that populations of many CWRs are best conserved *in situ* (Dullo *et al.,* 2010).

Concluding remarks

So far, no active *in situ* conservation measures have been developed for wild relatives of potato occurring in Bolivia. Our results help to identify and prioritize appropriate areas for such measures. Considering the wide distribution of wild potato species in Bolivia and the often limited resources for germplasm conservation, this study provides guidelines to direct *in situ* conservation efforts to priority areas with a higher concentration of species and with a relatively low level of threat. We prioritized eight areas of about 50 km^2 for *in situ* conservation, but only one is situated in a protected area, i.e. Area Natural de Manejo Integrado de Apolobamba, where three species are known to occur. A high number of wild potato species is predicted to occur in the protected area "El Palmar" in north Chuquisaca. A field inventory should be carried out to assess the number of wild potato species occurring in that area. Sound management strategies need to be developed for prioritized *in situ* conservation areas in order to ensure maintenance of viable populations.

Most Bolivian wild potato species are well represented in 10 gene banks in different countries. However, of the 21 endemic species, three are poorly represented in these gene banks, whereas they hold no samples of two additional species. The protected area "Area Natural de Manejo Integrado de Apolobamba" has the highest priority for additional collecting because three of these five species occur in this park. Other priority areas for targeted collecting missions include La Paz (Province Inquisivi), Cochabamba (Province Ayopaya), the Cochabamba-Santa Cruz and Potosi-Chuquisaca border areas.

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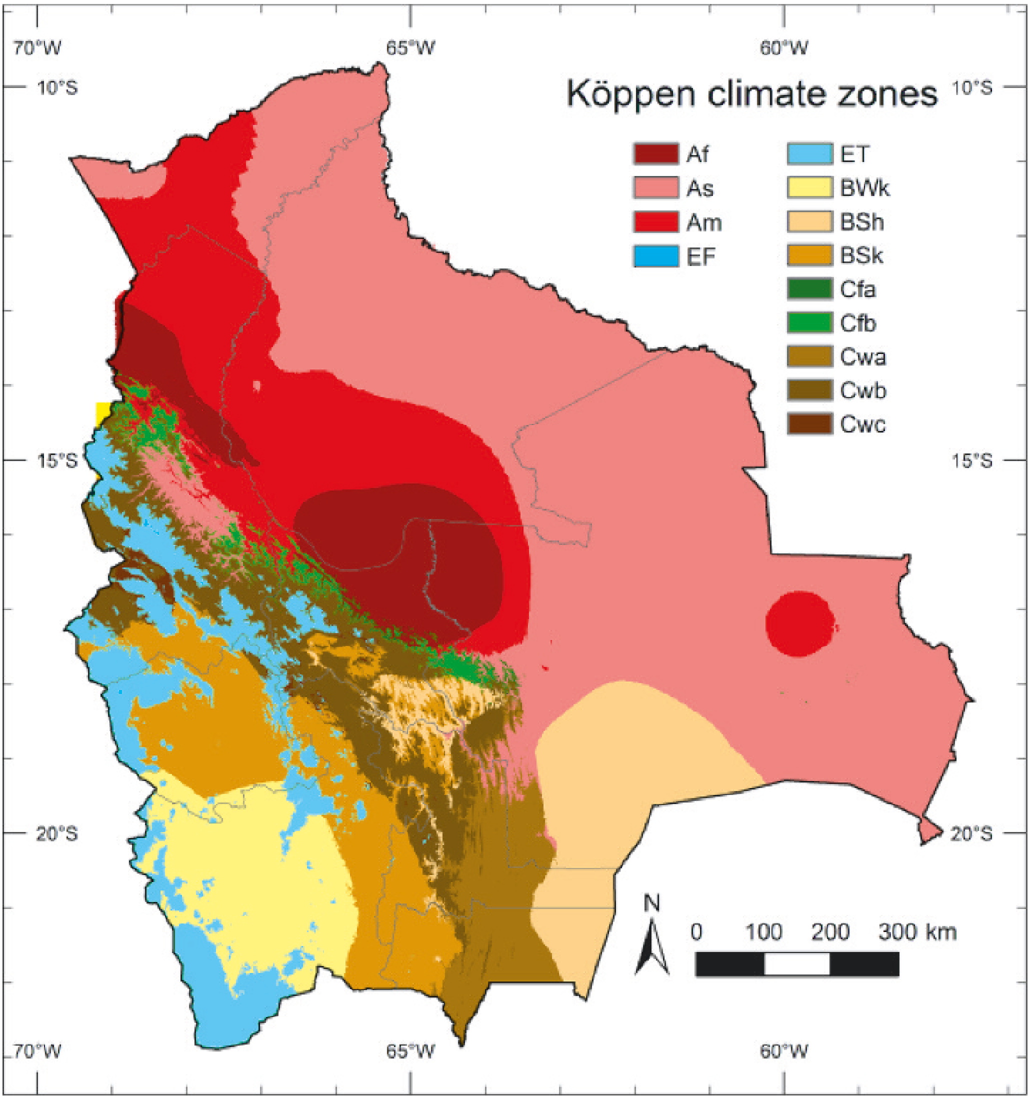
Appendix A. *Differences between two taxonomies for Bolivian wild potato species.*

No.	Wild potato taxa accepted for Bolivia by Spooner and Salas (2006)	Endemic	No.	Wild potato taxa as given by the Solanaceae source website (http://www.solanaceaesource.org)	Endemic
1	*Solanum acaule* Bitter		1	*Solanum acaule* Bitter	
2	*S. achacachense* Cárdenas	*		Synonym of *S. candolleanum* Berthault	
3	*S. alandiae* Cárdenas	*		Synonym of *S. brevicaule* Bitter	
4	*S. arnezii* Cárdenas	*		Synonym of *S. chacoense* Bitter	
5	*S. avilesii* Hawkes and Hjrt.	*		Synonym of *S. brevicaule* Bitter	
6	*S. berthaultii* Hawkes	*	2	*S. berthaultii* Hawkes	
7	*S. boliviense* Dunal	*	3	*S. boliviense* Dunal	
8	**subsp. astleyi (Hawkes and Hjert.) D.M. Spooner, M. Ugarte, and P.M. Skoch***				
9	*S. bombicynum* Ochoa	*	4	*S. bombicynum* Ochoa	*
10	*S. brevicaule* Bitter	*	5	*S. brevicaule* Bitter	
11	*S. candolleanum* Berthault		6	*S. candolleanum* Berthault	
12	*S. chacoense* Bitter		7	*S. chacoense* Bitter	
13	*S. circaeifolium* Bitter	*	8	*S. circaeifolium* Bitter	*
14	var. *capsicibaccatum* (Cárdenas) Ochoa*				
15	*S. ×doddsii* Correl (aln x chc)	*	9	*S. doddsii* Correl	*
16	*S. flavoviridens* Ochoa	*		Awaiting *Solanum* status designation	(*)
17	*S. gandarillasii* Cárdenas	*		Awaiting *Solanum* status designation	(*)
18	*S. hoopesii* Hawkes and K.A. Okada	*		Synonym of *S. brevicaule* Bitter	

No.	Wild potato taxa accepted for Bolivia by Spooner and Salas (2006)	Endemic	No.	Wild potato taxa as given by the Solanaceae source website (http://www.solanaceaesource.org)	Endemic
19	*S. infundibuliforme* Phil.		10	*S. infundibuliforme* Phil	
20	*S. leptophyes* Bitter			Synonym of *S. brevicaule* Bitter	
21	*S.* ×*litusinum* Ochoa (ber x tar)	*		Synonym of *S. berthaultii* Hawkes	
22	*S. megistacrolobum* Bitter			Synonym of *S. boliviense* Dunal	
23	**subsp.** *toralapanum* **(Cárdenas and Hawkes) R.B. Giannattasio and D.M. Spooner**				
24	*S. microdontum* Bitter		11	*S. microdontum* Bitter	
25	var. *montepuncoense* Ochoa (mcd x vio) *				
26	*S. neocardenasii* Hawkes and Hjert.	*	12	*S. neocardenasii* Hawkes and Hjert.	*
27	*S. neovavilovii* Ochoa	*	13	*S. neovavilovii* Ochoa	*
28	*S. okadae* Hawkes and Hjert.		14	*S. okadae* Hawkes and Hjert.	
29	*S. oplocense* Hawkes			Synonym of *S. brevicaule* Bitter	
31	*S. soestii* Hawkes and Hjert.	*		Synonym of *S. circaeifolium* Bitter	
32	*S. sparsipilum* (Bitter) Juz. and Bukasov			Synonym of *S. brevicaule* Bitter	
33	*S.* ×*sucrense* Hawkes (adg x opl)	*		Synonym of *S. brevicaule* Bitter	
34	*S. tarijense* Hawkes			Synonym of *S. berthaultii* Hawkes	
35	*S. ugentii* Hawkes and K.A. Okada	*		Synonym of *S. brevicaule* Bitter	
36	*S. vidaurrei* Cárdenas			Synonym of *S. brevicaule* Bitter	
37	*S. violaceimarmoratum* Bitter	*	15	*S. violaceimarmoratum* Bitter	*
38	*S. virgultorum* (Bitter) Cárdenas and Hawkes	*		Synonym of *S. brevicaule* Bitter	
39	*S. yungasense* Hawkes			Synonym of *S. chacoense* Bitter	

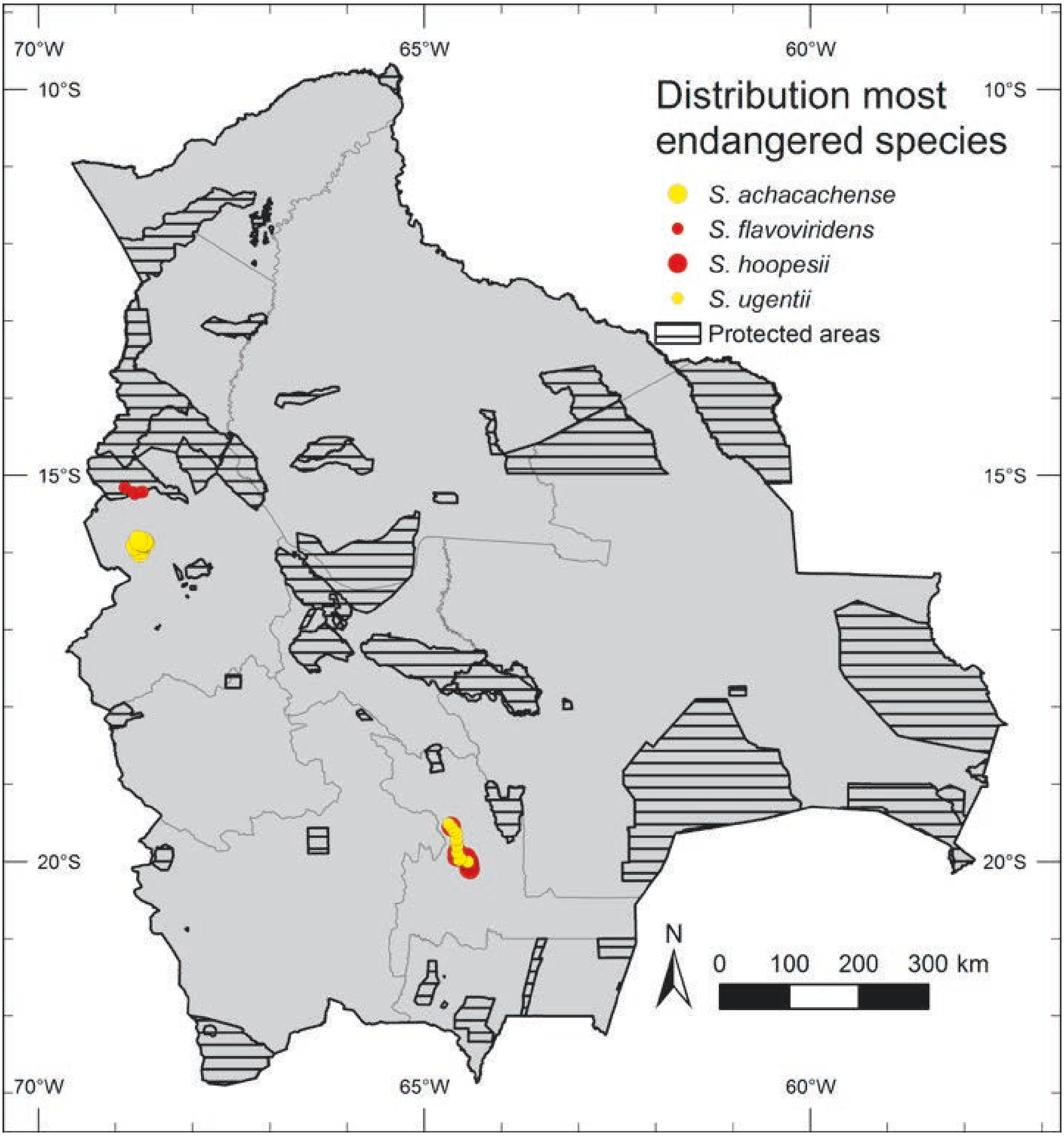
Appendix B. *Köppen climate classification on the basis of the criteria provided by Kottek et al., (2006) and calculated with 30-sec resolution monthly precipitation and mean temperature data from Worldclim. Af = equatorial rainforest, fully humid; As = equatorial savannah with dry summer; Am = equatorial monsoon; EF = tundra climate; ET = frost climate; BWk = cold desert climate; BSh = hot steppe climate; BSk = cold steppe climate; Cfa = warm temperature climate, fully humid and hot summers; Cfb = warm temperature climate, fully humid and warm summer; Cwa = warm temperature climate, dry winter and hot summer; Cwb = warm temperature climate, dry winter and hot summer; Cwc = warm temperature climate, dry winter and cool summer.*



Appendix C. *Summary of criterion B used to assess whether a taxon belongs to an IUCN Red List threat category.*

B. Geographic range in the form of either B1 (extent of ocurrence) AND/OR B2 (area of occupancy)			
	Critically Endangered	Endangered	Vulnerable
B1. Extent of ocurrence (EOO)	< 100 km^2	< 5,000 km^2	< 20,000 km^2
B2. Area of occupancy (AOO)	< 10 km^2	< 500 km^2	< 2,000 km^2
AND at least 2 of the following 3 conditions:			
Ȁ) ĀĀĀ(-9-5-1:Ā2537-,Ā&'Ā%82*-5Ā4.Ā14)70436Ā	!#Ā	;Ā$Ā	;Ā#"
(b) Continuing decline observed, estimated, inferred or projected in any of: (i) extent of ocurrence; (ii) area of occupancy; (iii) area, extent and/or quality of habitat; (iv) number of locations or subpopulations; (v) number of mature individuals			
(c) Extreme fluctuations in any of: (i) extent of ocurrence; (ii) area of occupancy; (iii) number of locations or subpopulations; (iv) number of mature individuals			

Appendix D. *Distribution of most endangered wild potato species.*



Appendix E. *Number of accessions per endemic wild species conserved ex situ in genebanks according to the potato germplasm conservation strategy (GCDT, 2006) and updated with new accessions collected by PROINPA.*

Species	INTA	BOL	CIP	PI	CGN	CPC	IPK	VIR	POL	CZE	Sum
S. achacachense		4		1	4		1				10
S. alandiae		20	15	17	13	2	8	6			81
S. arnezii		7		6	2		4				19
S. avilesii		17	3	3	3		3	5			34
S. berthaultii	1	31	33	62	34	12	12	41	1	1	228
S. boliviense	13	23	10	25	25	6	14	25			141
S. bombycinum		0									0
S. brevicaule	1	15	9	27	14	2	5	15			88
S. circaeifolium		20	9	15	16	3	11	7			81
S. ×doddsii		2	2	13	3	2	4	5			31
S. flavoviridens		4									4
S. gandarillasii		11	1	7	3	3	5	6			36
S. hoopesii		9	2	8	4		2				25
S. ×litusinum		0									0
S. neocardenasii		4	1	2	1	1	2	2			13
S. neovavilovii		2									2
S. soestii		1					1				2
S. ×sucrense		48	20	40	52	10	8	15			193
S. ugentii		3	2	5	3		2				15
S. violaceimarmoratum		8	8	8	5	1	4	7			41
S. virgultorum		6	1		7	1	2	1			18
Total:	**15**	**235**	**116**	**239**	**189**	**43**	**88**	**135**	**1**	**1**	**1062**

Where INTA= Estación Experimental Balcarce -Instituto Nacional de Tecnología Agropecuaria, Argentina; BOL=Bolivian potato collection; CIP=International Potato Centre, Peru; PI= Potato Introduction Project, USA; CGN=Centre for Genetic Resources, Netherlands; CPC=Common Wealth Potato Collection, UK; IPK=Institute of Plant Genetic Resources and Crop Plant Research, Germany; VIR= Vavilov Research Institute of Plant Industry, Russia; POL=Plant Breeding and Acclimatization Institute, Poland; CZE=Potato Research Institute, Czech Republic.

CHAPTER 4

Comparative assessment of the conservation status of wild potato species using two methodologies

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Abstract

The importance of crop wild relatives for the improvement of cultivated species is now broadly recognized at the national and international level. However, in contrast to the *ex situ* conservation efforts, *in situ* conservation measures for wild relatives are still incipient. Since resources for conservation actions are generally limited, it is essential to have sufficient, reliable information and proper arguments to prioritize actions. The current conservation status of Bolivian endemic wild potato species was assessed taking into account information on population, distribution area, habitat quality, estimation of threat, and presence / absence in protected areas. Twelve species were evaluated and categorized as having a high degree of threat (Critically Endangered, Endangered and Vulnerable) according to the IUCN criteria. A methodology with similar aims but applying objective scores to various evaluation criteria was developed within the framework of the UNEP/GEF-Crop Wild Relative Project (CWR Project). Applying this method yielded different results. However, the latter must be considered with care, since the method was developed for forest environments and some of the evaluation criteria and scoring should be revised in order to be more appropriate for potato species. Some considerations to improve the CWR methodology are discussed.

Introduction

Plant species considered to be wild relatives of cultivated plants are important genetic resources because they potentially carry valuable genes for crop improvement. espite their importance, crop wild relatives (CWR) have generally not attained high priority for inclusion in national conservation management plans. Recently however, international programs developed a number of global initiatives aimed at conserving CWR, such as the creation of web-based international platforms for the exchange of CWR information and data. These include the European platform "An Integrated European In Situ Management Work Plan: Implementing Genetic Reserves and On Farm Concepts" (AEGRO) (http://aegro.jki.bund.de/aegro/index.php?id=95 - last accessed July 2013) and the CWR Global Portal (www.cropwildrelatives.org) developed as part of the UNEP/GEF Crop Wild Relative Project, that provides access to CWR information and data at a global level (Dulloo *et al.*, 2010).

At present, in Bolivia, efforts and resources expended in the field of biodiversity conservation do not consider direct actions to conserve CWR. The conservation activities are mainly ecosystem based and intended to protect and manage large geographic areas rather than focus on an individual (group of) plant species, leading to a situation where threatened species are not seldom found outside the protected areas. Inventories of the flora in protected areas do not mention the presence of CWR because these are seldom identified as important species with either biological, ecological, environmental and / or cultural values within such areas (SERNAP, 2011; FAO-VMABC-INIAF, 2009). Recently, the Bolivia component of the UNEP/GEF-Crop Wild Relative Project (CWR Project), has contributed significantly to the generation and collection of information on CWR in Bolivia, and has concluded with a proposal to the Bolivian government on a National Strategy for CWR. This proposal is still being analysed and under negotiation for implementation (VMA-BIOVERSITY, 2010).

Resources to protect biodiversity are always limited and hence the need for sound information to support arguments leading to a well-founded prioritization of the conservation actions. A large number of CWR are endangered, mostly due to the loss of natural habitats (Jarvis *et al.*, 2003) often caused by an intensification of agriculture and/or application of modern agricultural practices (van de Wouw *et al.*, 2009), inadequate management of soil and water, burning and over grazing (FAO, 2010), or the effects of climate change (Jarvis *et al.*, 2008). These threats have profound implications for the *in situ* conservation of natural populations. However, since the collective CWR do not form a uniform group, a case by case analysis of different CWR groups should be considered. The Second Report on the State of the World's Plant Genetic Resources for Food and Agriculture (FAO, 2010) already mentions that, although the country reports cite the loss of local varieties, landraces and CWR, the situation regarding the true extent of genetic erosion is clearly very complex. While some reports have confirmed that in Bolivia diversity in farmers' fields and natural areas has indeed decreased (de Haan *et al.*, 2010; Coca Morante *et al.*, 2007), it is not possible to generalize that situation because other cases found no evidence that such decline has occurred at all (Iriarte *et al.*, 2009; Terrazas and Cadima, 2008; Willemen *et al.*, 2007; Quiros *et al.*, 1992).

The Global Strategy for Plant Conservation (CB , 2010) mentions the evaluation of the conservation status of individual plant species as a specific target, since it is an essential step prior to the development of protection strategies. This evaluation can be performed by determining the species-specific degree of threat using the categories and criteria of the IUCN (2012), and through complementary national / regional assessments of present conservation status, enabling the prioritization of plant species to be protected. The CWR project mentioned earlier, contributed to the development of the red book of the Bolivian CWR based on IUCN assessments. The book contains information of 152 species of different genera, 11 of the 12 wild potato species included in the book were categorized with some degree of threat (VMABCC-BIOVERSITY, 2009).

Methods developed to assess the state of conservation and to detect changes or ecosystem degradation often relate to forests and assume that in the field changes are difficult to detect and need the use of remote sensing (satellite image analysis and videography) (Hill and Suarez, 2010; Woodall *et al.*, 2010; Pinto, 2006; Steininger *et al.*, 2001). Therefore, complementary methods have been suggested focusing on measurable indicators and field observations such as changes in the composition and number of species, changes in forest structure, and key human impacts such as fire, overgrazing, recessed forest, selective logging and others (Navarro *et al.*, 2008; Stoll, 2007; Stork *et al.*, 1997).

The CWR Project designed a methodology to assess the current state of conservation for CWR based on the experience with forest environments. This methodology was designed for use in a wide range of CWR including herbaceous, shrub and tree species. In order to increase the efficiency of assessment and conservation efforts, specific criteria to *a priori* identify the priority CWR were developed. These criteria include: 1. egree of relatedness with the cultivated species (or cultigen), 2. Current and potential uses, 3. Endemism level, 4. egree of threat, and 5. Available information on the species. After the selection of priority species, the conservation status of each should be assessed. For this, the most appropriate and important criteria were selected, which are: 1. Population size and pattern, 2. istribution area, 3. Habitat quality, 4. Estimation of threat, and 5. Presence / absence in protected areas (VMA-BIOVERSITY, 2010).

The methodology designed by the CWR Project is a simple process of assessment to estimate and qualify the current conservation status and condition of CWR taking into account factors or circumstances that may affect their future occurrence. In order to assess the practicability of this tool and compare it with the globally accepted Red List methodology developed by the

IUCN, we applied both approaches to evaluate the conservation status of *in situ* conservation of wild potato species endemic to Bolivia.

Methodology

Two methodologies were used to compare evaluation results on the threat status of Bolivian wild potato relatives: the standard methodology applying the categories and criteria of the IUCN Red List (IUCN Standards and Petitions Subcommittee, 2014; IUCN Species Survival Commission, 2012; IUCN, 2009) and a second approach using the criteria developed by the CWR Project (VMA-BIOVERSITY, 2010). The 21 wild endemic potato species belonging to the section *Petota* within the genus *Solanum* and occurring in Bolivia (Spooner and Salas, 2006, Table 1) were subject to be assessed using both methodologies.

IUCN Red List Categories and Criteria

The IUCN has defined nine categories into which every taxon in the world (excluding micro-organisms) can be classified (IUCN Species Survival Commission, 2012). **Extinct** means that there is no reasonable doubt that the last individual has died. **Extinct in the Wild** means that the taxon is extinct in its natural habitat but survives *ex situ*. The following three categories, **Critically Endangered, Endangered** and **Vulnerable**, are assigned to taxa on the basis of quantitative criteria that are designed to reflect varying degrees of threat of extinction or serious decline. The category **Near Threatened** is applied to taxa that do not qualify as threatened now, but may easily become threatened when a specific condition changes. The category **Least Concern** is applied to taxa that do not qualify as threatened or near threatened. The remaining two categories do not reflect a threat status: **Data Deficient** highlights taxa with insufficient information to make a sound status assessment while the category **Not Evaluated** applies to taxa that have not yet been evaluated against the Red List Criteria (IUCN Species Survival Commission, 2012).

IUCN developed five quantitative criteria which are used to determine whether a taxon is threatened or not, and if threatened, to which category of threat it belongs (Critically Endangered, Endangered or Vulnerable). These criteria relate to biological features of the populations, such as rapid population decline or very small population size. Most of the criteria also include subcriteria that must be used to justify more specifically the listing of a taxon under a particular category. The five criteria are: **A**. Population size (past, present and/or projected), **B.** Geographic range (size, fragmentation, number of locations, decline or fluctuations), **C**. Small population size and decline (number of individuals), or fluctuations, **D**. Very small population or very restricted distribution (number of individuals, area of occupancy), and **E**. Quantitative analysis of extinction risk. To list a particular taxon in any of the categories of threat, the specific conditions defined within **only one** of the criteria, A, B, C, D, or E needs to be met (IUCN, 2009). In this study, we used criterion B (Fig. 1), because it has been designed to analyze populations based on distribution data only, possibly supplemented by data on extreme fluctuations (at present or in the near future) (IUCN Standards and Petitions Subcommittee, 2014).

Presence data was obtained using georeferenced passport data from existing gene bank databases, georeferenced herbarium records developed by Hawkes and Hjerting (1989), Ochoa (1990) and Hijmans and Spooner (2001), data from the Global Biodversity Information Facility (GBIF), and presence points obtained from recent collecting missions in the period 2006–2010. uplicates and inconsistencies were removed as described in Cadima *et al*., (2014) to obtain a final updated dataset of 21 endemic wild potato species in Bolivia. For each species we calculated the geographic range in the form of Extent of Occurrence (EOO, under criterion B1) and Area of Occupancy (AOO, under criterion B2), using ArcView 3.2 with the CATS tool (Willis *et al.*, 2003; Moat, 2007).

EOO is defined as the area within the shortest boundary that encompasses all occurrence sites. In this study we use the convex hull that encompasses all points which is taken as a measure of the distribution range of a taxon (IUCN Species Survival Commission, 2012). AOO is a parameter that estimates the area of suitable habitat for species occurrence within its EOO (IUCN Species Survival Commission, 2012). This is calculated as the area of all grid cells in which the species is present (IUCN Species Survival Commission, 2012). The size of the grid cells can be fixed to a certain value, or calculated as a function of the extent of the distribution range (IUCN Species Survival Commission, 2012; Moat, 2007). For each species, we defined the grid cell size by taking the 10% of the maximum geographic distance between two occurrences, following Willis et al. (2003). The relation between the EOO and AOO value gives information about the amount of suitable habitat available as well as about the scattering of the populations and hence their rarity.

The taxon must then meet the criteria as formulated under B1 and/or B2 and at least two of three other options listed for criterion B to qualify for the Critically Endangered, Endangered or Vulnerable conservation status (IUCN Species Survival Commission, 2012) (Fig. 1). The selection of the options was based on field observations (obtained during collecting missions) and number of locations according to collected points.

In this study, using all available additional data, we assess the IUCN threat status of the 21 endemic wild potato species and compare them to those published in the red book of Bolivian CWRs (VMABCC-BIOVERSITY, 2009).

Figure 1. *Summary of criterion B used to evaluate if a taxon belongs in an IUCN Red List threatened category*

B. Geographic range in the form of elther B1 (extent of ocurrence) AND/OR B2 (area of occupancy)			
	Critically Endangered	Endangered	Vulnerable
B1. Extent of ocurrence (EOO)	< 100 km^2	< 5,000 km^2	< 20,000 km^2
B2. Area of occupancy (AOO)	< 10 km^2	< 500 km^2	< 2,000 km^2
AND at least 2 of the following 3 conditions:			
À) ĀĀĀ(-9-5-1:Ā2537-,Ā&'Ā%82*-5Ā4.Ā1470436Ā	!#Ā	;Ā$Ā	;Ā#"
(b) Continuing decline observed, estimated, inferred or projected in any of: (i) extent of ocurrence; (ii) area of occupancy; (iii) area, extent and/or quality of habitat; (iv) number of locations or subpopulations; (v) number of mature individuals			
(c) Extreme fluctuations in any of: (i) extent of ocurrence; (ii) area of occupancy; (iii) number of locations or subpopulations; (iv) number of mature individuals			

Source: IUCN Species Survival Commission (2012)

CWR Project criteria

The CWR approach consists of two steps: first, to improve efficiency, five criteria are applied to select the species that should be assessed, then five further criteria are applied to assess the state of *in situ* preservation of those species. This method is presented below.

Selection of species

The five criteria to select the wild potato species to be subjected to evaluation of their *in situ* conservation status were:

1. Degree of relatedness with the cultivated species

Only wild potato species closely related to the crop were selected. The following concepts of this criterion assist in determining how close a wild species is to the cultivated species (Maxted *et al.*, 2006):

i) ***Crop Gene Pool Concept***

etermined by the crossability of the cultivated species with its wild relatives. This recognizes the following genetic sectors:

- Primary Gene Pool (GP 1): within-group crossability is easy using conventional breeding techniques, and the material belongs to the same species, within which GP-1A are the cultivated forms and GP-1B are the wild or weedy forms of the crop.
- Secondary Gene Pool (GP 2): which includes different but closely related species from which gene transfer to the crop is possible; at least some hybrids are produced using conventional breeding techniques.
- Tertiary Gene Pool (GP 3): which includes the species from which gene transfer to the crop is generally impossible unless special techniques, such as embryo rescue, somatic fusion or genetic engineering are applied.

ii) ***Taxon Group Concept***

When there is no information on patterns of genetic diversity and the ability of crossing, the degree of relatedness is determined by establishing the taxonomic hierarchy of species, as follows:

- Taxon Group 1a - crop, cultivated
- Taxon Group 1b - same species as crop, but wild/weedy
- Taxon Group 2 - same series or section as crop
- Taxon Group 3 - same subgenus as crop
- Taxon Group 4 - same genus as crop
- Taxon Group 5 - same tribe but different genus as crop

2. Current and potential uses

This criterion refers to CWR with some identified use that can be exploited as a source of genetic material for improving the crop in attributes like resistance to adverse weather conditions, pests and diseases, or an improved quality of the crop itself.

Wild potato species with identified current or potential use were candidates for the selection and conservation evaluation.

3. Endemism level

This criterion refers to species with a restricted distribution within the geographic boundaries of Bolivia. Only wild potato species endemic to Bolivia were selected for this study.

4. Presence of threat

For reasons of efficiency, only species with some degree of threat were considered. For this, the species categorized as Critically Endangered, Endangered and Vulnerable according the IUCN criteria described in previous paragraphs were subject to an analysis using the CWR approach.

5. Available information

In addition to the above criteria, it is desirable that a maximum amount of information to justify the assessment process is available for each species. Therefore, only wild potato species for which data about the following characteristics was available were selected:

- i) Taxonomy and within-species variation
- ii) Status of conservation or IUCN Red List category.
- iii) Distribution
- iv) Population size
- v) Reproductive biology
- vi) Major threats (past, present and future)
- vii) Uses (including local traditional knowledge)
- viii) Genetic variation
- ix) Present-day conservation, including ex situ conservation
- x) Other relevant information
- xi) Sources of information.

Species that met the five criteria were considered as high priority to follow the process of assessing their state of *in situ* preservation.

Criteria for the evaluation of in situ conservation

1. *Population size and pattern*

The methodology proposes a semi-quantitative estimation of population size according to an estimation of abundance / dominance of individuals of a species across its distribution area, according to the following score:

Estimated percentage of abundance/dominance	Population	Population pattern	Score
76 – 100 %	Population present continuously in most of its known distribution area	Populations or individuals clearly aggregated in colonies	2
51 – 75 %	Population present regularly in its known distribution area	Individuals moderately aggregated or growing in small groups	1,5
26 – 50 %	Population present scattered in its known distribution area	Population with scattered individuals	1
1 – 25 %	Population present in isolation in its known distribution area	Isolated individuals	0,5

2. *Distribution area*

This criterion takes into account that the smaller the distribution area of a species, the more susceptible or vulnerable to threat factors and any changes in its environment it will be. The calculation considers:

- Number of records per species, which should not be less than 10.
- Size of potential distribution area estimated through predictive models using MAXENT (www.cs.princeton.edu/~schapire/maxent/).
- Size of area of occupation (AOO) calculated using ArcView and the CATS tool (Willis *et al*., 2003; Moat, 2007).
- Presence of the species as a percentage calculated as follows:

$$\text{Percentage of Presence of species} = \frac{\text{(Occupation area)}}{\text{(Potential area)}} * 100$$

Using this equation the following score is applied:

Distribution	Score
Species occupies >75% of its potential distribution area	2
Species occupies 51-75% of its potential distribution area	1,5
Species occupies 26-50% of its potential distribution area	1
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3. Habitat quality

The quality and integrity of the habitat of a species can be analyzed visually in the field (sampling sites representative of the habitat of the species) or with the help of satellite images, or also by the qualitative assessment of ecological indicators such as fragmentation, degradation and reduction of habitat where the wild species occur. Fragmentation and reduction of plant cover can be estimated through analysis and visual estimates, overlaying the modeled potential distribution area on satellite images. Based on these analyses, the following assessment can be applied:

Quality of habitat	Ecological indicators	Score
In >76% of the potential distribution area or >76% of points sampled / field areas tested the habitat where the species occurs is in good condition or ecologically suitable	- No evidence of habitat fragmentation - Good structure, vegetation cover closed to semi-closed (some perforated) - The ecosystem is the landscape matrix	2
In 51-75% of the potential area or 51-75% of points sampled/ field areas tested the habitat where the species occurs is in good condition or ecologically suitable	- Some evidences of habitat fragmentation - Moderate structure, vegetation cover is semi-open - The ecosystem is no longer a matrix, but a noticeable element of the landscape	1,5

Quality of habitat	Ecological indicators	Score
In 26-50% of the potential area or 26-50% of points sampled/ field areas tested the habitat where the species occurs is in good condition or ecologically suitable	- Evidently fragmented ecosystem - Low structure, coverage of vegetation in the ecosystem is notoriously open - The ecosystem is being replaced by a mosaic of serial stages and has areas with different types of human use, e.g. crops, roads, urbanization.	1
In < 25% of the potential area or < 25% of points sampled/ field areas tested the habitat where the species occurs is in good condition or ecologically suitable	- Almost total fragmentation - Original ecosystem has been replaced mostly by other vegetation (transformed) - With little or no core habitat of the species.	0,5

4. Estimation of threats

In this methodology, threats are limited to human activities that have a negative impact on the vegetation and affect the normal development of wild populations in their natural habitats, such as:

- Presence or proximity and size of human settlements
- Existence of clearing and burning activities on small or large scale
- Presence or proximity to agricultural and large-scale livestock activities
- Presence of roads or other access routes (paths) across the distribution area
- Presence of industry, mining exploration, pipelines or other comparable disturbances
- Exploitation of other inorganic resources
- Presence of extractive use of native biotic resources, such as logging, harvesting of wildlife
- Erosion or removal of soil incipient or advanced
- Presence of large-scale tourism or recreational activities without adequate control

Threats are considered significant or severe depending on the proximity to the species or populations, and the criterion is applied with the following score:

Threats	Score
<25% of the potential area or <25% of points sampled or field evaluations are severely or significant threatened	2
26-50% of the potential area or 26-50% of points sampled or field evaluations are severely or significant threatened	1,5
51-75% of the potential area or 50-75% of points sampled or field evaluations are severely or significant threatened	1
More than 76% of the potential area or more than 76% of points sampled or field evaluations are severely or significant threatened	0,5

5. Presence/Absence in Protected Areas

The percentage of the potential distribution area within a protected area was calculated in ArcGIS 10 (ESRI, Redlands, California, USA). The protected area layer was derived from the World Database on Protected Areas (WDPA) (UNEP-WCMC, 2010). The results were scored as follows:

Presence in Protected Areas	Score
>76% of the potential distribution area is included within a protected area.	2
51-75% of the potential distribution area is included within a protected area.	1,5
26-50% of the potential distribution area is included within a protected area.	1
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Final estimate of conservation status

The sum of all scores allocated during the evaluation process according to the criteria 1 to 5, is then used to estimate the corresponding conservation status of each species. The qualifications of “very good”, “good”, “regular” and “bad” were applied to a total score of 8-10, 5.5-7.5, 3-5 and 0-2.5, respectively.

Results

Preliminary IUCN conservation status of Bolivian endemic wild potato species

Table 1 shows the results of the evaluation of 21 Bolivian endemic wild potato species applying the IUCN Red List Categories and Criteria. According to this approach twelve species have a tentative designation to an IUCN Red List category of threat following criterion B. The arguments for such designations are described below.

Five species with a tentative Critically Endangered (CR) status:

S. avilesii has a restricted range. It is only known from the Vallegrande province (west Santa Cruz department) in Bolivia at an altitudinal range of 2560 to 3000 m., where the total extent of occurrence (EOO) is approximately 59 km^2 (<100 km^2). Its natural habitat in the Bolivian-Tucuman Biogeographic Province is declining due to rapid expansion of agriculture (mainly for commercial potato culture) and the increasing number of cattle and goats.

S. bombycinum is found in a very small area. It is known from only one location in the Franz Tamayo province (La Paz department). Its area of occupancy (AOO) is only 4.9 km^2 (<10 km^2). Its natural habitat in the Yungas Biogeographic Province is expected to decline because new roads are opening the natural forest favoring human settlements.

S. flavoviridens is found in a restricted area within the boundaries of three provinces in the La Paz department. Its extent of occurrence (EOO) is approximately 67 km^2 (<100 km^2). Plants of this species occur in the Yungas Biogeographic Province in small populations near or even within human settlements and cultivated fields.

S. neocardenasii is registered in a restricted area in three provinces of the Santa Cruz department. Its extent of occurrence (EOO) is approximately 49.7 km^2 (<100 km^2). Its habitat in the Bolivian-Tucuman Biogeographic Province is declining because large natural areas are cleared for intensive vegetable cultivation and grazing cattle and goats. Therefore, natural populations of this species are severely fragmented due to the strong pressure of agriculture.

S. soestii is found in only one location in the Inquisivi Province (La Paz department). Its area of occupancy (AOO) is 6 km^2 (<10 km^2). Its habitat (steep slopes) in the Yungas Biogeographic Province is declining due to an uncontrolled clearing of vegetation for land cultivation, which is producing landslides and destruction of natural populations.

Four species with an Endangered (EN) status:

S. achacachense is found in a restricted area in two provinces in the La Paz department and has an area of occupancy (AOO) of 28.5 km^2 (<500 km^2). Small populations of this species are found forming isolated patches. Overgrazing of sheep and Andean camel (lama) is the main reason for the decline in habitat quality of this species in the Puna Mesophytic Biogeographic Province.

S. oopesii and ***S. ugentii*** are known only from two provinces in the Chuquisaca department and their areas of occupancy (AOO) are 263.5 km^2 and 323.9 km^2 (<500 km^2) respectively. Their habitat at the Bolivian-Tucuman Biogeographic Province is being heavily altered by the clearing of land for cultivation and grazing of sheep and goats.

S. neovavilovii is found only in the Franz Tamayo Province (La Paz department). Its area of occupancy (AOO) is 61.2 km^2 (<500 km^2). Its natural habitat in the Yungas Biogeographic Province is expected to decline because new roads are opening the natural forest favoring human settlements (same habitat as *S. bombycinum*).

Three species with the Vulnerable (VU) status:

S. arnezii is found in five provinces of two departments and has an extent of occurrence (EOO) of approximately 5,488 km^2 (<20.000 km^2). Its habitat in the Bolivian-Tucuman Biogeographic Province is declining due to the presence of voracious goats feeding on all herbaceous and shrub vegetation in the area.

S. boliviense is registered in six provinces of two departments and has an extent of occurrence (EOO) of approximately 10,076 km^2 (<20.000 km^2). Its habitat in the Bolivian-Tucuman Biogeographic Province is declining due to an aggressive expansion of agriculture and pesticide use affecting wild potato populations.

S. gandarillasii is found in seven provinces of three departments and has an extent of occurrence (EOO) of approximately 12,308 km^2 (<20.000 km^2). The habitat of this species was verified in one of the departments (Santa Cruz), observing a continuous decline because many of the natural areas are increasingly used for grazing cattle and goats, which are factors of pressure on wild populations of the species.

Table 1. *Tentative threat status using IUCN categories and conservation status according to the Red Book of crop wild relatives in Bolivia*

Species	AOO (km²)	EOO (km²)	Tentative IUCN threat status **	CWR in Bolivia IUCN threat status***
*Solanum achacachense**	28.5	129.1	EN B2ab(iii)	EN B2ab(iii)
Solanum alandiae	6873.9	20586.1	NT	VU B1ab(i,iii)
*Solanum arnezii**	5123.5	5487.5	VU B1ab(iii)	EN B1ab(iii)
*Solanum avilesii**	38.4	59.0	CR B1ab(iii)	CR B2ab(ii,iii)
Solanum berthaultii	25085.1	36307.0	NT	Not analysed
*Solanum boliviense**	5204.6	10076.2	VU B1ab(iii, iv)	EN B2ab(iii)
*Solanum bombycinum**	4.9	0.3	CR B2ab(iii)	Not analysed
Solanum brevicaule	111659.0	105673.0	LC	Not analysed
Solanum circaeifolium	42094.8	46386.0	NT	VU B1ab(iii)
*Solanum flavoviridens**	38.5	67.0	CR B1ab(iii)	VU B2ab(iii)
*Solanum gandarillasii**	2913.0	12307.6	VU B1ab(iii)	VU B1ab(iii)
*Solanum hoopesii**	263.5	429.9	EN B2 ab(iii)	EN B2ab(iii)
*Solanum neocardenasii**	36.9	49.7	CR B1 ab(iii)	EN B2ab(iii)
*Solanum neovavilovii**	61.2	180.4	EN B2 ab(iii)	Not analysed
*Solanum soestii**	0.8	3.2	CR B2 ab(iii)	Not analysed
*Solanum ugentii**	323.9	401.4	EN B2 ab(iv)	Not analysed
Solanum violaceimarmoratum	8830.2	13701.9	NT	VU B1ab(iii)
Solanum virgultorum	18791.5	25034.7	NT	Not analysed
Solanum ×doddsii	3267.9	11984.6	VU?	Not analysed
Solanum ×litusinum	1662.9	10161.3	VU ?	Not analysed
Solanum ×sucrense	25435.6	48283.7	NT	Not analysed

* Species selected for evaluation of their *in situ* conservation status in this study
**CR: Critically Endangered; EN: Endangered; VU: Vulnerable; LC: Lower Concern; NT: Not threatened
*** VMABCC-BIOVERSITY, (2009)

The extent of occurrence (EOO) of ***S. ×doddsii*** and the area of occupancy (AOO) of ***S. ×litusinum*** were calculated as 11,985 km² (<20.000 km²) and 1,663 km² (<2.000 km²), respectively. Based on these outcomes both species could have a Vulnerable (VU) status, but verification of population size and habitat quality was not possible, so they were not included in the further analyses.

Comparing this IUCN assessment with that reported in the Red Book of wild relatives of Bolivia (VMABCC-BIOVERSITY, 2009), *S. arnezii* and *S. boliviense* would change from Endangered (EN) to Vulnerable (VU), *S. bombycinum* and *S. soestii* from Not Analysed to Critically Endangered (CR), *S. flavoviridens* from Vulnerable (VU) to Critically Endangered (CR), *S. neocardenasii* from Endangered (EN) to Critically Endangered (CR), and *S. neovavilovii* and *S. ugentii* from Not Analysed to Endangered (EN).

In situ conservation status according to CWR approach

The same twelve endemic species that received an IUCN category of threat above, were selected to apply the CWR approach. Belonging to *Solanum* sect. *Petota*, these species are all relatives of the cultivated potato, and according to the crop gene pool concept (Maxted *et al*., 2006), they are in the secondary gene pool (GP 2). Crosses of wild relatives with cultivated species in order to transfer genes into crop are possible in principle, but might require adjustments in breeding techniques (eg. dihaploid production, duplication of chromosomes) (Estrada Ramos, 1984).

The state of knowledge on the 12 species is presented in Table 2 showing extensive information which supports the selection of these species for the CWR approach.

Table 2. *The state of knowledge on 12 wild potato species endemic to Bolivia*

i) Taxonomy **- Scientific name** **- Common/native name**	***S. achacachense*** Wild potato	***S. arnezii*** Wild potato	***S. avilesii*** Wild potato, "papa del zorro"	***S. boliviense*** Wild potato
ii) IUCN conservation status	Endangered (EN)	Vulnerable (VU)	Critically Endangered (CR)	Vulnerable (VU)
iii) Distribution - Information about locality	La Paz (Prov. Larecaja, Omasuyos.) Altitudinal range: 3800 – 4130 m	Chuquisaca (Prov. Tomina, Azurduy, Zudañez), Santa Cruz (Prov. Vallegrande). Altitudinal range: 1700 – 3000 m	Santa Cruz (Prov. Vallegrande). Altitudinal range: 2200-2900 m.	Chuquisaca (Prov. Oropeza, Yamparáez, Nor Cinti), Potosí (Prov. Linares, Chayanta, Saavedra). Altitudinal range: 2770-3520 m.
- Potential distribution map (extend of occurrence)	129.1 km^2	5487.5 km^2	59 km^2	10076 km^2
- Current presence in protected areas	No	No	No	No
iv) Population (trends)	Decline	Stable	Stable	Decline
v) Ecology **- Biogeographic province**	Puna Mesophytic, high-Andean level	Bolivian-Tucumán, montane level	Bolivian-Tucuman, montane level	Bolivian-Tucumán, Pre-puna level
- Characteristics of habitat	Bioclimate orotropical pluviseasonal	Bioclimate mesotropical pluviseasonal	Bioclimate mesotropical pluviseasonal	Bioclimate xeric, mesotropical and lower supratropical

- Ecological data and dominant vegetation	Plants growing in steep cliffs surrounded by rocks.	Plants growing in forests and humid shrublands with *Polylepis neglecta, Polylepis tomentella, Podocarpus parlatorei, Prunus tucumanensis, Myrcianthes callicoma*. It is also found in areas with low forest potential in prepuna with *Prosopis ferox*. In low moisture condition, with *Jacaranda mimosifolia* and *Tipuana tipu.*	Mosaics of scrub and forest grasslands with P*odocarpus parlatorei* and *Myrcianthes callicoma.* It can be found also in transitional zones to heads of valleys with *Jacaranda mimosifolia* and *Tipuana tipu.* It grows in clay soil, between rocks or along roads.	Plants growing in forests of *Polylepis tomentella,* also in areas with *Prosopis ferox*, as well as in shrublands such as *Acacia feddeana* and *Cercidium andicola* accompanied by numerous cacti on stony. Also in open areas near field crops and vegetation replacement such as shrubs and grasses
vi) Reproductive biology	Diploid outcrossing species	Diploid outcrossing species	Diploid outcrossing species	Diploid outcrossing species
vii) Main threats	Overgrazing	Overgrazing of goats, climate variability (droughts)	Expansion agriculture activities	Expansion agriculture activities
viii) Uses (might include traditional knowledge)	Potato breeding for resistance to nematode *Globodera pallida.*	Potato breeding for resistance to *Phytophthora infestans, Erwinia carotovora, Meloidogyne* sp., *Globodera pallida.*	Potato breeding for resistance to *Phytophthora infestans, Erwinia carotovora, Meloidogyne* sp., *Globodera pallida* and insects (*Epitrix cucumeris, Macrosiphum euphorbiae*)	Potato breeding for resistance to *Phytophtora infestans, Synchytrium endobioticum, Erwinia carotovora, Ralstonia solanacearum*; virus (PVY), insects (*Leptinotarsa* sp., *Myzus persicae, Epitrix* spp.), nematodes (*Globodera* spp., *Meloidogyne* spp.); frost.
ix) Other information - Conservation activities (including ex situ)	10 accessions conserved in 4 genebanks (CGN, IPK, PI, Bolivia)	19 accessions conserved in 4 genebanks (CGN, IPK, PI, Bolivia)	34 accessions in 6 genebanks (CGN, IPK, VIR, CIP, PI, Bolivia)	141 accessions in 8 genebanks (Balcarce, CIP, PI, CGN, CPC, IPK, VIR, Bolivia)
x) Sources of information	Hawkes and Hjerting 1989; Ochoa, 1990; Databases of CGN, IPK, PI and Bolivia	Hawkes and Hjerting 1989; Ochoa, 1990; Databases of CGN, IPK, PI and Bolivia	Hawkes and Hjerting 1989; Ochoa, 1990; Databases of CGN, IPK, PI and Bolivia	Hawkes and Hjerting 1989; Ochoa, 1990; Databases of CGN, IPK, PI and Bolivia

i) Taxonomy - Scientific name - Common/native name	***S. bombycinum*** Wil potato	***S. flavoviridens*** Wild potato/ "khipa choque"	***S. gandarillasii*** Wild potato/"alqo papa"	***S. hoopesii*** Wild potato/"papa perdiz"
ii) IUCN conservation status	Critically Endangered (CR)	Vulnerable (VU)	Vulnerable (VU)	Endangered (EN)
iii) Distribution **- Information about locality**	La Paz (Prov. Tamayo). Altitudinal range: 2000-3200 m.	La Paz (Muñecas, Saavedra, Larecaja). Altitudinal range: 1150 - 2800 m.	Santa Cruz (Prov. Vallegrande, Florida), Cochabamba (Prov. Campero, Carrasco), Chuquisaca (Prov. Oropeza). Altitudinal range: 1400-2500 m.	Chuquisaca (Prov. Azurduy, Zudañes). Altitudinal range: 2300-3450 m.
- Potential distribution map (extend of occurrence)	0.3 km^2	67 km^2	12308 km^2	430 km^2
- Current presence in protected areas	Yes	Yes	No	No
iv) Population (trends)	Unknown	Stable	Decline	Stable
v) Ecology **- Biogeographic province**	Yungas, montane level	Yungas, Subandean zone	Bolivian-Tucumán, sub-andean and valley zone	Bolivian-Tucuman, montane zone
- Characteristics of habitat	Bioclimate supratropical and orotropical	Bioclimate thermotropical pluviseasonal	Bioclimate thermotropical pluviseasonal	Bioclimate mesotropical pluviseasonal

- Ecological data and dominant vegetation	In the high-montane level, plants can be found in areas associated in evergreen rainforests with *Polylepis triacontandra, Buddleja montana* and *Styloceras columnare.* In the montane associated with *Cytharexilum laurifolium.*	Plants associated in seasonal evergreen forests with Ceiba Bolivian, *Astronium urundeuva, Juglans Ladenbergia, Bolivian oblongifolia,* in organic soils and also under fruit trees.	Plants associated with scrub and serial forests of *Polylepis tomentella, Jacaranda mimosifolia* and *Tipuana tipu.* It is also common in xeric forest areas of *Schinopsis haenkeana, Cardenasiodendron brachypterum* and *Neocardenasia herzogiana.* Growing in poor soils, clay and dry	Plants are in the midst of shrubs and forests of *Polylepis neglecta* and *Polylepis tomentella,* also under bushes in forest of *Jacaranda mimosifolia* and *Tipuana tipu.* Grow in well-drained soils high in organic matter
vi) Reproductive biology	Diploid outcrossing species	Diploid outcrossing species	Diploid outcrossing species	Tetraploid outcrossing species
vii) Main threats (past, present and future)	Disturbed natural areas by new open roads	Expansion agriculture activities	Expansion agriculture activities, cattle and goats	Expansion agriculture activities
viii) Uses (might include traditional knowledge)	No reports	Potato breeding for resistance to insects (*Myzus persicae, Leptinotarsa* sp., *Tetranichus* sp., *Empoasca* sp., *Lyriomiza* sp.)	Potato breeding for resistance to drought, *Globodera* spp.	No reports
ix) Other information				
- Conservation activities (including ex situ)	No materials under ex situ conservation	4 accessions collected for ex situ conservation	36 accessions in 7 genebanks (Bolivia, CIP, PI, CGN, CPC, IPK, VIR)	25 accessions in 5 genebank (Bolivia, CIP, PI, CGN, IPK)
x) Sources of information	Databases of CGN, IPK, PI and Bolivia	Ochoa, 1990.	Coleman, 2008; Hawkes and Hjerting 1989; Ochoa 1990; Databases of CGN, IPK, PI and Bolivia	Hawkes and Hjerting 1989; Ochoa, 1990; Databases of CGN, IPK, PI and Bolivia

i) Taxonomy **- Scientific name** **- Common/native name**	***S. neocardenasii*** Wild potato/"atoj papa"	***S. neovavilovii*** Wild potato	***S. soestii*** Wild potato	***S. ugentii*** Wild potato
ii) IUCN conservation status	Critically Endangered (CR)	Endangered (EN)	Critically Endangered (CR)	Endangered (EN)
iii) Distribution **- Information about locality**	Santa Cruz (Prov. Vallegrande, Florida). Altitudinal range: 1400 - 1700 m.	La Paz (Prov. Tamayo). Altitudinal range: 2500-3500 m.	La Paz (Prov. Inquisivi). Altitudinal range: 2700-2900 m.	Chuquisaca (Prov. Azurduy, Zudañes). Altitudinal range: 2500-3750 m.
- Potential distribution map (extend of occurrence)	50 km^2	180 km^2	3 km^2	401 km^2
- Current presence in protected areas	No	Yes	No	No
iv) Population (trends)	Decline	Stable	Decline	Stable
v) Ecology **- Biogeographic province**	Bolivian-Tucumán, valley zone	Yungas, Ceja de monte	Yungas, montane level	Bolivian-Tucuman, montane zone
- Characteristics of habitat	Bioclimate thermotropical pluviseasonal	Bioclimate supratropical	Bioclimate mesotropical pluviseasonal	Bioclimate mesotropical pluviseasonal
- Ecological data and dominant vegetation	Plants growing in xeric forests of *Schinopsis haenkeana, Neocardenasia herzogiana* and *Samaipaticereus corroanus,* also in forests of *Jacaranda mimosifolia* and *Tipuana tipu;* in clay soils, dry and rocky.	Plants associated with evergreen forests, growing in mountain streams, stone walls or over large rocks covered with moss and succulent plants.	Plants grow in evergreen forests, characterized by *Gynoxys asterotrich* and *Polylepis pepei.* Also among herbaceous plants, protected by spiny shrubs and rocks under partial shade of trees.	Plants are under shrubs and forests of *Polylepis neglecta* and *Polylepis tomentella*, also under bushes in forest of *Jacaranda mimosifolia* and *Tipuana tipu*. Grow in well-drained soils high in organic matter and on road borders.

vi) Reproductive biology	Diploid outcrossing species	Diploid outcrossing species	Diploid outcrossing species	Tetraploid outcrossing species
vii) Main threats (past, present and future)	Expansion agriculture activities, cattle and goats	Disturbed natural areas by new open roads	Presence of catlle and landslides	Expansion agriculture activities
viii) Uses (might include traditional knowledge)	Potato breeding for resistance to insects (*Myzus persicae, Macrosiphum euphorbiae, Empoasca favae, Epitrix* cucumeris, Tetranichus urticae), and drought	No reports	Potato breeding for resistance to *Phytophtora infestans, Erwinia carotovora*, nematodes (*Globodera* spp.) and high temperatures	Potato breeding for resistance to *Phytophtora infestans,* nematodes (*Globodera* spp.)
ix) Other information - Conservation activities (including ex situ)	13 accessions in 7 genebanks (Bolivia, CIP, PI, CGN, CPC, IPK, VIR)	2 accessions collected for ex situ conservation	1 accession collected for ex situ conservation	15 accessions in 5 genebank (Bolivia, CIP, PI, CGN, IPK)
x) Sources of information	Hawkes and Hjerting 1989; Ochoa, 1990; Databases of CGN, IPK, PI and Bolivia	Databases of CGN, IPK, PI and Bolivia	Databases of CGN, IPK, PI and Bolivia	Hawkes and Hjerting 1989; Ochoa, 1990; Databases of CGN, IPK, PI and Bolivia

After applying the five evaluation criteria to the 12 selected species, the following observations can be made:

1. *Population size and pattern*

The species *S. achacachense* and *S. bombycinum* present small populations and isolated individuals covering less than 25% of their distribution area. Populations of *S. arnezii, S. boliviense, S. neocardenasii, S. soestii* and *S. ugentii* show scattered individuals covering 26-50% of their distribution area. *S. avilesii, S. flavoviridens, S. gandarillasii, S. hoopesii,* and *S. neovavilovii* present individuals moderately aggregated or growing in small groups covering 51-75% of their current distribution area.

2. *Distribution area*

Table 3 shows the estimated presence of species as percentages of their potential distribution. *S. arnezii* and *S. boliviense* occupy 26-50% of their potential distribution area. *S. achacachense, S. avilesii, S. bombycinum, S. flavoviridens, S. gandarillasii, S. hoopesii, S. neocardenasii, S. neovavilovii, S. soestii, S. ugentii* and *S. violaceimarmoratum* occur in less than 25 % of their potential distribution area.

Table 3. *Percentage of presence of species in potential area and percentage of potential area in protected areas for twelve wild potato species endemic to Bolivia*

Species	% presence of species in potential area	% potential area in protected areas
S. achacachense	1.25	0.00
S.	*arnezii*	30.41
S. avilesii	3.05	0.00
S. boliviense	33.90	0.15
S. bombycinum	0.01	66.13
S. flavoviridens	0.27	41.07
S. gandarillasii	18.71	3.26
S. hoopesii	19.58	0.00
S. neocardenasii	2.19	2.81
S. neovavilovii	0.64	74.11
S. soestii	0.003	0.00
S. ugentii	4.79	0.00

3. Quality of the habitat

Between 26-50% of the occurrences of *S. achacachense, S. boliviense, S. gandarillasii, S. hoopesii, S. neocardenasii* and *S. ugentii* are in a habitat where the ecosystem has an open vegetation coverage, which is being replaced by a mosaic of serial stages (secondary vegetation as a result of ecosystem disturbance) and has areas with different types of human use such as farm land, roads and housing developments. Between 51 – 75 % of occurrences of *S. avilesii, S. arnezii, S. flavoviridens* and *S. soestii* are in a habitat of good condition, although there is evidence of fragmentation and the vegetation cover of the ecosystem is moderately perforated and semi-open (clear). Over 76% of the occurrences of *S. bombycinum* and *S. neovavilovii* are in a habitat with a good structure and the vegetation cover of the ecosystem is closed to semi-closed (only somewhat perforated).

4. Estimation of threats

Between 51 – 75% of the occurrences of *S. achacachense* and *S. boliviense* are in areas severely or significantly threatened due to agricultural and livestock activities. In the case of *S. achacachense*, the main threat is overgrazing and for *S. boliviense* it is the expansion of agriculture and use of pesticides. The situation for *S. arnezii, S. gandarillasii, S. hoopesii, S. neocardenasii,* and *S. ugentii* is that 26-50% of the evaluated areas are severely or significantly threatened, mainly due to expansion of agriculture activities, grazing and fires, the latter particularly in the areas of *S. gandarillasii* and *S. neocardenasii. S. avilesii, S. bombycinum, S. flavoviridens, S. neovavilovii* and *S. soestii* are in areas where less than 25% of the habitat is severely or significant threatened, due to new open roads.

5. Presence/Absence in Protected Areas

The potential distribution areas of *S. achacachense, S. avilesii, S. soestii* and *S. ugentii* are not within any protected area. Less than 25% of the potential distribution area of *S. arnezii*, *S. boliviense*, *S. gandarillasii* and *S. neocardenasii* is included within a protected area. Between 26-50% of the potential distribution area of *S. flavoviridens* is included within a protected area. Only two species, *S. bombycinum* and *S. neovavilovii*, have between 51-75% of their potential distribution area within a protected area (Table 3).

After applying the five evaluation criteria, the species *S. arnezii, S. avilesii, S. bombicynum, S. flavoviridens* and *S. neovavilovii* fall within the ‘good’ status of *in situ* conservation, while *S. achacachense, S. boliviense, S. gandarillasii, S. hoopesii, S. neocardenasii, S. soestii* and *S. ugentii* have a 'regular' status (Table 4).

Table 4. *Assessment of the* in situ *conservation of 12 species of wild potato species endemic to Bolivia following the CWR Project methodology*

Species/Criteria	ach	arz	avl	bol	bmb	flv	gnd	hps	ncd	nvv	sst	ugt
Population	0.5	1	1.5	1	0.5	1.5	1.5	1.5	1	1.5	1	1
Distribution area	0.5	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Quality habitat	1	1.5	1.5	1	2	1.5	1	1	1	2	1.5	1
Threats	1	1.5	2	1	2	2	1.5	1.5	1.5	2	2	1.5
Presence/Absence PA	0	0.5	0	0.5	1.5	1	0.5	0	0.5	1.5	0	0
Total Valuation	3	5.5	5.5	4.5	6.5	6.5	5	4.5	4.5	7.5	5	4
Conservation status	R	G	G	R	G	G	R	R	R	G	R	R

Where species are: ach= *S. achacachense*, arz=*S. arnezii*, avl=*S. avilesii*, bol=*S. boliviense*, bmb=*S. bombycinum*, flv=*S. flavoviridens*, gnd=*S. gandarillasii*, hps=*S. hoopesii*, ncd=*S. neocardenasii*, nvv=*S. neovavilovii*, sst=*S. soestii*, and ugt=*S. ugentii*. PA=protected area.

Conservation status: R=regular, G=good.

Comparison of *in situ* conservation status assessment using the two approaches

According to the IUCN criteria, 12 species are listed in three threatened categories. According to the criteria of CWR seven of these would be categorized as regular, the rest as good.

Five of the species are Critically Endangered (CR) according to the IUCN red list. In contrast, according to the CWR criteria, none of the species is in the lowest category (bad) and three of them are even assessed as having a 'good' conservation status.

Discussion

A conservation status analysis often implies the definition of categories of rare and/or threatened species. The most well-known of these is probably that developed by the IUCN (IUCN Species Survival Commission, 2012), serving as a basis for most of the "Red ata Lists". Other experiences show the application of adapted, more simple and coarse system when not enough ecological or biological knowledge is available (Hawthorne and Abu Juam, 1995; Sosef *et al.*, 2004). In the present study we carried out two different assessments, one developed by IUCN and another developed within the CWR Project, to estimate the state of *in situ* conservation of a group of Bolivian endemic potato wild relatives.

The use of any methodology for evaluating taxa against different criteria is often plagued by considerable uncertainty. Such uncertainty can arise from any one or all of the following four factors: natural variation, vagueness in the terms and definitions used, measurement errors, and lack of sufficient amount of data (IUCN Species Survival Commission, 2012). Natural variability results from the fact that species' life histories and the environments in which they live change over time and space. Semantic uncertainty arises from a lack of sufficient detail in the definition of terms (e.g. the term "colonies" used to describe population pattern). Measurement errors arise from the lack of sufficiently detailed information about the parameters used in the criteria, which may also be due to inaccuracies in estimating the values (IUCN Species Survival Commission, 2012).

We discuss here the implications of the use of either a standard methodology (IUCN) or an adapted one (CWR Project) to potato wild relatives aiming at a critical revision of each step of both methodologies. We also address reasons why the two approaches resulted in a different conservation status in the case of the 12 endemic Bolivian species.

Selection of species

In principle, all wild potato species (belonging to *Solanum* section *Petota*) are candidates to assess their conservation status because they are potentially useful for the genetic improvement of cultivated potato. Some of these characteristics have been identified for most of the studied species (except for *S. bombycinum, S. hoopesii* and *S. neovavilovii*) and have been reported in several studies and databases (Table 2). However, for this study we restricted the assessment to a group of wild potato species considered endemic to Bolivia with sufficient historical information and recent field observations. Based on such data we could establish a global level assessment using the IUCN Red List Categories and Criteria (IUCN Species Survival Commission, 2012).

Twelve out of the original 21 endemic species were endangered or vulnerable according to the IUCN criteria and these were subjected to the CWR approach.

In the CWR approach, endemism is one of the five criteria for the selection of species. But since some endemic species have a wide distribution (several departments and provinces covering hundreds of kilometers) and others are very restricted (only in micro-ecologies) (Guarino *et al.*, 2002; Hawkes and Hjerting, 1989; Ochoa, 1990), this criterion could be improved by avoiding the term 'endemic' but define species with a restricted distribution in terms of a range of square kilometers of the EOO. Special attention for conservation evaluation should be given to the highly restricted species because they are likely to be in a condition more susceptible to threats than others. The term 'endemic' does keep its relevance within the context of the global responsibility of local or national governmental organizations for the survival of species endemic to their territories (Press, Doak & Steinberg, 1996).

Population size

For the *in situ* conservation of genetic resources of CWR, monitoring populations is the first step in order to estimate their levels of genetic diversity, detect changes in such populations and determine further actions leading to the maintenance of diversity and the assurance of the viability of the populations from a demographic, genetic and ecological perspective (Iriondo *et al.*, 2008). What to measure and how to measure are the key questions before starting any monitoring process (Hunter and Heywood, 2011; Elzinga *et al.*, 1998). To monitor vegetation, techniques will vary according to the type of vegetation. For herbs (such as wild potatoes), and shrubs, field techniques to measure population size include qualitative measurements such as presence/absence of the species at a site, and estimates of the population size based on visual evaluation. The key disadvantage of qualitative monitoring is that because of inconsistency among observers, only large changes can be monitored with confidence (Elzinga *et al.*, 1998).

More accurate techniques include quantitative measurements, such as census, sample and demographic monitoring. Counting the number of individuals of tree species in forests or major strata of vegetation in a given area (Stoll, 2007; Stork *et al.*, 1997) is a laborious task, but it is possible and repeatable in successive years. These data can be complemented with satellite images. Such a method may, however, not be appropriate for other situations, for example for species with long-lived seed banks (e.g. tubers and rhizomes), a very short or very long lifespan, an episodic reproduction, multiple stems and a mat-like morphology, or high densities and large populations in heterogeneous habitats (Elzinga *et al.*, 1998; Hunter and Heywood, 2011).

Quantitative parameters could be impractical or almost impossible to obtain in the case of herbaceous species such as wild potatoes. Most species are small plants and grow hidden under trees and shrubs, or in inaccessible places to avoid voracious animals. Also, weather conditions determine the population size: while in dry years populations are very small, in wet years, that favor the growth of hidden seed in the ground, the population of the same species at the same location may be abundant in number of individuals. ifferent authors already stressed that quantitative attributes are not an adequate measure for CWR populations that fluctuate dramatically in numbers from year to year (Iriondo *et al.*, 2008; Elzinga *et al.*, 1998). That is why the criterion A to evaluate if a taxon belongs in an IUCN threatened category is hardly used for this type of vegetation and why population size should be measured over a period of at least 10 years or at least three generations, whichever is longer (IUCN Species Survival Commission, 2012).

Iriondo *et al*. (2008) suggest some alternatives for population monitoring, such as estimating frequency (percentage of plots or sampling units occupied by the target species within a sampled area), density (number of individuals per unit area) and cover (percentage of plot area or sampling unit that falls within the vertical projection of the plants of the target species). When frequency is the parameter used, great changes can be expected from year to year in potato wild species with an annual life form. Frequency values will mainly be affected by changes in spatial distribution. If density is used, then also year to year changes can be expected because density values of many potato species turn out to be affected by changes in environmental conditions (see also chapter 2 “The state of natural populations of Bolivian wild potato species” of this document). If cover is used, then it is affected by changes both in plant density and vigor. Cover values will be greatly affected by annual changes in environmental conditions. On the other hand, some wild potatoes can behave as herbaceous perennials with too many individuals to count. Such species experience fewer spatial changes, and for this kind of situation canopy cover is the most common measure (Iriondo *et al*., 2008).

Thus, the most useful criterion for selecting the appropriate measuring technique will vary by species and situation, including the availability of resources and time (Hunter and Heywood, 2011).

In this study, population measurements were used only in the CWR Project methodology. A semi-quantitative estimate of population size was used with an estimation of abundance / dominance of individuals of a species across its distribution area. Since the assignment to a certain size category is not well described and, moreover, involves two principally unrelated aspects (population size and pattern), this process is prone to misapplication of the evaluation criteria, especially when the distribution area is interpreted as the extent of the population. Notably, the area considered for the estimation of abundance is an area determined by the evaluator within the optimal habitat of the species following Braun-Blanquet (1979), where different population patterns may be present.

The criterion of population size for wild potato in this study is a coarse estimate in a given year and at a certain time during the reproductive cycle of the plants, but even if it were possible to count the number of individuals in a given area, the data would also be questionable because of the large variation from one year to another.

Geographic range and distribution area

Because of the availability of data on presence points, the criterion B or geographic range in the form of either extent of occurrence (EOO) and/or area of occupancy (AOO) was used to assign wild potato species to IUCN threat categories. An individual species/taxon must also meet at least two of three other conditions in order to qualify for the Vulnerable, Endangered or Critically Endangered status (IUCN Species Survival Commission, 2012; Fig, 1). These conditions suggest an intensive monitoring of specific populations, but our field observations in different seasons in the periods 2006–2010 only allowed coarse estimations of the changes in population sizes and habitat quality. The data collected are, therefore, no more than estimates of the condition of wild populations in their natural environment and this leads to an uncertainty in the classification of threatened wild species. However, as the IUCN states, the absence of high-quality data should not deter attempts at applying the IUCN criteria, as methods involving estimation, inference and projection are considered acceptable to assign a threat category (IUCN Species Survival Commission, 2012).

Within the CWR approach, the criterion distribution area considers an estimate of the presence of the species (current occupation) in its potential distribution area as result from a species distribution modeling analysis. The score of this criterion should be taken with caution, because although GIS tools used to model the potential distribution take into account the presumable environmental conditions where the species occur (van Zonneveld *et al.*, 2009; Ferrier and Guisan, 2006; Scheldeman *et al.*, 2008), in some validation studies of modeled areas it was not possible to find species in expected places. For example, the potential distribution of the wild tomato would include Bolivia (modeled with the GIS tools MaxEnt and Bioclim), but field surveys in the potential areas did not locate the species (Patiño, 2011). However, other studies modeling potential distribution areas successfully indicated new occurrences such as for *Passiflora* species in Bolivia (elanoy *et al*., 2006). The usefulness of these tools has not yet been fully validated in the field, and important aspects for modeling such as the quality and number of data points and the consideration of other variables like seed dispersal, topography, species interaction, etc., should be incorporated as well.

A potential distribution model is generally thought to be more accurate when based on more occurrence points. In this study, only 9 of the 12 species had 10 or more points, but the proposed methodology suggests 10 as the minimum number for using in distribution models (VMA-BIOVERSITY, 2010). According to Scheldeman and van Zonneveld (2010) there is no standard in terms of the minimum number of points required, as this will often relate to the nature of the species. Consequently, we included species with less than 10 points, but as a consequence we were not able to assess the reliability of their models.

A modeled potential distribution area can either be an overestimation, because it shows a geographical area with climatic and soil conditions favorable for a species which does not necessarily mean that the species actually occurs in this area, or be an underestimation, because our collection points do not necessarily indicate the entire ecological range of the species (Scheldeman and van Zonneveld, 2010; Raes *et al*. 2009).

Finally, scoring based on the sizes of habitat occupancy is complicated by problems of spatial scale. The finer the scale at which the distributions or habitats of taxa are mapped, the smaller the area will be that they are found to occupy (IUCN Species Survival Commission, 2012). Mapping at finer scales reveals more areas in which the taxon is unrecorded. Conversely, coarse-scale mapping reveals fewer unoccupied areas, resulting in range estimates that are more likely to exceed the thresholds for the threatened categories (IUCN Species Survival Commission, 2012). Also, in species with only few occurrences, it is likely that these do not fully cover the ecological preferences of the species, and hence the modeled area is likely to be smaller than the actual potential one.

Habitat quality

For some species, such as wild potatoes (especially the annual species), that fluctuate dramatically in number of individuals from year to year, habitat monitoring may be more sensitive to detecting undesirable changes than monitoring the plant species directly (Elzinga *et al*., 1998). Because of that, in this study change in habitat quality, rather than change in population size, was deemed an important criterion to evaluate the threat status according to both methodologies.

Some parameters for habitat quality are based on the qualitative assessment of ecological indicators such as fragmentation, degradation and reduction of the original habitat area (Navarro *et al*., 2008). Such measurements are very complex and could even be subjectively interpreted by different researchers, but they are relevant when tree species are the target and support tools such as satellite images can be used, to detect changes in vegetation cover. However, in the case of wild potato species that grow in a variety of environments, from primary or slightly disturbed habitats to fully modified and open habitats such as roadsides and agricultural fields (Hijmans *et al*., 2002; Hawkes and Hjerting, 1989), some ecological indicators described in the CWR Project approach are not the most appropriate to define their habitat quality. For instance, an open vegetation cover is not a negative element for wild potatoes, since a number of species perform well on or are even favored by open ground (Hawkes, 1990).

Threats

In the CWR Project approach, the threat criterion only refers to the present threats observed in field surveys. Threats are understood as all human activities that have negative impact on vegetation and affect the normal development of wild populations in their natural habitats. epending on the number of points recorded for each species in its range, the threats may be misinterpreted. A low number of points collected in an area where threats are not very obvious, may lead to underestimating the magnitude of threat and could be a source of inconsistency and bias.

In general, none of the habitats where wild potato species occur are free from disturbances. Some habitats show greater disturbance than others, but the assessment does not reach 75% with severe threats in any species. This may be due to the ability of wild potatoes to occupy a wide range of habitats (Hijmans *et al*., 2002), making them more resilient than other CWR. It is not the case in for example wild peanut species that can face severe disturbance such as expansion of agricultural areas and construction of roads causing population fragmentation and size reduction or even local extinction (Jarvis *et al*., 2003).

Presence/Absence in protected areas

The criterion of presence/absence in protected areas is not explicitly considered by the IUCN methodology, but is in fact incorporated in the continuous decline condition. It is an important element of the CWR Project approach, where it is seen in relation to the potential distribution area of the species. Seven species have a potential area extending to protected areas, but if one takes into account only the actually registered localities, that number of species is reduced to three. The presence percentages are quite low (less than 4%) for *S. arnezii, S. boliviense, S. gandarillasii* and *S. neocardenasii.* According to their recorded data these species have no current collecting locality in protected areas. Species that were recorded within protected areas are *S. bombycinum, S. neovavilovii,* and *S. flavoviridens*. According to their potential distribution area, the first two could also grow in other areas outside the protected areas (34% and 25%, respectively, of the potential area).

Although the condition of being present in protected areas is a positive aspect for the conservation of a wild species, assigning a positive value based on the potential distribution does not guarantee that the species is actually protected. Moreover, potential distribution calculations are neither a guarantee that the species really occurs in such areas, even when we followed recommendations in order to have strength models using Maxent to avoid overestimation of the modelled distribution ranges as is detailed in Cadima *et al*. (2014). A few occurrence data may be sensitive to over-prediction in Maxent (case of *S. bombycinum, S. flavoviridens* and *S. soestii*), although Maxent may even produce useful models with only 5–10 observations if these species have a narrow distribution (Hernandez *et al.*, 2006).

Final considerations

According to the CWR Project approach the sum of values for all the criteria applied to each of the 12 species identified seven species having a regular conservation status and five that apparently are in a good conservation status. The CWR Project methodology combined criteria of assessing the status of wild populations in the field with the criteria of the IUCN. Species classified with a high degree of threat (Critically Endangered CR, Endangered EN and Vulnerable VU) according to the IUCN categories, were classified differently using the CWR Project criteria. Three species categorized as CR (*S. avilesii, S. bombycinum* and *S. flavoviridens*) showed a good condition according to the CWR Project method. This is probably due to the fact that estimating the threat status using the IUCN guidelines was calculated taking into account the geographic range based on presence data of the species and decline estimations of populations and quality of habitat, whereas in the CWR methodology other data were included such as estimation of population size, indicators of habitat quality, threats. For many habitats the environment (quality) is in good condition and this improves the resilience of the species. However, since some of these CWR Project approach criteria should be revised so as to apply to wild potato habitats, the evaluation results are only an estimate of the actual situation.

Additionally, the rating of the conservation status according to the CWR Project criteria should be modified, since the “bad” category can only be assigned to species that fall in the lowest category in ALL five criteria. We therefore suggest to broaden the “bad” category to values 2.5-4, the “regular” category to 4.5-6, the “good” to 6.5-8, and the “very good” category to values 8.5-10.

Another important aspect that needs consideration, is the taxonomic classification assumed in this study. According to Spooner and Salas (2006) there are 34 species of wild potato in Bolivia, of which 21 are endemic. This is the taxonomic treatment currently in use for the Bolivian potato collection. However, recently a new taxonomic treatment has been published (http://www.solanaceaesource.org) which suggests many species accepted in the first as proper taxa, are in fact to be included in others. For example 11 species that were part of the "brevicaule complex" are now treated as a single species. According to this new classification, the number of accepted species occurring in Bolivia is reduced to 15, with only 6 endemics and an uncertain status for *S. flavoviridens* and *S. gandarillasii*. The species *S. achacachense, S. arnezii, S. avilesii, S. boliviense, S. hoopesii, S. soestii* and *S. ugentii* would no longer be considered endemic and their threat situation at global level would not exist anymore. Therefore the analysis of the *in situ* conservation would yield completely different results. This shows the great influence of taxonomic revisions on a threat status assessment, which in our case may have led to an overestimation of the level of threat that wild potatoes are facing in Bolivia, at least at national level.

The estimated degree of threat assessment of wild potato species with the IUCN criteria and categories should be considered as a first attempt to determine the conservation status of these species. According to this first attempt, species can be prioritized for a second and more thorough evaluation considering additional and more detailed parameters, hopefully allowing the application of IUCN criteria other than criterion B, which is in fact the simplest one.

Elements of the CWR Project methodology could be refined for wild potato species. It is clear that counting or measuring all individuals of a population is not practical, but monitoring some aspects of the habitat or site condition such as potential disturbances, associated vegetation, or site characteristics might serve as indicators of species success. For example, to detect a reduction of herbaceous vegetation in comparison with bushy vegetation in the habitat may indicate that the target population is decreasing. This assumes that the measured change in characteristics of the habitat can be directly related to population change (Iriondo et al., 2008). Other indicators could be species composition identifying typical, keystone or indicator species, health and viability of the population, richness or diversity, plant community patterns, and ground-, shrub- and tree-layer topography (Hunter and Heywood, 2011).

Within the CWR Project method, a distinction should be made between the presence of an actual, ongoing threat or a potential future threat. Species or populations with actual threats would rank higher than those with potential threats.

Obviously, it is important to have accurate results and data of the highest possible quality, but this depends, to a large degree, on the timing and frequency of the monitoring. Monitoring depends in turn partly on the life history of the plant, its phenology, its growth form and the season when it is most easily measured (Hunter and Heywood, 2011).

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CHAPTER 5

Genetic change during *ex situ* conservation of wild potato species from Bolivia and a comparison with re-collected *in situ* populations

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Abstract

Potato wild relatives are important sources of novel variation for the genetic improvement of the cultivated potato. Consequently, many populations were collected and deposited as accessions in gene banks around the world. Part of the accessions originating from the Bolivian Andes is maintained in the gene bank of the Centre for Genetic Resources, the Netherlands (CGN), and in 2004 this collection was repatriated to the National Gene bank of Andean Tubers and Roots in Bolivia. Here we investigate how the genetic variation of the received material compares to that of the originally collected material, and how this relates to the diversity maintained under *in situ* conditions. To answer these questions material from seven selected species was screened using microsatellite markers. Genetic changes between different generations of *ex situ* germplasm were not observed for the species *Solanum leptophyes* and *S. megistacrolobum*, but were detected for *S. neocardenasii* and *S. okadae*, and partially for *S. acaule, S. avilesii* and *S. berthaultii*. Potential causes of these changes include genetic drift and contamination resulting from human error during regeneration. Re-collected populations of six of the studied species showed highly significant genetic differences with the *ex situ* accessions due to changes during *ex situ* maintenance, sampling effects during collecting and *in situ* genetic change over time. The implications of the results for the maintenance of wild potato species in *ex situ* collections and for the development of *in situ* conservation policies are discussed.

Introduction

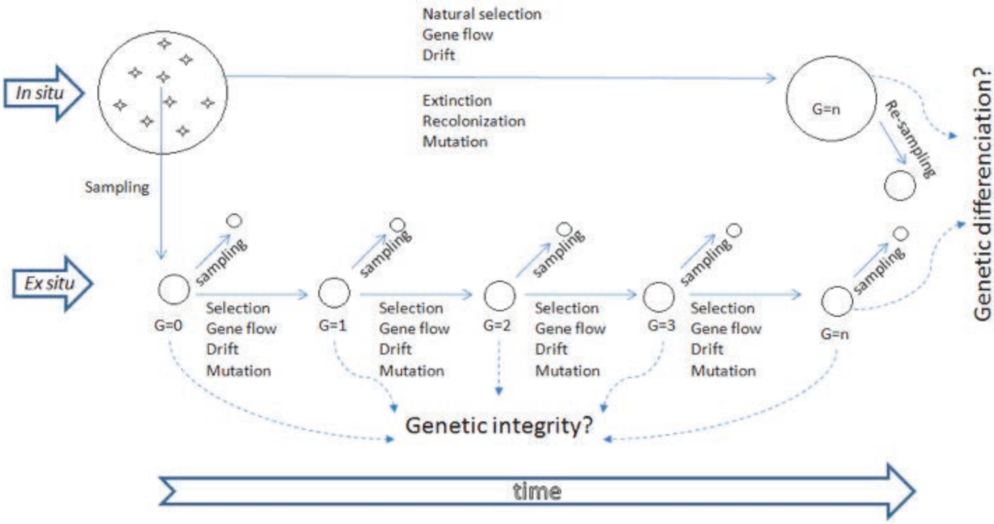
The wild relatives of potato contain a large genetic diversity and hence are an important reservoir of genetic resources for crop improvement. Wild species have been used, in particular, as sources of resistance to pests and diseases caused by bacteria, fungi, insects, nematodes and viruses, and to abiotic stress such as drought and frost (Gabriel, 2010; Jacobs *et al.*, 2010; Spooner *et al.*, 2009; Coleman, 2008; Jansky *et al.*, 2008; Gabriel *et al.*, 2007; Bradshaw *et al.*, 2006; Bradshaw and Ramsay, 2005; Hijmans *et al.*, 2003; Estrada Ramos, 2000, 1984).

Wild tuber-bearing *Solanum* species are found in the Americas from the southwestern United States to central Argentina and Chile (Hijmans and Spooner, 2001; Hijmans *et al.*, 2002). Potato genetic resources have been collected extensively in the past and these collections are conserved worldwide in at least 23 gene banks (GCDT, 2006). Due to taxonomic reconsiderations, the number of species has been gradually reduced from 232 (Hawkes, 1990) to 188 (Spooner and Salas, 2006) to around 100 (http://www.solanaceaesource.org). However, the resulting synonymy has not yet been fully implemented by all gene banks. The Centre for Genetic Resources, the Netherlands (CGN) maintains a potato collection of 2,700 accessions comprising 127 accepted species names (CGN, 2014). Part of this potato collection was originally collected in Bolivia. The earliest Bolivian material mentioned in the CGN database was collected in 1941, while the most recent collections were added in 2004. The total collection of Bolivian wild potato material was repatriated in 2004 to join the potato collection of the National Gene bank of Andean Tubers and Roots.

Gene banks are concerned with the maintenance of the genetic integrity of preserved materials. Fig. 1 shows the potential factors influencing genetic variation in *ex situ* and *in situ* populations. *Ex situ* management includes the multiplication of seeds from an initial generation sampled from the field (G0), representing the source for the next generation (G1), which in its turn may be the source of later regenerations (G2, etc.). The frequency of regeneration activities may vary between accessions, depending on seed depletion and loss of viability. Ideally, the effect of regeneration on genetic variation is minimized. However, as a result of regeneration the genetic integrity of an accession can be compromised by unintentional selection, mutation, genetic drift and gene flow, the latter either through seed contamination or unwanted cross pollination between different accessions (Rao *et al.*, 2007; Bacchetta *et al.*, 2008; Bamberg and Del Rio, 2006).

How the genetic variation and integrity of gene bank accessions relates to that of the current *in situ* populations not only depends on the potential influences experienced during *ex situ* maintenance, but also on the collecting procedures used to sample the original *in situ* populations and the processes that *in situ* populations may have experienced since they were sampled. Whether representative samples were collected for *ex situ* storage depends on the sample size, the type of material sampled (seeds, tubers or whole plants) and the extent of spatial variation at the sampled location. After collecting for *ex situ* storage, *in situ* populations may experience natural selection, mutation, genetic drift, gene flow and extinction/re-colonization events (Fig. 1). Besides that, there is a growing concern that genetic variation may be negatively influenced through changes in environmental conditions, such as climatic change (Jarvis *et al.*, 2008, 2010).

Figure 1. *Schematic representation of the possible factors influencing population genetic variation under* in situ *and* ex situ *conditions over time. G = 0, G = 1, etc. indicate different regeneration cycles.*



Extensive discussions have been conducted on the issue whether *ex situ* conservation in gene banks or *in situ* conservation in their natural habitat is the most appropriate method to preserve wild species (Maxted *et al.*, 1997, 2008; Scarascia-Mugnozaa and Perrino, 2002;). It has been argued that crop wild relatives are best preserved under *in situ* conditions because of the potential to evolve and adapt to changing environmental conditions resulting in new variation that may be exploited in crop improvement (ulloo *et al.*, 2010). However, it is clear that *ex situ* collections are of enormous importance as they generally provide well-identified material that is immediately available for plant breeding purposes.

In the present study, microsatellite markers were used to investigate potential genetic changes in *ex situ* conserved wild potato species, while these accessions were also compared to recently re-collected *in situ* populations. Microsatellites, also known as simple sequence repeat (SSR) markers, were chosen for this study because of their qualities such as high resolving power, high reproducibility and simplicity of use (Kantartzi, 2013). Previously, microsatellites have been used in potato to study phylogeny (Ghislain *et al.*, 2009a; Merino Méndez, 2006), taxonomy (Spooner *et al.*, 2007; Lara-Cabrera and Spooner, 2005), genetic diversity (de Haan *et al.*, 2010; Ghislain *et al.*, 2009b, 2004) and disease resistance (Ghislain *et al.*, 2001). Results from our microsatellite study are discussed within the context of the future conservation management of Bolivian wild potato species.

Materials and methods

Plant material

This study included the wild potato species *Solanum acaule* Bitter, *S. avilesii* Hawkes & Hjerting, *S. berthaultii* Hawkes, *S. leptophyes* Bitter, *S. megistacrolobum* Bitter, *S. neocardenasii* Hawkes & Hjerting and *S. okadae* Hawkes & Hjerting, comprising a total of 12 accessions represented by 37 seed samples. The study material was selected based on their origin in Bolivia, the availability of several regenerated populations of the same accession, and the availability of sufficient documentation to enable re-collecting of the original *ex situ* material. The selected species represent different breeding systems (self-pollinating and outcrossing), comprise examples of taxa with both wide and limited distributions in Bolivia, and originate from different ecological niches including from areas more or less disturbed by man.

The taxonomic names are those used by CGN, although we are aware of the recently proposed synonymy (http://www.solanaceaesource.org) which questions the distinction of the species belonging to the brevicaule-complex (*S. leptophyes* and *S. avilesii*). Given the objectives of the present study, such taxonomic changes are considered inconsequential, since the main interest is the detection of possible genetic differences between regenerations of the same accession, and between *ex situ* and *in situ* populations.

This study comprises three types of populations, i.e. seed samples from the originally collected material (G0), seed samples from *ex situ* regenerations (G1, G2), and seed samples re-collected *in situ.* Unfortunately, complete time series (G0 → G1 → G2) were unavailable. The accessions were denoted by the former gene bank (BGRC) code number because some had not yet received a CGN accession number. Populations were denoted by the generation number (G0, G1, G2) and the year of collecting or regenerating. For some accessions multiple populations were available per regeneration. Re-collected *in situ* populations are denoted by "New", where applicable followed by an identifier of the location, and re-collecting year (Table 1). These re-collected populations were obtained in the period 2008-2010 through re-sampling of the original location (or as close as possible to this location). Unfortunately, the newly collected populations of *S. okadae* did not survive.

In the context of this study, the term “accession” is used for germplasm collected at a specific location at a certain time and all the populations derived from it through germplasm management in gene banks. Study accessions were acquired from CGN. Its potato collection is the continuation of the utch-German Potato Collection, which was established in 1974 as the Braunschweig Genetic Resources Collection (BGRC) which formed a merger of the two German national collections (Lange, 1976). Some of the samples used in this study originated from the German Erwin Bauer Sortiment (EBS), the collection of the University of Birmingham (BIRM) or the utch Wageningse Aardappel Collectie (WAC), from which only very limited details on the rejuvenation history are available.

To rejuvenate *ex situ* accessions, CGN aims to obtain 20-25 plants per accession from 50 to 60 botanical seeds. If necessary, these plants are first tested for quarantine diseases by the Dutch Plant Protection Service and then grown in a greenhouse or transplanted into the field in isolated plots. When no fruits are produced by natural pollination in field plots, hand-pollination is carried out. In the greenhouse they are hand-pollinated with bulked pollen mixtures from all the flowering plants of the accession. Per accession a similar number of berries is harvested per plant and subsequently bulked for seed extraction. A similar procedure is used in subsequent seed multiplications, either using the original seed-lot or the previous generation as parents. This methodology is commonly used by gene banks to regenerate wild potato species (Salas *et al.*, 2008). Table 1 specifies the regeneration site and the number of harvested plants per regeneration, where available.

Table 1. *Summary of the study material. Accessions maintained at CGN are denoted by BGRC code. G0 refers to the original seed sample, while G1 and G2 denote the first and second regeneration of each studied accession together with the generation site and number of harvested plants between brackets. Geographic information on the original collecting site is provided by the name of the department (province) and a short description of the locality. "New" represents material recollected at or in the vicinity of the locality of the accession origin. Only samples in gray color were available for the study.*

Species	Code	Original seed sample (G0)	1st regeneration (G1)	2nd regeneration (G2)	Other seed samples	Department (Province)	Locality
acaule	7976	1971	1972 (WAC)	1990 (BGRC, 24); 1994 (BGRC, 18)		La Paz (Ingavi)	Ruins of Tiwanacu.
acaule	15473 New	1959	1976 (BGRC, 10)			La Paz (Ingavi) La Paz (Ingavi)	at or near Tiahuanaco. Ruins of Tiwanacu
acaule	Tiw	2010					
acaule	7973	1971	1972 (WAC)			Potosí (Frias)	15km from Potosi on road to Sucre
acaule	New Pot	2008				Potosí (Frias)	12 km from Potosí to Sucre
avilesii	31184	1980	1981 (BIRM); 1985 (BGRC, 20) 1991 (BGRC, 20, from 1981 seed)			Santa Cruz (Vallegrande)	26km from Valle Grande to Pucara
avilesii	31185	1980	1981 (BIRM); 1988 (BGRC, 13)			Santa Cruz (Vallegrande)	28km from Valle Grande to Pucara.
avilesii	31186	1980	1981 (BIRM); 1988 (BGRC, 18)			Santa Cruz (Vallegrande)	28km from Valle Grande to Pucara.
avilesii	New	2008				Santa Cruz (Vallegrande)	Varios points on the rout Vallegrande to Pucara
avilesii	New	2012				Santa Cruz (Vallegrande)	Varios points on the rout Vallegrande to Pucara

berthaultii	10063	1959	1960 (EBS)		1961 (EBS, either G1 or G2) 1962 (EBS, either G1 or G2) 1975 (BGRC, 18, possibly regenerated from a mixture of 1959-1962) 1986 (BGRC, 26, regenerated from 1975 seed)	Cochabamba (Cercado)	Cerro San Pedro
berthaultii	New	2009				Cochabamba (Cercado)	Cerro San Pedro
leptophyes	8222	1971	1972 (WAC)	1998 (CGN, 17)		Potosí (Frias)	56-63 km from Potosi to Oruro
leptophyes	New	2008				Potosí (Frias)	54Km rout Potosí-Oruro
megistacrolobum	27115	1980	1991 (BGRC, 23)			Cochabamba (Tiraque)	76km from Cochabamba to Santa Cruz, Koari
megistacrolobum	New	2010				Cochabamba (Tiraque)	Koari
megistacrolobum	8234	1971	1973 (WAC)	2001 (CGN, 21)		Cochabamba (Tiraque)	Est. Exp. de Toralapa.
megistacrolobum	New	2010				Cochabamba (Tiraque)	Est. Exp. de Toralapa.
neocardenasii	28001	1980	1983 (BGRC, 13)			Santa Cruz (Vallegrande)	1.5 km from Mataral to Valle Grande.
neocardenasii	New	2008				Santa Cruz (Vallegrande)	Varios points on rout Vallegrande to Mataral
neocardenasii	New	2010				Santa Cruz (Vallegrande)	Varios points on rout Vallegrande to Mataral
okadae	27040	1980	1989 (BGRC)	1992 (BGRC, 14)		La Paz (Inquisivi)	6km from Quime to Licoma

Abbreviations used: BGRC = Braunschweig, Germany; EBS = German Erwin Bauer Sortiment; WAC = Dutch Wageningse Aardappel Collectie and BIRM = University of Birmingham

DNA isolation

In 2009, 24 populations were raised from true seed (twenty five individuals per population) and planted in a greenhouse at PROINPA, Bolivia. An additional 13 populations were grown in 2010 at CGN. Leaves collected in The Netherlands were dehydrated using silica gel and sent to PROINPA for NA extraction. Collected leaves were ground in liquid nitrogen, and approximately 100 mg per ground tissue sample was stored at -20°C until NA extraction.

DNA was extracted from young leaves using the protocol of Doyle and Doyle (1990) with slight modifications (Ghislain *et al.*, 1999). DNA concentration was estimated on 1% agarose gels using a size ladder of 200 to 10,000 bp (SmartLadder, Eurogentec) as a reference, and SYBR Green for visualization. The number of individuals from which NA was extracted is listed per species in Table 2.

Selection of microsatellite markers

Initially, 30 microsatellites were selected based on high polymorphic information content, and high quality of amplicons as determined by clarity and reproducibility according to previous SSR fingerprinting of cultivated potato (Ghislain *et al*., 2009b; Feingold *et al.*, 2005; Milbourne *et al.*, 1998). The selected markers were tested in the laboratory of PROINPA with a group of seven randomly selected plants of *S. leptophyes, S. neocardenasii, S. avilesii, S. berthaultii* and *S. acaule* to assess their ability to detect genetic variation in wild potato species. PCRs were performed in 15 µl volumes containing 20 ng DNA, 1.5 µl 1X PCR buffer (15mM MgCl2, 500mM KCl, 100mM Tris HCl 1M pH 8.3), 0.2 mM of each dNTP, 1 pmol/µl of forward and reverse primer, and 1 U of Taq polymerase. The amplifications were performed in a MJ Research PTC 100 thermocycler using the following thermal profile: one initial denaturation cycle at 94°C for 5 min; 35 cycles at 94ºC for 1 min, 30 sec at annealing temperature, 30 sec at 72ºC; 1 final extension cycle at 72ºC for 5 min. Successful amplification was checked on 1.8% agarose gels using SYBR green for staining. Seventeen markers were selected based on the quality of the amplified products and were used to screen the 37 study populations; two additional markers, STI0012 and STM1064, were used in *S. leptophyes* only (Table 2). Details about the markers, such as repeat motif, primer sequences and annealing temperatures, can be obtained from the references indicated in Table 2.

Table 2. *Microsatellite markers used in the analyses. The number of individuals is indicated between brackets after the species code. The range of allele sizes observed for each species is presented with the number of alleles per marker between brackets.*

Microsatellite*	Potato species**						
	acl (154)	avl (159)	ber (126)	lph (57)	mga (98)	ncd (65)	oka (35)
STG 0001	111-158 (8)	146-158 (4)	151-165 (7)		147-151 (4)	135-176 (4)	149-155 (4)
STG 0010	169-196 (5)	168-189 (12)	169-188 (6)	176-184 (5)	168-188 (10)	168-188 (5)	168-188 (6)
STG 0016	138-159 (3)	138-171 (10)	140-159 (6)	150-168 (6)	134-167 (5)	135 (1)	156-171 (4)
STG 0025	213-214 (2)	216-217 (2)	217-218 (2)	217-218 (2)	213-217 (2)	216-218 (3)	217 (1)
STI 0001	201-203 (3)	200-216 (8)	199-213 (6)	199-208 (3)	192-207 (5)	199-210 (5)	202-208 (2)
STI 0004	86-95 (2)	93-107 (6)	98-104 (5)	92-104 (4)		89-101 (6)	92-104 (3)
STI 0012				187 (1)			
STI 0014	136-152 (2)	136-146 (3)	139 (1)	139-151 (6)	136-158 (6)	136-139 (2)	139-145 (3)
STI 0030	107 (1)				99-119 (4)	106-157 (9)	102-125 (3)
STI 0032	130-139 (3)	125-150 (6)	130-139 (4)	133-143 (4)	124-158 (9)	122-139 (4)	133-139 (3)
STI 0033	129-159 (4)	117-162 (7)	129-149 (4)	138-155 (5)	129-162 (5)	138-159 (2)	139-155 (4)
STM 0019	177-212 (4)						128-208 (3)
STM 0037	79-102 (7)	79-120 (15)	79-106 (12)	79-114 (12)	87-112 (10)	60-118 (16)	104-116 (5)
STM 1052	227 (1)	214-234 (7)	224-230 (4)	215-227 (2)	225-227 (2)	225-235 (4)	197-227 (2)
STM 1053	189-192 (2)	187-192 (4)		191-197 (2)	192 (1)	191 (1)	192 (1)
STM 1064				199-209 (2)			
STM 1106	169-178 (4)	142-213 (14)		161-215 (6)	166-222 (16)		158-178 (4)
STM 5114	302-305 (2)	302-320 (6)	305-332 (6)	302-314 (4)	302-314 (4)	308 (1)	308-320 (4)
STPoAc58	249-267 (6)	251-265 (6)	251-259 5)	251-257 (4)	247-293 (16)	251-253 (2)	249-257 (5)

* Details about the microsatellites can be found in Ghislain *et al* (2009), except for STM 0019 which was published by Milbourne *et al* (1998).
**acl = *S. acaule*, avl = *S. avilesii*, ber = *S. berthaultii*, lph = *S. leptophyes*, mga = *S. megistacrolobum,* ncd = *S. neocardenasii*, oka = *S. okadae*

Selection of microsatellite markers

Initially, 30 microsatellites were selected based on high polymorphic information content, and high quality of amplicons as determined by clarity and reproducibility according to previous SSR fingerprinting of cultivated potato (Ghislain *et al*., 2009b; Feingold *et al.*, 2005; Milbourne *et al.*, 1998). The selected markers were tested in the laboratory of PROINPA with a group of seven randomly selected plants of *S. leptophyes, S. neocardenasii, S. avilesii, S. berthaultii* and *S. acaule* to assess their ability to detect genetic variation in wild potato species. PCRs were performed in 15 µl volumes containing 20 ng DNA, 1.5 µl 1X PCR buffer (15mM MgCl2, 500mM KCl, 100mM Tris HCl 1M pH 8.3), 0.2 mM of each dNTP, 1 pmol/µl of forward and reverse primer, and 1 U of Taq polymerase. The amplifications were performed in a MJ Research PTC 100 thermocycler using the following thermal profile: one initial denaturation cycle at 94°C for 5 min; 35 cycles at 94ºC for 1 min, 30 sec at annealing temperature, 30 sec at 72ºC; 1 final extension cycle at 72ºC for 5 min. Successful amplification was checked on 1.8% agarose gels using SYBR green for staining. Seventeen markers were selected based on the quality of the amplified products and were used to screen the 37 study populations; two additional markers, STI0012 and STM1064, were used in *S. leptophyes* only (Table 2). Details about the markers, such as repeat motif, primer sequences and annealing temperatures, can be obtained from the references indicated in Table 2.

PCR and electrophoresis

SSR genotyping was performed at the Genotyping Services Laboratory of ICRISAT-Patancheru, India. The M13 primer sequence 5'CACGACGTTGTAAAACGAC3' was used as a pigtail extension at the 5' end of each forward primer (Oetting *et al.*, 1995). PCR was performed in 5 µl reaction volume with 5 ng of DNA, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1X PCR buffer, 0.006 pM of M13-tailed forward primer, 0.09 pM of M13-tailed forward primer labelled with either 6-Fam, Vic, Ned or Pet (Applied Biosystems), 0.09 pM of reverse primers and 0.1 U of *Taq* DNA polymerase (SibEnzyme Ltd., Russia). A GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, USA) was used to carry out PCR according to the following thermal profile: initial denaturation at 94˚C for 3 min, followed by 10 cycles of denaturation at 94˚C for 1 min, annealing at 61˚C for 1 min (temperature reduced by 1˚C after each cycle) and extension at 72˚C for 1 min.

This touchdown PCR was followed by 40 cycles of denaturation at 94˚C for 1 min, annealing at 54˚C for 1 min and extension at 72˚C for 1 min, ending with final extension of 10 min at 72˚C. Based on the expected size of the amplification product, PCR products were multiplexed together with an internal size standard (GeneScan™ 500 LIZ® from Applied Biosystems) and capillary electrophoresis was carried out using ABI 3730*xl* Genetic Analyzer (Applied Biosystems, USA). Resulting data were analysed using the software package Genemapper (Applied Biosystems, USA) and fragment size was scored in base pairs (bp) relative to the migration of the internal size standard.

Data analysis

Microsatellite peak patterns were manually verified for clarity and consistency. Ambiguous microsatellite scores were removed from the dataset. In most cases, the samples showed one or two alleles per microsatellite. In cases where three or four unambiguous microsatellite peaks were observed, more than two alleles were recorded. Nine samples were removed from the data set as they clearly represented misidentifications. Microsatellite data were transformed to binary data by denoting the presence of an allele as one and the absence as zero. The binary data were used to calculate the Jaccard's similarity coefficient between individuals. These were subsequently used to perform a UPGMA cluster analysis. Relationships between individuals were graphically represented by dendrograms, both for the entire set of material and for each separate species. All cluster analyses were performed using NTSYS_pc 2.10 (Rohlf, 2000).

To test the genetic difference between two populations for statistical significance, a non-parametric resampling procedure with replacement was used. For this purpose, the binary microsatellite data were used to calculate the mean Jaccard similarity value between all pairs of individuals from different populations. The individuals of the two populations were then pooled and two simulated populations with a size equal to that of the original population were constructed by randomly drawing individuals from the pooled set. Subsequently, the mean Jaccard similarity value between the two simulated populations was calculated. This procedure was repeated 10,000 times, and the proportion of simulated similarity values smaller or equal to the original similarity value was recorded. This value was denoted by P, representing the probability of erroneously rejecting the null hypothesis of genetic uniformity. Statistical analyses, using a tailor-made program written in Turbo Pascal, were carried out per microsatellite locus as well as for the combined set of markers.

Results

Genetic diversity among and within species

Among the 694 examined individuals, a total of 236 alleles were identified for the set of 19 microsatellite markers. The UPGMA cluster analysis showed that the microsatellites used in this study were able to discriminate the seven wild potato species involved (Fig. 2), despite the fact that the markers were developed for cultivated potatoes (Ghislain *et al.,* 2009b). In the UPGMA dendrogram, the five representatives of series *Tuberosa* (*S. leptophyes, S. avilesii, S. okadae, S. berthaultii* and *S. neocardenasii*) grouped together into a large cluster, separate from a second cluster formed by *S. acaule* and *S. megistacrolobum.* Within the cluster of series *Tuberosa* the two members of the so-called 'brevicaule-complex', i.e. *S. avilesii* and *S. leptophyes* (van den Berg *et al*., 1998) form a subgroup. The topology of the tree thus confirms to the generally accepted taxonomic classification.

The dendrogram shows a wide genetic variability within each species, particularly in *S. avilesii* and *S. megistacrolobum*. In order of increasing similarity range, observed Jaccard values were 0.17-1.00 for *S. megistacrolobum*, 0.18-1.00 for *S. avilesii*, 0.27-0.91 for *S. leptophyes*, 0.37-1.00 for *S. berthaultii*, 0.41- 0.73 for *S. okadae*, 0.52-1.00 for *S. neocardenasii* and 0.59-1.00 for *S. acaule*.

The observed number of alleles also differed among the studied species. *Solanum avilesii* showed the highest number of alleles (110), followed by *S. megistacrolobum* (99). For *S. acaule* with 59 and *S. okadae* with 57, the lowest number of alleles was recorded (Table 2). In all species, STM0037 was found to be the most polymorphic microsatellite, presenting more alleles than each of the other markers (Table 2).

Figure 2. *UPGMA dendrogram based on 17 microsatellite markers of the study samples from the wild potato species* Solanum acaule, S. avilesii, S. berthaultii, S. leptophyes, S. megistacrolobum, S. neocardenasii *and* S. okadae.

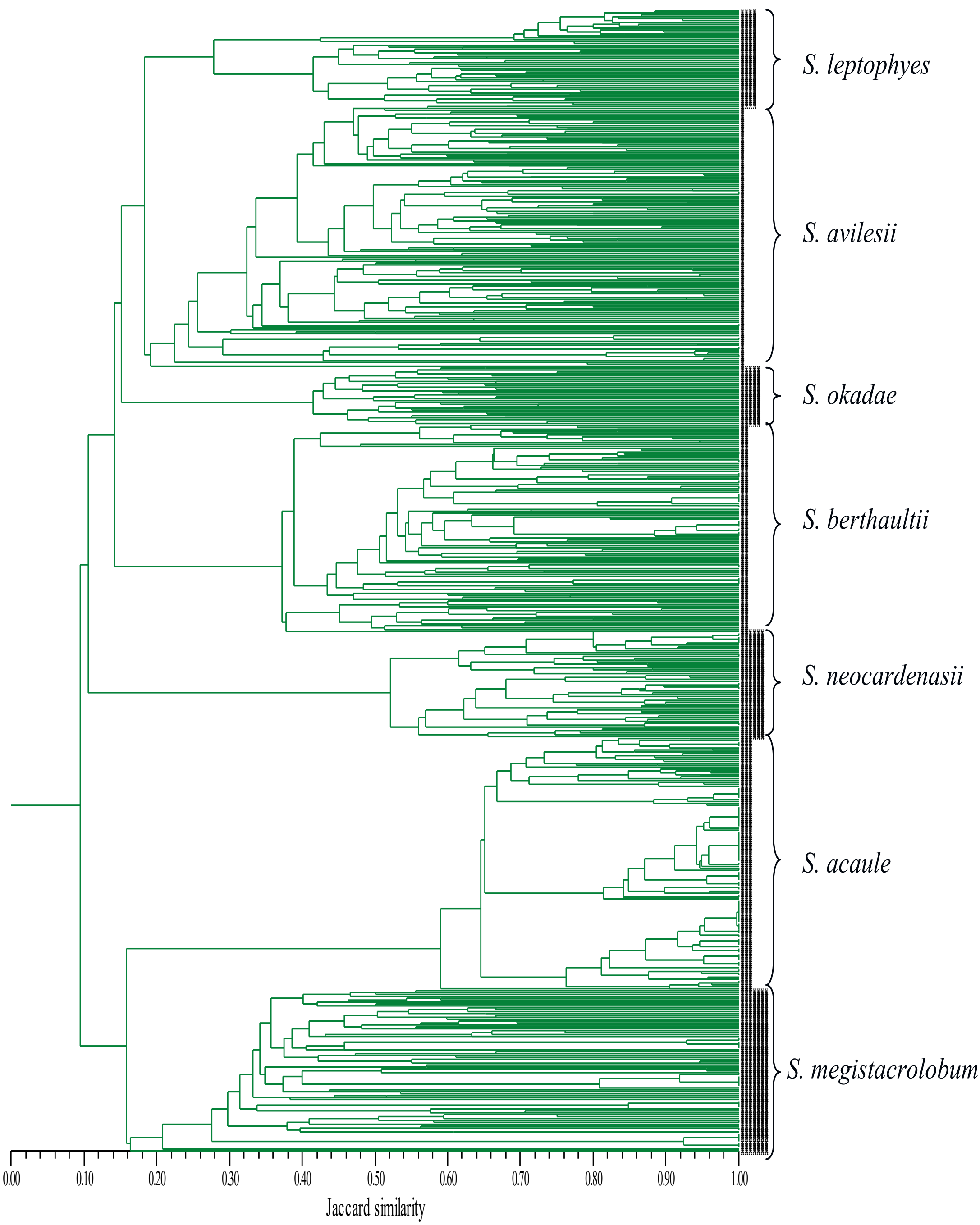


Table 3. *Pairwise comparison of microsatellite differences between different populations of gene bank accessions. Populations are denoted by their generation number ('x' when unknown) with the production year given between parentheses. The number of private alleles per population as well as the number of shared alleles is presented for each comparison. accard denotes the mean similarity value of all pairwise comparisons between individuals from different populations, while the corresponding probability of erroneously rejecting the null hypothesis of genetic homogeneity is presented by the P-value (* P<0.05, ** P<0.01, *** P<0.005).*

Accession	Population 1	Population 2	Number of private alleles Population 1	Number of private alleles Population 2	Number of shared alleles	Jaccard	P-value
S. acaule							
BGRC7976	G2 (1994)	G2 (1990)	1	6	25	0.861	0.001***
BGRC7976	G2 (1994)	G1 (1972)	1	6	25	0.869	0.000***
BGRC7976	G2 (1990)	G1 (1972)	0	0	31	0.939	0.413
BGRC15473	G1 (1976)	G0 (1959)	2	5	30	0.918	0.099
S. avilesii							
BGRC31184	G2 (1991)	G1 (1985)	15	17	30	0.395	0.012*
BGRC31184	G2 (1991)	G1 (1981)	15	9	30	0.373	0.000***
BGRC31184	G1 (1985)	G1 (1981)	14	6	33	0.462	0.098
BGRC31185	G1 (1988)	G1 (1981)	1	5	35	0.532	0.011*
BGRC31186	G1 (1988)	G1 (1981)	11	2	35	0.503	0.298
S. berthaultii							
BGRC10063	Gx (1986)	Gx (1975)	9	3	35	0.515	0.331
BGRC10063	Gx (1986)	Gx (1962)	15	13	29	0.369	0.000***
BGRC10063	Gx (1986)	G1 (1960)	17	18	27	0.464	0.039*
BGRC10063	Gx (1986)	G0 (1959)	15	6	29	0.396	0.000***
BGRC10063	Gx (1975)	Gx (1962)	7	11	31	0.430	0.000***
BGRC10063	Gx (1975)	G1 (1960)	12	9	26	0.527	0.097
BGRC10063	Gx (1975)	G0 (1959)	11	8	27	0.452	0.001***
BGRC10063	Gx (1962)	G1 (1960)	14	7	28	0.366	0.000***
BGRC10063	Gx (1962)	G0 (1959)	18	11	24	0.336	0.000***
BGRC10063	G1 (1960)	G0 (1959)	9	9	26	0.489	0.017*
S. leptophyes							
BGRC8222	G2 (1998)	G1 (1972)	0	3	49	0.472	0.058
S. megistacrolobum							
BGRC8234	G2 (2001)	G1 (1973)	13	18	44	0.401	0.126
S. neocardenasii							
BGRC28001	G1 (1983)	G0 (1980)	13	10	28	0.637	0.013*
S. okadae							
BGRC27040	G2 (1992)	G1 (1989)	5	3	49	0.437	0.012*

Genetic integrity of material conserved **ex situ**

Based on Jaccard similarity, significant genetic differences were observed between the different *ex situ* generations of *S. neocardenasii* and *S. okadae*, but not between those of *S. leptophyes* and *S. megistacrolobum*. For *S. acaule*, *S. avilesii* and *S. berthaultii* mixed results were obtained, as some comparisons showed significant differences while others did not. Private alleles were observed in all but 3 populations. Compared to the private alleles the number of shared alleles was higher in all cases. Comparisons between generations showing no significant differences also showed less private alleles in relation to comparisons with significant differences, indicating small genetic changes (Table 3).

Genetic differentiation between **ex situ** *and* **in situ** *populations*

All genetic differences between *ex situ* and *in situ* populations were significant for each of the studied species. In the majority of cases there is a high number of private alleles, in *S. avilesii* sometimes even higher than the number of shared alleles (Table 4).

Observed differences between *in situ* and *ex situ* populations were most pronounced for *S. leptophyes*, where significant effects were observed for the two comparisons at 13 and 12 marker loci, respectively.

Samples from the *in situ* populations of *S. avilesii* and most of *S. acaule* harbored more private alleles than those of the *ex situ* populations, while opposite results were observed for *S. berthaultii, S. leptophyes* and *S. megistacrolobum* (Table 4).

***Table 4.** Pairwise comparison of microsatellite differences between ex situ populations of gene bank accessions with re-collected in situ populations.* Ex situ *populations are CGN accessions denoted by the BGRC code followed by the generation year between parentheses.* In situ *populations are denoted by "New" followed by the year of recollection. The number of private alleles per population as well as the number of shared alleles is presented for each comparison. accard denotes the mean similarity value of all pairwise comparisons between individuals from different populations, while the corresponding probability of erroneously rejecting the null hypothesis of genetic homogeneity is presented by the P-value (* P<0.05, ** P<0.01, *** P<0.005).*

Population 1 (in situ)	Population 2 (ex situ)	Number of private alleles Population 1	Number of private alleles Population 2	Number of shared alleles	Jaccard	P-value
S. acaule						
New (2008)	BGRC7973 (1972)	7	12	31	0.654	0.000***
New (2010)	BGRC7976 (1994)	15	0	26	0.725	0.000***
New (2010)	BGRC7976 (1990)	12	2	29	0.771	0.000***
New (2010)	BGRC7976 (1972)	12	2	29	0.782	0.000***
New (2010)	BGRC15473 (1976)	11	2	30	0.724	0.000***
New (2010)	BGRC15473 (1959)	9	3	32	0.760	0.000***
S. avilesii						
New (2010)	BGRC31184 (1991)	21	17	28	0.240	0.000***
New (2010)	BGRC31184 (1985)	23	21	26	0.244	0.000***
New (2010)	BGRC31184 (1981)	26	16	23	0.240	0.000***
New (2010)	BGRC31185 (1988)	27	14	22	0.232	0.000***
New (2010)	BGRC31185 (1981)	25	16	24	0.242	0.000***
New (2010)	BGRC31186 (1988)	20	17	29	0.263	0.000***
New (2010)	BGRC31186 (1981)	24	12	25	0.291	0.001***
New (2008)	BGRC31184 (1991)	44	11	34	0.248	0.000***
New (2008)	BGRC31184 (1985)	46	15	32	0.248	0.000***
New (2008)	BGRC31184 (1981)	49	10	29	0.259	0.000***
New (2008)	BGRC31185 (1988)	48	6	30	0.237	0.000***
New (2008)	BGRC31185 (1981)	45	7	33	0.254	0.000***
New (2008)	BGRC31186 (1988)	43	11	35	0.250	0.000***
New (2008)	BGRC31186 (1981)	48	7	30	0.261	0.000***
S. berthaultii						
New (2009)	BGRC10063 (1986)	8	17	27	0.448	0.000***
New (2009)	BGRC10063 (1975)	10	13	25	0.526	0.004***
New (2009)	BGRC10063 (1962)	10	17	25	0.395	0.000***
New (2009)	BGRC10063 (1960)	13	13	22	0.503	0.001***
New (2009)	BGRC10063 (1959)	10	10	25	0.440	0.000***
S. leptophyes						
New (2008)	BGRC8222 (1998)	15	19	30	0.287	0.000***
New (2008)	BGRC8222 (1972)	15	22	30	0.302	0.000***

Population 1 (in situ)	Population 2 (ex situ)	Number of private alleles Population 1	Number of private alleles Population 2	Number of shared alleles	Jaccard	P-value
S. megistacrolobum						
New Koa (2010)	BGRC27115 (1991)	9	30	31	0.312	0.000***
New Tor (2010)	BGRC8234 (2001)	15	16	41	0.365	0.000***
New Tor (2010)	BGRC8234 (1973)	20	26	36	0.359	0.003***
S. neocardenasii						
New (2010)	BGRC28001 (1983)	6	19	22	0.510	0.000***
New (2010)	BGRC28001 (1980)	7	17	21	0.541	0.000***
New (2008)	BGRC28001 (1983)	15	13	28	0.495	0.000***
New (2008)	BGRC28001 (1980)	18	13	25	0.499	0.000***

Discussion

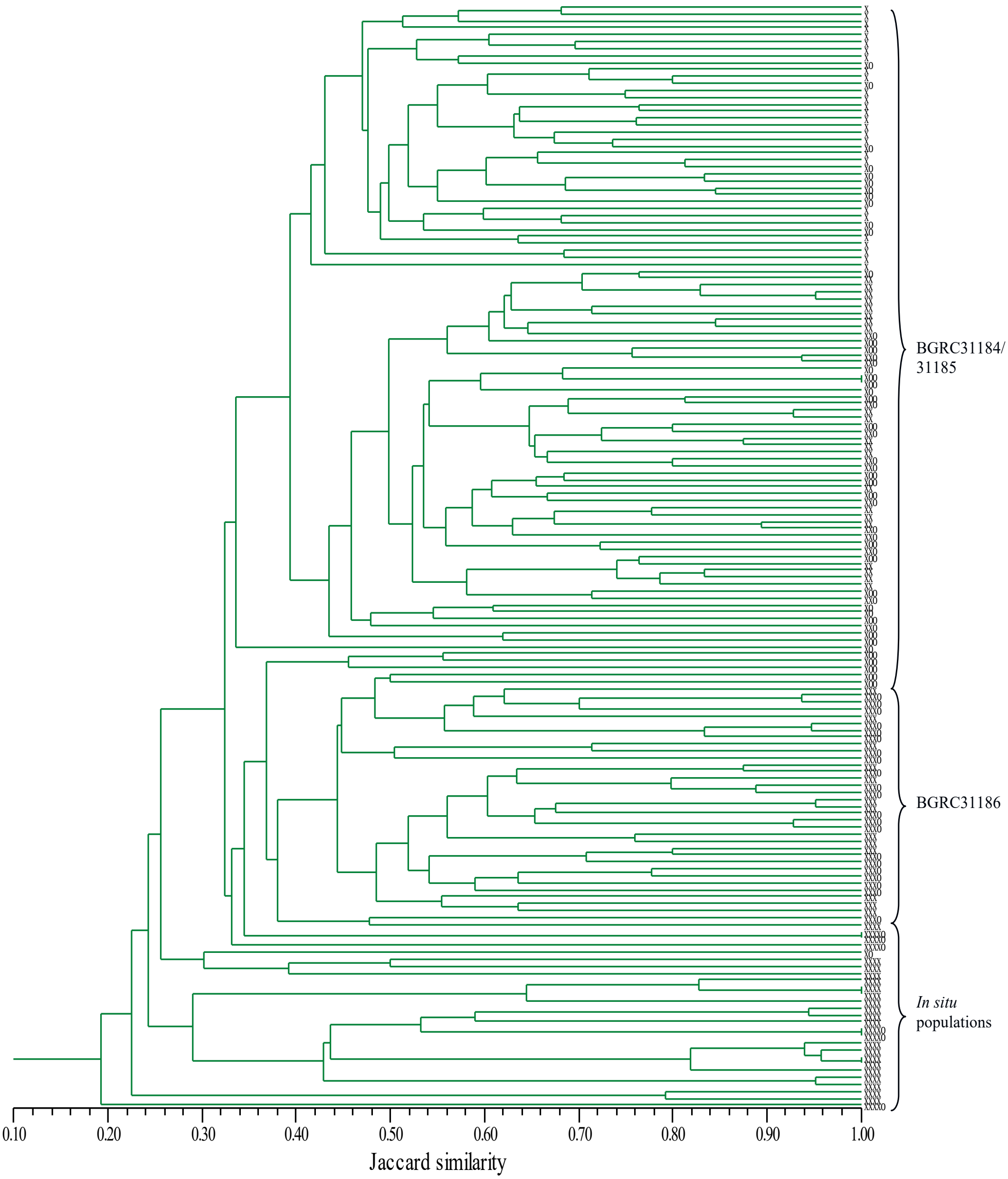
The continuous demand for increasing agricultural productivity requires new sources of genetic variation. Gene banks play an important role in this context, making germplasm available for present and future users. Therefore, proper maintenance of the genetic integrity of accessions is the main challenge during *ex situ* conservation. The genetic integrity may be compromised when gene bank accessions are regenerated to produce new seed stocks (van Hintum *et al.*, 2007, Chebotar *et al.*, 2003, Börner *et al.*, 2000). Ideally, the effect of seed management on the genetic integrity of accessions should be minimal (Rao *et al.*, 2007). However, several processes may affect the genetic composition of accessions during regeneration, including genetic drift, selection, gene flow among different accessions and handling errors. Another important issue is the extent to which gene bank accessions represent the diversity occurring *in situ*. This degree of representativeness depends on several factors, including sampling procedures in natural populations and genetic changes that have occurred *ex situ* as well as *in situ* (Del Rio *et al.*, 1997b). In the present study, genetic changes in gene bank accessions of Bolivian wild potato species were examined, while also a comparison was made with *in situ* re-collecting.

Genetic change in accessions during ex situ *conservation*

As genetic drift is expected to affect all loci to more or less the same extent, selection is generally inferred from a strong differentiation at specific loci, while other loci exhibit no or smaller changes (e.g. van Hintum *et al*., 2007). The present study did not provide clear support for the influence of selection as the observed significant changes did not systematically involve the same loci. However, it cannot be ruled out that selection plays a role as it may operate on genes that are unlinked to the marker loci used in this study (Ghislain *et al*., 2009b). As multiple private alleles were observed in nearly all comparisons, it seems more likely that the examined accessions have been affected by genetic drift. Depending on the extent of genetic drift, accessions may have changed significantly during a single regeneration or through accumulating effects over multiple regenerations. An example of accumulating effects was found for *S. berthaultii*, assuming that the 1986 sample originated from the 1975 seeds, and the latter from the 1960 material. The extent of genetic drift increases when population size is reduced. In several cases where significant genetic changes were observed, the regeneration history indicated a reduced population size. For example, the regeneration of *S. okadae* in 1992 was performed in a greenhouse in autumn. Compared with summer conditions the plants grew under reduced light intensity and shorter day length. As a result, only 15 plants flowered and fruits could be collected from 14 plants. For *S. neocardenasii*, generation G1 was obtained from only 13 plants. Furthermore, in both cases plants may have produced a disproportional quantity of seeds (some large others small amounts). In seed bags intended to be used for future regeneration, each regenerated plant is represented with the same number of seeds, but balanced bulks are not made for “user bags”, which were used for the current study. In the case of *S. leptophyes* and *S. megistacrolobum* the regeneration history did not indicate protocol deviations, while seeds were harvested from 17 and 21 plants, respectively, during regeneration. In both cases, no significant changes were observed.

In addition to genetic drift also handling errors have most probably contributed to the observed genetic changes. For example, fixations for different alleles were observed in successive generations of accession BGRC7976 of *S. acaule*, which are unlikely to be explained by genetic effects but rather point towards a substitution of accessions. In *S. avilesii*, the G2 generation of accession BGRC31184 differed significantly from the two G1 samples. Surprisingly, the G2 generation of BGRC31184 was not significantly different from the populations of BGRC31185, suggesting that generation G2 of BGRC31184 was erroneously derived from BGRC31185. This is supported by the UPGMA analysis, showing that samples of generation G2 of BGRC31184 are intermixed with those of G1 of BGRC31185 (Fig. 3).

Figure 3. *UPGMA dendrogram based on 15 microsatellite markers of the samples of* Solanum avilesii. Ex situ *samples were from three CGN accessions: BGRC31184 denoted by X (=G1/1981), X0 (=G1/1985) and X00 (=G2/1991), BGRC 31185 denoted by XX (=G1/1981) and XX0 (=G1/1988), and BGRC 31186 denoted by XXX (=G1/1981) and XXX0 (=G1/1988).* In situ *samples are denoted by XXXX (=2008) and XXXX0 (=2010).*



Compared to autogamous species, the level of genetic variation in populations of allogamous species is generally much higher, and hence populations of outbreeders are more prone to genetic changes during regeneration (Chebotar *et al.*, 2003; Bamberg and el Rio, 2004), although earlier studies on the genetic integrity of time-series generations showed no significant changes both in selfing and outcrossing potato species (el Rio *et al.*, 1997a). In self-pollinating wheat, no genetic changes were observed in accessions after long-term maintenance in the Gatersleben gene bank (Börner *et al.*, 2000). The single autogamous species included in the present study was *S. acaule*. Apart from the comparisons involving G2 of BGRC7976, which seemed to involve a substitution of accessions, no significant genetic changes were observed for *S. acaule*.

Comparison with* in situ *re-collecting

In the present study genetic differences between *ex situ* conserved accessions and *in situ* re-collected material were significant in all comparisons. Unfortunately, G0 was not available in all cases, which makes it difficult to infer whether the observed differences were due to *ex situ* or *in situ* processes. Nevertheless, the observed changes during *ex situ* conservation can be expected to have contributed to the differences found between *ex situ* and *in situ* material. An example is provided by *S. neocardenasii* for which also significant differences were found between the G0 sample and the *in situ* populations, suggesting that *in situ* genetic changes and/or sampling effects have occurred as well.

Sampling effects have most likely contributed to the genetic differences observed between the *ex situ* and *in situ* material. In *S. acaule*, the observed differences were most likely caused by sampling the same heterogeneous population in different ways. The two examined gene bank accessions were collected from the same location (ruins of Tiwanacu), respectively 40 and 52 years ago. Our SSR data revealed that the two *ex situ* accessions were significantly different (data not shown) and that the re-collected material from Tiwanacu actually consisted of two subgroups, each clustering with the material of one of the two original accessions (Fig. 4). Apparently, BGRC15473 and BGRC7976 represent genetically different subpopulations from the Tiwanacu area, while the recollected material from 2010 included both subpopulations. Previous studies support the finding that *S. acaule* may display wide genetic variation within a limited area (McGregor et al., 2002;

van Treuren et al., 2004). In those studies BGRC7976, collected from Tiwanacu ruins in the La Paz epartment appeared very similar to most of the other *S. acaule* accessions from the same department, whereas BGRC15473, which also originates from the Tiwanacu ruins, clustered with *S. acaule* accessions collected in southern Bolivia (e.g. BGRC7973 from the Potosi epartment) and northern Argentina. In *S. avilesii*, material recollected in 2008 and 2010 captured many alleles that were not observed in the *ex situ* samples. Although it cannot be ruled out that alleles may have been lost during *ex situ* regeneration, the scale of resampling may have been an important underlying cause. Also in *S. avilesii*, the original area may have been resampled to a larger extent as exact coordinates of the geographic origin of the gene bank accessions were missing. Strong genetic differentiation over short geographic distances was also observed for *S. megistacrolobum*, not only between the collecting sites at Toralapa and Koari, which are only 3 km apart, but also within the Koari location itself (Fig. 5). In such cases, variation in collecting methods can easily result in genetically different samples. For *S. berthaultii* (Fig. 6) and *S. megistacrolobum* (Fig. 5) tubers instead of seeds were collected during the recent expeditions, which explains the finding of a comparatively high number of identical genotypes based on the SSR analysis. This difference in sampling method also explains the lower levels of variation found for these species in the *in situ* populations as compared to the *ex situ* samples. The strong difference in the number of sampled genotypes has most likely contributed to the significant differences observed between the *in situ* and *ex situ* material of these species.

Figure 4. *UPGMA dendrogram based on 17 microsatellite markers of the* Solanum acaule *populations. Samples were collected in Potosí and the Tiwanacu ruins in the Department La Paz. Potosí* ex situ *samples are denoted by X and* in situ *samples by XX. Tiwanacu* ex situ *samples from BGRC 15473 are denoted by XXX (=G0/1959) and XXX0 (=G1/1976),* ex situ *samples from BGRC 7976 are denoted by XXXX (=G1/1972), XXXX0 (=G2/1990) and XXXX00 (=G2/1994), and the Tiwanacu* in situ *samples by XXXXX.*

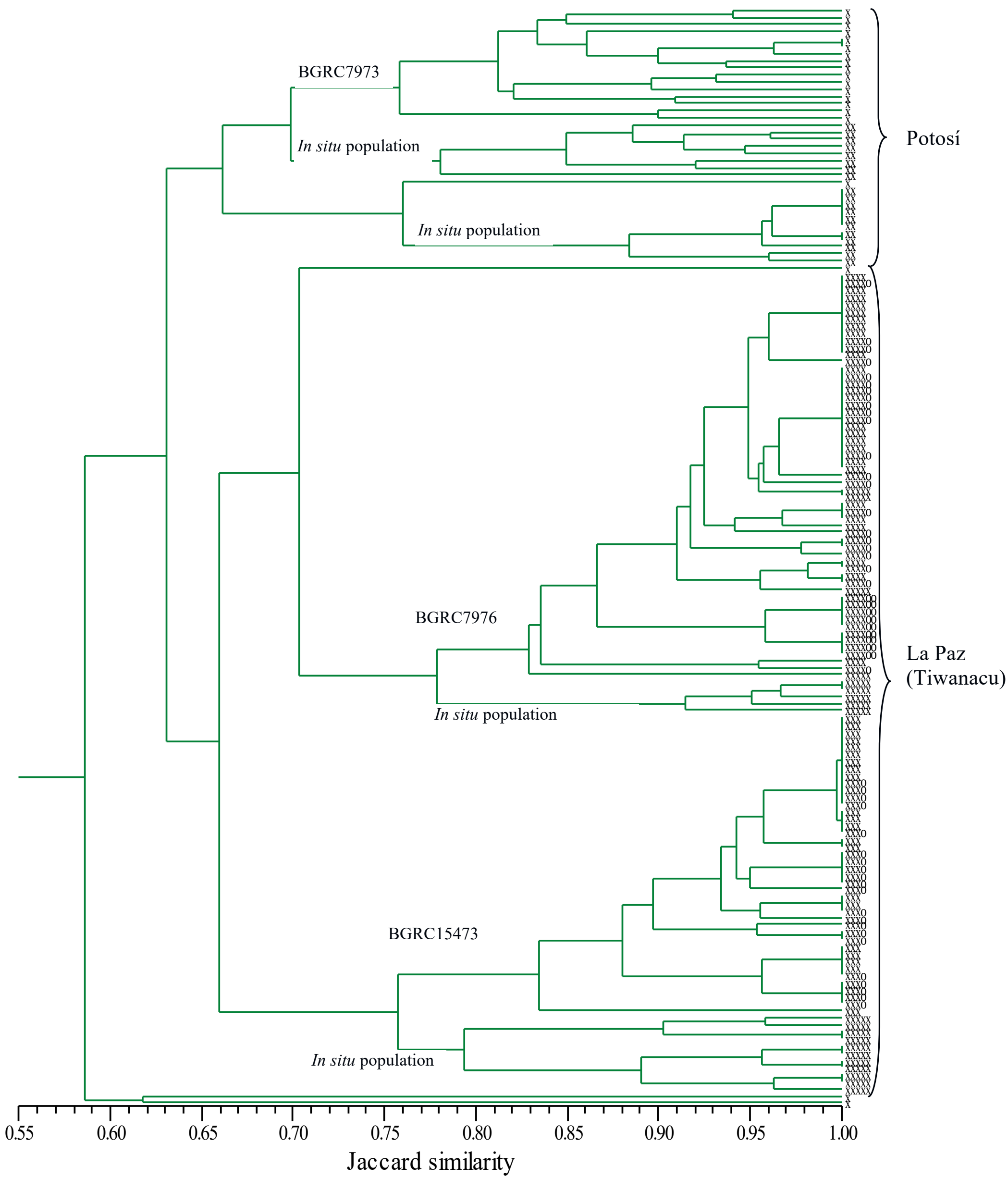


Figure 5. *UPGMA dendrogram based on 15 microsatellite markers of the samples of* Solanum megistacrolobum. Ex situ *samples were from two CGN accessions (BGRC8234 and BGRC 27115), indicated by Tor1973, Tor2001 (Toralapa) and Koa1991 (Koari), respectively.* In situ *samples from the recollected populations are indicated by Tor2010 and Koa2010.*

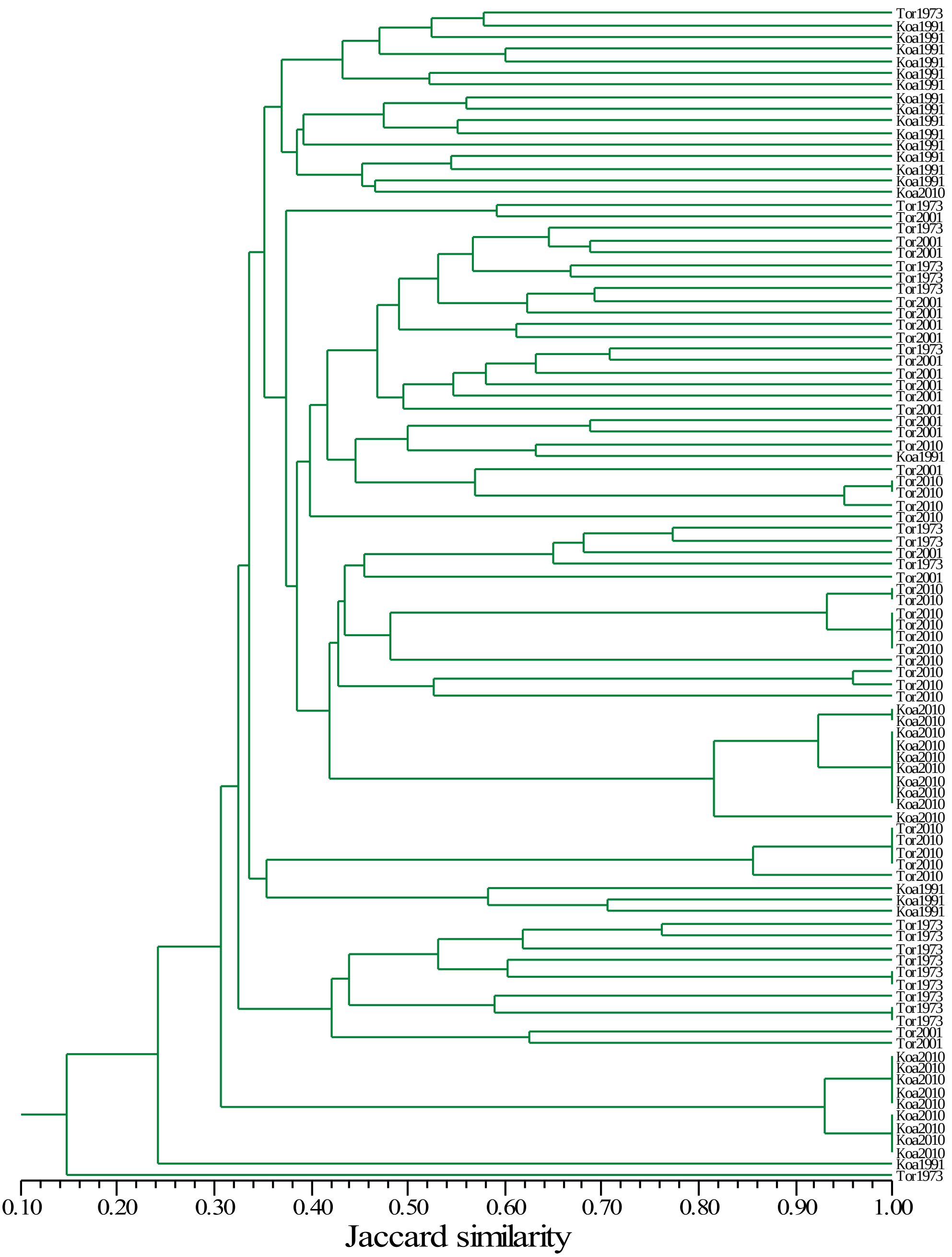
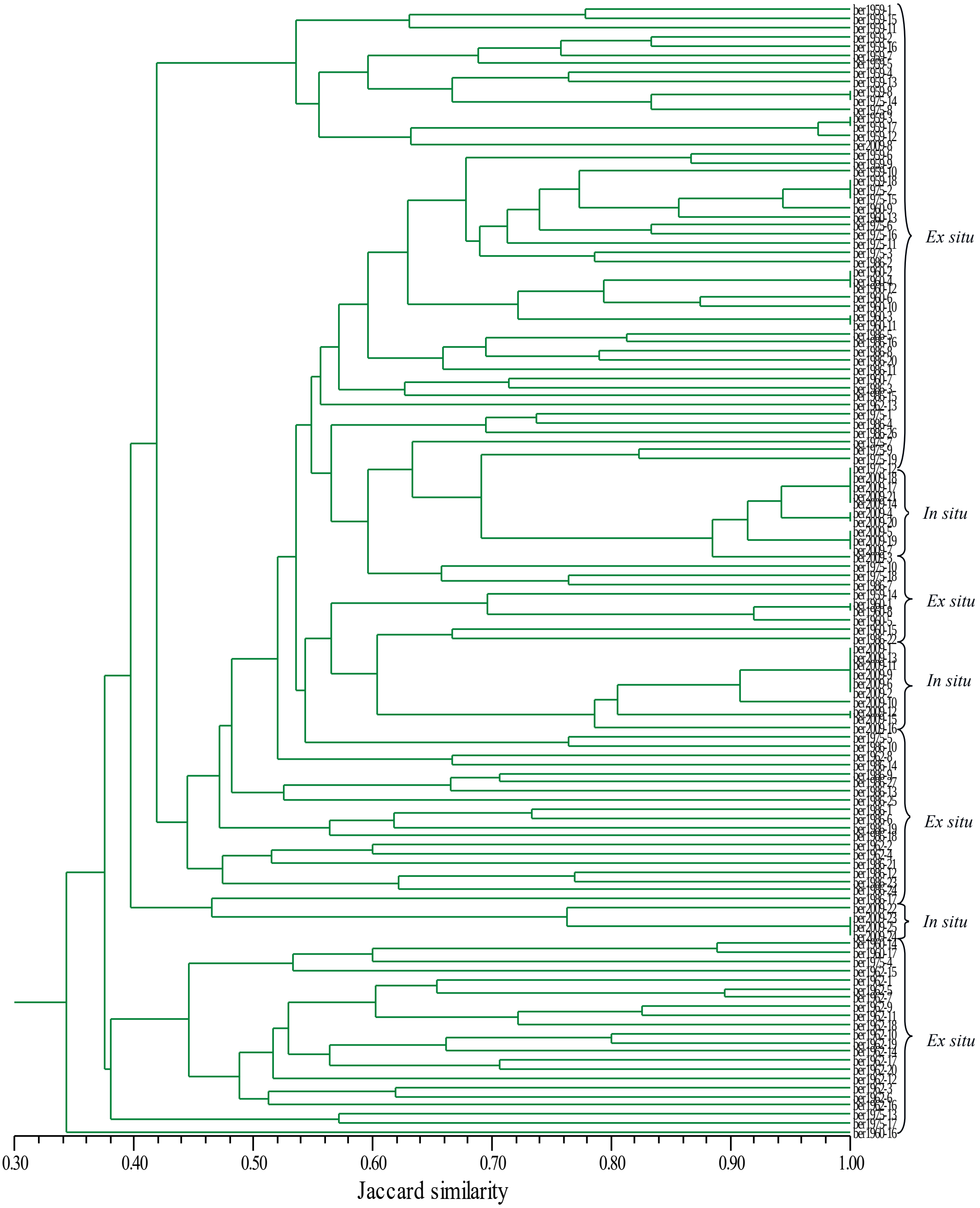


Figure 6. *UPGMA dendrogram based on 13 microsatellite markers of the samples of* Solanum berthaultii. Ex situ *samples were from 1959, 1960, 1962, 1975 and 1986.* In situ *samples originate from the recollected population in 2009.*



It can be expected that natural populations will experience genetic change over time. However, to infer that genetic differences between *ex situ* and recently collected *in situ* samples are due to developments in natural populations requires the availability of the original seed sample (G0), while also the influence of sampling effects has to be ruled out (Fig. 1). Unfortunately, this appeared difficult in the present study, because G0 was not available for all accessions and detailed original collecting data were largely missing. Also el Rio et al. (1997b) were unable to ascribe the observed differences between *ex situ* and *in situ* materials of the wild potato species *S. jamesii* Torr. and *S. fendleri* A. Gray to either *in situ* genetic changes or sampling effects. In the present study, *in situ* developments were observed that may have contributed to genetic changes in natural populations. For example, Cerro San Pedro, the type locality of *S. berthaultii*, was an area of natural vegetation, while to date this population is suffering from habitat destruction caused by ongoing urbanization as it is situated within the city of Cochabamba. Reduction of the population size since 1959 may have strongly reduced the level of genetic variation, which may have contributed to the observed difference with the *ex situ* samples.

Genetic differences between *ex situ* and *in situ* samples were most pronounced for *S. leptophyes,* as significant differences were observed for nearly all investigated markers, while also fixations for different alleles were found. Several explanations may account for these findings. First, the recollecting may not have been performed exactly at the same location as the original material and substantial spatial variation may occur within the area. Second, the *in situ* population may have been strongly disturbed by the effects of agricultural activities during the last 40 years which may have led to the loss of alleles and the introduction of other alleles by gene flow (hybridization) from nearby diploid cultivated potatoes. Third, although the taxonomic identity of the recollected material was confirmed by the curator of the wild potato gene bank of CIP, the pronounced differences between *in situ* and *ex situ* populations may indicate that the recollected material might even be another species with similar morphological characteristics. ifferent taxonomic authorities repeatedly mentioned the difficulty in discerning species of the "brevicaule complex" (van den Berg *et al.,* 1998) and recent studies have already agreed in the recognition of only two species within this complex, one representing a northern group and another a southern group with great variability, to which *S. leptophyes* belongs (Jacobs *et al*., 2011; Jacobs *et al.,* 2008; Solanaceae Source website http://www.solanaceaesource.org).

Implications for conservation

In the present study, private alleles were observed in nearly all comparisons of *ex situ* samples, indicating that alleles may easily be lost during regeneration. The probability of losing genetic variation by genetic drift depends on the frequency of alleles in the population and the effective population size during regeneration (Chebotar *et al*., 2003; Crossa, 1995). The latter is determined, amongst others, by the number of plants used for seed production. The higher the number of plants, the higher the probability that alleles are maintained in the population. For perennial allogamous species, it has been suggested to use at least 100 plants for regeneration (Johnson *et al*., 2002). Substantial lower numbers of plants have been used to regenerate accessions of the outbreeding potato species examined in the present study. Therefore, increasing the size of the regeneration population can be expected to reduce the effects of genetic drift. In practice, capacity limitations (both in human resources and field availability) often drive gene banks to accept suboptimal regeneration sizes. Inevitably, this will cause the loss of alleles, in particular those occurring at low frequency. However, genetic drift may be less relevant to functional genes, in particular those that are subject to selection, as such genes can be expected to be less variable than (neutral) SSR loci.

eviations from the commonly used regeneration protocol and handling errors during *ex situ* maintenance contributed to the loss of genetic integrity of the examined accessions. These effects can be minimized by introducing commonly agreed minimum standards (FAO, 2013; ulloo *et al*., 2008; FAO/IPGRI, 1994) for the regeneration of potato germplasm and by operating according to an international quality management system. Such a system should include detailed documentation on operating procedures and the independent monitoring of practices in order to increase efficiency and effectiveness.

Our study suggested the existence of extensive geographical and temporal genetic variation for the examined wild potato species. Therefore, material collected for *ex situ* conservation may represent only a small fraction of the genetic diversity that occurs in time and space. The representativeness of the genetic diversity in *ex situ* collections may be improved by wider sampling of the distribution area and resampling of earlier collecting sites. Species that are under-represented in gene banks and/or are threatened *in situ*, such as *S. avilesii* and *S. neocardenasii*, could be prioritized in order to keep *ex situ* collections manageable. In addition, a broader conservation strategy (at national or regional level) could be developed for the *in situ* conservation of crop wild relatives, placing emphasis on endemic species that show restricted distribution, such as *S. avilesii* and *S. neocardenasii*. This will provide a complementary strategy for preserving genetic diversity already present in gene banks and new diversity that is evolving under *in situ* conditions.

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CHAPTER 6

General Discussion

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The wild relatives of potatoes form the genetic reservoir for the improvement of the cultivated potato. While wild potatoes can be found from the south-western United States to central Argentina and Chile, their centre of diversity is located in the Peruvian and Bolivian Andes (Hawkes and Hjerting, 1989; Hijmans *et al.*, 2002). Bolivia harbours 39 wild taxa, 21 of which are endemic species (Spooner and Hijmans, 2001; Spooner and Salas, 2006). Because of the high importance of potato for human well-being, vast efforts have been made to conserve these genetic resources, particularly *ex situ* in gene banks (van Soest, 2006). In Bolivia, *in situ* conservation of wild potato has been incidental, rather than being the result of a planned policy. This study aimed to evaluate to what level the current management efforts have adequately conserved the genetic diversity of Bolivian wild potato species, and what recommendations can be formulated for improvement.

In the following sections, the implications of the results and the encountered gaps in our knowledge are discussed. This chapter concludes with recommendations for a general conservation strategy of wild potatoes in Bolivia based on the lessons learnt from this study.

Why devote efforts to the conservation of wild potatoes?

The use of genes available from crop wild relatives (CWR) to improve crop performance is well established. Their utility was recognized in breeding programs of major crops since the 1940s (FAO, 2010; Hajjar and Hodgkin, 2006; Meilleur and Hodgkin, 2004). Hajjar and Hodgkin (2006), in their survey of the introduction of genes from CWR into cultivars of 13 crops of major importance to global food security during the period of the mid-1980s to 2005, observed that the awareness of the importance of CWR grew, although their contribution to the development of new cultivars remain less than expected. Wheat and tomato are the crops for which the wild gene pool has been used extensively, mainly as a source for pest and disease resistance, followed by rice and potato (Hajjar and Hodgkin, 2006). That study shows that in the case of potato, only six wild species (from more than one hundred) have contributed beneficial traits to potato varieties. In the other nine crops of global importance between one to five wild species have been used in released cultivars (Hajjar and Hodgkin, 2006). espite the availability of improved procedures for intercrossing species with biological constraints, breeders continue to prefer to re-use old cultivars, landraces or advanced breeding lines with known resistances to develop new cultivars in order to save time and to be able to release new cultivars faster (Gabriel *et al*., 2011, Estrada Ramos, 2000). This being the case, there is a broad array of the CWR gene pool that is still underutilized. In the case of the Bolivian wild potatoes, for example, at least 16 endemic species known to harbour resistances to a wide range of abiotic and biotic stresses (chapter 3) were identified, none of which have been used for potato breeding. This indicates that the utilization of CWR in plant breeding is likely to continue growing in order to improve agricultural productivity. Logically, the importance of their effective *in situ* and *ex situ* conservation will also increase.

Databases and information management

Because of the high global importance of potato as a crop, many efforts to explore the taxonomic diversity and to collect genetic material were made. Such material was deposited in herbaria and gene banks worldwide (Hawkes, 2003; Ochoa, 1990; Spooner and Hijmans, 2001; Spooner *et al.*, 1991a; Spooner *et al.*, 1991b). Based on this material, a large amount of information, including distribution range, taxonomy, phylogenetic relationships, responses to biotic and abiotic stress, etc., has been generated and is available to users (van Soest, 2006).

Since information availability and management is essential for decision making regarding conservation plans, the quality of the data provided by taxonomic studies and gene banks is of utmost importance. In this study we focus on gene bank information, and the first step in selecting species for the assessment of the *in situ* and *ex situ* conservation status of Bolivian wild potatoes was retrieving information on former collecting sites using databases of different institutes (Centre for Genetic Resources, the Netherlands (CGN), United States Potato Genebank (USPG), Institute of Plant Genetics and Crop Plant Research (IPK) of Germany, International Potato Center (CIP) of Peru). In the past, these gene banks made efforts to standardize information about the germplasm maintained and to solve missing and conflicting data, resulting in the establishment of the Intergenebank Potato Database (IPD) (Huamán *et al.*, 2000).

Our analysis of the information included in these databases (chapter 2) showed that, despite all previous efforts to clean the data, there are still many uncertainties and imprecisions, which makes it difficult to process and interpret the information. It was necessary to clean passport data, identify duplicate accessions, add geographic coordinates of locations, etc., and even correct erroneous information. The latter was only possible based on information collected during recent field trips, and by using expertise on taxonomy, geographic distribution and ecology. The present study showed that for future initiatives, possibly including an ever wider range of species, greater efforts are required to resolve questionable data and standardize the information available in global databases. This can be a highly demanding process in terms of time and costs, but is crucial to strive for reliable data. Our effort has now resulted in a reliable database for the group of endemic wild potato species occurring in Bolivia. However, a similar approach still needs to be carried out for the non-endemic Bolivian potato species, and most likely for the remainder of wild potato species. Most probably, such data cleaning is also necessary for other gene banks holding potato germplasm, and even for gene bank data on other crops.

Implications of the new taxonomic status of Bolivian wild potatoes

Regarding potato taxonomy, we decided to follow the classification suggested by Spooner and Salas (2006), which is until now still used by the Bolivian germplasm bank. This classification includes thirty-four wild potato species occurring in Bolivia, with 24 endemic taxa (21 species, 1 subspecies and 2 varieties).

The most recent taxonomic treatment of wild potato, made available in 2011 through the Solanaceae source website (http://www.solanaceaesource.org), synonymizes various species of the "brevicaule complex" (van den Berg *et al.*, 1998) and others. According to this new treatment, the number of wild potato species for Bolivia would be reduced to 15, plus an additional two species (*S. flavoviridens* and *S. gandarillasii*) awaiting a final status designation.

This reduction has not yet been adopted in practice, neither in the databases of *ex situ* collections nor in *in situ* analyses. It seems obvious that taking into account the new taxonomic treatment would affect the status of various species, notably those identified as poorly conserved or still absent from gene banks. For example, the species *S.* × *litusinum* (no germplasm in any *ex situ* collection) and *S. soestii* (poorly represented in gene banks) would lose collecting priority when synonymized to the well-represented *S. berthaultii* and *S. circaeifolium,* respectively. Under the new taxonomy, only three species maintain their collecting priority (*S. bombycinum, S. flavoviridens* and *S. neovavilovii*). Similarly, using the more conservative taxonomic treatment of Spooner and Salas (2006) the IUCN threat status of the Bolivian endemic species shows at least 14 of them to have some degree of threat (CR, EN or VU), but when applying the new taxonomic classification only five species with some threat status would remain. The new taxonomy drastically reduces the importance to conserve some species, such as *S. avilesii* (which has some populations resistant to many stress factors) considered Critically Endangered. Similarly, *S.* × *litusinum*, resistant to Colorado beetle (*Leptinotarsa* spp.) and having the Vulnerable status, would lose its conservation importance and with that the priority to collect or protect a valuable source of resistance for future breeding programs.

Adopting the new taxonomic classification may also have implications for diversity studies (where the assessment of the diversity of an area is based on the number of taxa present in the area) and germplasm management in *ex situ* conservation. Considering that today gene banks are increasingly concerned with the improvement of the composition of their collections rather than increasing their size, several methods to optimize collection composition have been proposed (van Treuren *et al*., 2009; van Hintum *et al*., n/d). These include for example improved efficiency by optimizing the number of accessions for each species, which means finding and removing redundancy. Accepting the proposed synonymy would lead to a reduction in the number of species and an increment of the number of accessions in the remaining species. The acceptance of such increment will depend on the gene bank capacity (van Treuren *et al*., 2009), which will depend on the objectives set for their collection and limited by the available (financial) resources. Accessions beyond the capacity established for the collection might be considered redundant and be discarded.

For the reasons exposed before, this study suggests to maintain the conservative taxonomic classification, so as to avoid discarding valuable germplasm collections. But, at the same time, to maintain the priority in future collecting efforts on covering a greater ecogeographical range of Bolivian wild potatoes with a focus on those species poorly or non-represented in gene banks, since the latter will likely represent a disproportionally high addition to the genetic diversity of wild potatoes.

In situ conservation status

We have used two complementing methods, i.e. spatial analysis and field observations, to evaluate the threat status of wild potato species in Bolivia (chapter 3).

The spatial analysis, using occurrence data of species in combination with threat maps, emphasized the possible sources of threat that may endanger species in specific areas. Following this approach, seven endemic species were identified as the most threatened ones. Although the threat maps are at a continental scale (Jarvis *et al.*, 2010), they are relevant as a starting point to estimate the threat sources at a local level. The relevance of the analysis using threat maps is to obtain an approximation of the threat that the species will encounter in the near future (2-5 years), and the results assist in defining conservation actions to be taken in order to avoid or reduce risks. The (preliminary) assignment of an IUCN Red List category applying the well standardized IUCN criteria leads to an internationally accepted threat level for each species.

Since the occurrence of wild potatoes under *in situ* conditions is dynamic in time and space, the results of spatial analyses based on information of databases should be interpreted with caution because in most cases the information in these databases comprises different time periods and may thus under – or over – estimate the threat status of a particular species due to the presence of a large number of occurrence points (e.g. in *S.* × *sucrense*). In such cases, the species could be affected by recent threats that do not appear in the information and hence not in the results. Also, many of these points or localities of occurrence may well represent populations that have disappeared recently. On the other hand, knowledge about population occurrences might be absent because they occur in inaccessible environments.

Monitoring and assessing the *in situ* conservation status of wild potato species in Bolivia, using complementing field observations and tools for spatial analysis, showed that the occurrence of populations of wild potatoes is determined by climatic conditions and the influence of human activities. In several cases, the latter have favoured the dispersal of plants, but in general the increased pressure on wild populations by human activities will cause the disappearance of some species with small populations and narrow distribution areas. Such decline can also be caused by severe weather changes. Therefore, monitoring wild species populations should ideally be performed by using data that was systematically collected over time to detect changes timely, such as populations size, increasing threats, etc. (Iriondo *et al.*, 2008). Such actions are justified within national *in situ* conservation programs of crop wild relatives.

According to the observations made in this study, the threat level of the species is highly correlated with the biogeographic province where they occur. In the Yungas province, the environmental conditions and less disturbed habitat have favoured the conservation of the species. However, three of the 13 species reported from this province have been categorized as Critically Endangered following the IUCN criteria. This IUCN category was determined by a low number of collections, indicating a small population size, all in a very narrow distribution area. In this particular case, a number of presence points refer to a situation that existed long time ago, and so are less relevant in determining the conservation category. More expeditions are required to confirm the present status of these three species and the extent of occurrence, and also to test the hypothesis that the availability of a good quality habitat is more important to assess the conservation state of these wild species.

In Bolivia, the highest number of wild potato species is reported from the Bolivian-Tucuman province, and it also harbours the largest number of species assigned to a IUCN threat category (VU, EN or CE). Field observations and spatial analyses indicated the fragility of the ecosystems, mainly due to the effect of intense human activities but also because in this province the influence of climate change seems to be more severe in terms of increased temperatures and prolonged droughts.

Jarvis *et al.* (2008), using projected future climate data, showed that the predicted effects of climate on the distribution and richness of wild potato are strong. In Bolivia, endemic species occurring in the Bolivian-Tucuman biogeographic province seem to be most affected. In this province at least one endemic species was predicted to go extinct due to the complete loss of climatically suitable areas, while another two species will experience area losses above 80% (Jarvis *et al.*, 2008). When the analysis of future climate change effects is combined with other evaluations of future conditions, e.g. alteration of habitats, anthropogenic impacts and other threats, the status of many of the potato species will probably appear as being highly threatened, and measures to conserve the genetic resources will need to be expanded.

In the Puna Mesophytic province only one out of the six reported species requires more attention due to its threatened situation determined by its restricted distribution and because it is seriously affected by the effect of overgrazing, agriculture and fires. No threat condition was assigned to the other species because of their comparatively wide distribution area. However, evidence of the rapid increase of degraded ecosystems in this province, due to livestock grazing, wood extraction, forests replaced by grasses and weeds (chapter 2), shows that in the medium to short term the resilience of widespread species is likely to be affected and these may acquire a threat condition as well.

In the Puna Xerophytic province, the most common species is the frost-resistant *S. acaule*. Despite severe weather conditions in this province, field observations showed that *S. acaule* forms abundant populations in semi- to heavily disturbed environments. Because it is a non-endemic species with a wide distribution, a threat maps assessment was not considered, but according to Jarvis *et al*. (2010) montane xeric shrublands are expected to be the least threatened of all ecosystems in South America. Therefore, populations of this species are likely to remain safe in the near future.

The preceding paragraphs show that the combined use of field evaluation methods with geographic information systems should be preferred to evaluate the threat status of target species because both are complementary. However, field evaluations mean having extensive human resources with expertise in different areas, as well as sufficient funds and time (to have reliable data, more than a year of assessment may be required). Although a GIS-based methodology will be more efficient in this respect, there is a risk that it will over- or under-estimate threat status. However, it still offers valuable means for targeting conservation efforts, saving time and money in the process, and when limited resources are available, GIS tools are the best option to evaluate an *in situ* conservation status.

Ex situ conservation status

Species representation in gene banks

Representative samples of Bolivian wild potato species are being preserved *ex situ* in at least 10 gene banks worldwide (van Soest, 2006). Some of these are being maintained in the gene bank of the Center for Genetic Resources, the Netherlands (CGN), and in 2004 this collection was repatriated to the country of origin to be inserted in the National genebank of Andean tubers and roots at Cochabamba. The collection was subsequently supplemented with new collecting during 2006 to 2009, making a total of 618 accessions of 30 wild species, of which 235 accessions correspond to 19 endemic species. These numbers do not yet cover all of the Bolivian potato taxa, because this country harbours 34 wild potato species of which 21 are endemic (Spooner and Salas, 2006; Hawkes and Hjerting, 1989).

Gene banks aim to cover the maximum amount of genetic variation and the entire range of environmental adaptation of the target species. In order to reach this the objective for most collecting expeditions is to fill in the gaps and correct some biases in the collection. This method is described by Hijmans *et al.* (2000) for the Bolivian potato collection. Still, our own as well as other collecting explorations often contribute to an increased redundancy, i.e. collecting of samples of species that were already over-represented in the Bolivian potato *ex situ* collection, except for three species (*S. avilesii, S. neocardenasii* and *S. neovavilovii*) for which the number of samples in the collection has increased 3 to 6-fold. Two other species (*S. soestii* and *S. flavoviridens*) still remain poorly represented in the *ex situ* collection and of two species there is no genetic material (*S. bombycinum* and *S.* ×*litusinum)*.

The representativeness of *ex situ* collections can be measured in taxonomic, genetic or ecogeographical terms. The taxonomic representation can simply be assessed in terms of the percentage of the target species (or taxa such as subspecies and varieties) covered. The genetic representation can be assessed by comparing total and sampled genetic diversity. Morphological descriptors, molecular markers or agronomic evaluation data can be used to assess or estimate the genetic diversity in collections. However, estimation of the total genetic diversity of the existing species in nature can be an unattainable task (Parra-Quijano *et al*., 2012).

Alternatively, ecogeographical representativeness can indirectly reflect genetic representativeness, and thus genetic diversity can also be assessed by comparing total and sampled ecogeographical range of *ex situ* collections. In order to facilitate ecogeographical representativeness assessments different methods are described by Parra-Quijano *et al*. (2012). Advances in ecogeographical land characterization maps (Parra-Quijano *et al*., 2008), gap analysis (Scheldeman and van Zonneveld 2010) and species distribution models (Segura *et al*., 2003) render it possible to detect ecogeographical gaps and locate potential collecting sites. The representativeness of *ex situ* collections can be increased in both quantitative (number of accessions) and qualitative (genetic and ecogeographical range) terms. In the case of the Bolivian potato collection, the gap analysis was used to identify new areas for future collecting expeditions. However, the application of complementary tools such as ecogeographical land characterization maps that include climatic, edaphic and geophysical variables, could also be considered. In this sense genetic material from marginal environments of the species' range, would provide plant breeders with a very important source of traits (genes) related to extreme abiotic tolerance, and also would improve the representativeness of the potato collection, not only in quantitative but also qualitative terms.

Preserving genetic integrity and genetic differentiation

An important concern of *ex situ* collections is to preserve the genetic integrity of the material originally collected. When the Bolivian gene bank repatriated the wild potato collection from the Netherlands, most of the accessions were more than 40 years old and the immediate question was whether the received material still retained its genetic integrity and whether it was still representative of the diversity existing in the original collecting sites.

The test on the genetic integrity, using microsatellite markers, was performed on plant material that went through a process of seed regeneration and multiplication during *ex situ* conservation. Genetic changes between different generations of *ex situ* germplasm were observed in the samples of five out of the seven species considered in the study (see chapter 5). Potential causes of these changes include genetic drift and contamination resulting from human error during regeneration.

Because changes were detected during the *ex situ* conservation process, strict monitoring techniques should be implemented in germplasm management protocols to assess genetic changes and track possible human errors. The method used in this study allowed the detection of genetic changes between generations. However, those changes were not quantified, i.e. we could not tell whether there was loss of genetic diversity between generations and to what extent. Multiple private alleles were observed in nearly all comparisons, some populations lose while others gain alleles. However, the detection of private or rare alleles highly depends on the sample size used for the analysis. The use of a small sample bears the risk that rare alleles will not be detected even though they are still present in the population. Because of that, Chebotar *et al.* (2003) suggested to reconsider the evaluation data obtained from different samples over the years in order to detect true genetic changes. It is therefore important to not only know if there have been genetic changes, but also to quantify them (Ferdinandez *et al.*, 2005). Furthermore, a better understanding of the nature of the genetic changes would contribute to make decisions, for example in the modification of the standard protocol for the regeneration of wild potato that suggests to take at least 25 plants for seed production (Salas *et al.*, 2008). The higher the number of plants for seed production, the less probability to lose low frequency alleles. ulloo *et al.* (2008) suggest that 90–210 seeds are needed to retain low frequency alleles (with a frequency of 0.003 to 0.05) for 10-150 loci with a probability of 90–95%, but this number of plants can be impractical in terms of available time and field surface.

In order to determine the genetic integrity of gene bank collections compared to the original wild populations, populations of six species generated under *ex situ* conditions were also compared with re-collected *in situ* populations from the same location or area as the original collection (see chapter 5). The results showed highly significant differences in all cases. Potential causes for these differences are changes during *ex situ* maintenance, sampling effects during collecting and *in situ* genetic change over time.

The study showed wide genetic differences in time and space between materials originating the same collecting area, and indicated that the variability present in a population cannot be captured by a single germplasm collection composed of a few samples. We must conclude that the genetic diversity of germplasm distributed by a gene bank does not necessarily represent that found in the source population in the wild. It is now widely accepted that complementary or integrated conservation involving a combination of both *in situ* and *ex situ* techniques, each with their advantages and disadvantages, is most likely to secure diversity for future use (Iriondo *et al.*, 2008). *In situ* preservation may be important as a back-up of diversity already present in gene banks and for the preservation of new diversity which can be accessed in future re-collections (el Rio *et al.*, 1997b). Furthermore, germplasm in gene banks can also be considered as an *ex situ* safety duplicate of *in situ* conserved material, because if for some reason the original population declines or goes extinct, the manager can always obtain seeds from the gene bank in an attempt to restore the natural population using samples from the original population (ulloo *et al.*, 2010; Hunter and Heywood, 2011; Iriondo *et al.*, 2008).

Recommendations for a general conservation strategy

As discussed earlier, the conservation of genetic resources of wild potatoes must be approached integrally, i.e. by *in situ* and *ex situ* plans. In line with the outcomes of this and other studies, some basic ideas for the conservation strategy of wild potatoes in Bolivia are mentioned here.

In situ conservation

Underlying principle: Move from a passive to an active *in situ* conservation of wild potatoes.

Main steps:

1. Establish priorities: target species and areas for conservation

Financial restrictions prevent the implementation of conservation plans in each ecosystem where wild potatoes are present, and render it necessary to direct efforts to prioritized areas. The spatial analysis applied in our study identified eight priority areas for *in situ* conservation of the 21 Bolivian endemic wild potato species. In these areas we predict a high concentration of species and a relatively low level of threat. This is a first step to direct conservation efforts of wild potato species, although this prioritization still needs a field validation to determine the actual species density and threat level, as well as an assessment of the feasibility of carrying out conservation actions. When the method proves sufficiently reliable, it can be extended to other species groups and areas. Furthermore, identifying conservation areas does not automatically guarantee that conservation actions can be performed effectively in these locations. The combination of different factors from political, social, economic to technical and environmental should be considered before suggesting conservation plans (Hunter and Heywood, 2011).

2. Design conservation plans for the target species and areas

Appropriate and tailor-made conservation plans should be designed according to the type of area identified. For example, in this study the highest priority areas were located in two different biogeographic provinces: Bolivian-Tucuman and Yungas. While the target area in Yungas is inside a protected area with its own management plans, the one in the Bolivian-Tucuman province is strongly influenced by agricultural activities. Thus, in the latter area the focus should be more on on-farm conservation involving the agroecosystem which includes useful species (crops, forages and agroforestry) as well as their wild relatives growing in nearby areas.

The design of a tailor-made conservation plan for wild potatoes, would include a number of actions, such as:

- determining the distribution of wild potato species in each priority area (i.e. updating and detailing the information so far obtained by ecogeographic data;
- determining the patterns of infra-specific diversity;
- updating the status of conservation (threat status);
- determining the feasibility of implementing recovery actions in the case of species with some degree of threat (e.g. through assisted migration or re-introduction of populations);
- determining the extent of the threats affecting the conservation status of the species;
- detailing actions that will be required to contain, reduce or eliminate the threats and ensure the maintenance of viable populations of the species;
- establishing a program of systematic monitoring of wild species in order to detect changes in populations timely.

3. Involvement of local stakeholders for the conservation of wild relatives

From the above list of necessary actions to arrive at a sound *in situ* conservation plan it is clear that such will not be possible without the involvement and support of local communities in both protected areas and agro-ecosystems. Such plans require the active promotion of the sustainability of the target species and maintenance of the natural, semi-natural or agricultural environment. The involvement of local communities through participatory methods seems crucial to help minimize human threats (e.g. overuse of pesticides and overgrazing) leading to genetic diversity loss. From our experience we learned that local communities are often aware of the existence of wild crop relatives but the benefits and importance of those species are often not obvious. Therefore, it is essential to raise awareness on these issues at different levels, from schools to local authorities, and construct partnerships in order to combine resources and work for the common purpose of strengthening the *in situ* conservation of potato wild relatives, and come up with a clear benefit model.

***Ex situ* conservation**

Underlying principle: conservation of the maximum amount of genetic variation and maintenance of genetic integrity.

Main steps:

1. efinition of target areas and species for new collecting efforts in order to improve the representativeness of the potato collection

After repatriation from the Netherlands and additional collecting, an important wild potato collection is maintained by the Bolivian gene bank of Andean tubers. However, even in this impressive *ex situ* collection not all species are well represented. Of the 21 Bolivian endemic species, three are poorly represented and there is no material (no accessions) from an additional two species. To improve the taxonomic coverage of the collection targeting of selected priority sites for collecting samples is required. This study used Species Distribution Modelling to identify the most appropriate areas and to guide targeted germplasm collecting trips. The species distribution model is explained mainly by climatic factors, which generally are the most important parameters influencing species distribution (Scheldeman and van Zonneveld, 2010; Scheldeman *et al*., 2007; Segura *et al*., 2003). Some validation studies of modelled areas were not possible to find species in expected places but others successfully indicated new occurrences (Patiño, 2011; Delanoy *et al*., 2006; Jarvis *et al.,* 2005). This indicates that modelling is an important tool but needs to be validated in the field, and one should attempt to integrate additional factors for modelling such as ecological (notably seed dispersal and pollination) and even historical parameters such as past vegetation changes, past climate features, or even phylogenetic constraints to a presence in a specific area.

2. Definition of a sampling strategy

When collecting wild potatoes, it is necessary to review a sampling strategy that optimizes the amount of collected genetic diversity. The FAO drafted gene bank standards that recommend to collect seeds from a minimum number of 30-60 plants (depending on the breeding system of the target species) in order to capture at least one copy of 95 percent of the alleles that occur within the target population with a frequency greater than 0.05 (FAO, 2013). The number of populations to be sampled depends on the distribution of genetic variation among populations, but this information is generally not available for most species, including wild potatoes. In order to ensure a sufficient coverage of the genetic diversity of a potato population, in general more than one visit will be needed, especially after dry years, because the occurrence of plants in a given year may depend on specific weather conditions during that year, as we observed during recent collecting of wild potato in Bolivia. Where possible, true seeds should be collected (as well as a herbarium specimen to verify taxonomic identity, even after changes in the taxonomic classification). If it is not possible to collect seeds, then other materials (tubers or plants) must be collected in the field, and replanted elsewhere to obtain seeds, but this is not preferred.

3. Re-collecting

This study has shown the existence of a wide differentiation between *ex situ* generations of the same accession, and also between germplasm material collected in different years from the same population. Therefore, to make sure that *ex situ* material provides a good representation of the *in situ* genetic variability, regular re-collecting of material is necessary. Unfortunately, this is virtually impossible for all accessions because of obvious time and capacity constraints. We recommend gene banks to develop a clear re-collecting strategy so as to prioritize species with few accessions (and therefore less variability), species that are endangered *in situ*, and species with known or potential favourable traits.

4. Revision of international and national regulations for acquisition of germplasm

New acquisitions have to be made in accordance with relevant international and national regulations. This is especially sensitive in countries that harbour centers of origin and diversity. Currently, many of the countries with high biodiversity, such as Bolivia, are subject to strong regulatory processes governing the movement of genetic resources. For collecting trips organized by domestic institutions, special care must be taken and appropriate permits obtained to collect in protected areas. If such collecting involves international organizations, the procedures are relatively long and complicated. Collaborative work between foreign institutions and national collections could help to facilitate field expeditions and the collecting of genetic material.

Ratifying the International Treaty on Plant Genetic Resources for Food and Agriculture will provide a sound framework for permits to collect and also to exchange germplasm, particularly for potato and other crops listed in Annex 1 (FAO, 2009). Bolivia is studying the possibility to ratify this Treaty.

5. Adoption of FAO gene bank standards

For general gene bank management procedures, any organization in charge of *ex situ* conservation should follow the FAO gene bank standards. These were recently revised by the Commission on Genetic Resources for Food and Agriculture (FAO, 2013). The implementation of the FAO gene bank standards requires strong national commitment and continuous financial support, and therefore gene banks should be monitored by a national body responsible for genetic resources.

6. Monitoring the genetic integrity of *ex situ* material

The FAO gene bank standards (FAO, 2013) provide guidelines for an optimal maintenance of seed viability and genetic integrity. These standards define the level of performance of routine gene bank operations below which there is a high risk of losing genetic integrity. The routine operations include acquisition of germplasm, drying and storage, seed viability monitoring, regeneration, characterization, evaluation, documentation, distribution, safety duplication and personnel security (FAO 2013). The maintenance of genetic integrity is mentioned as one of the underlying principles of the gene bank standards but the actual monitoring of this integrity is not yet part of the routine gene bank operations. This study provides arguments to consider the implementation of periodic monitoring of the genetic integrity as part of good practices during regeneration procedures in order to detect possible changes and help to combat human errors. As demonstrated by different authors, various molecular markers could facilitate such assessments (Börner *et al.*, 2000; Chebotar *et al.*, 2003; el Rio *et al.*, 1997a; Ferdinandez *et al.*, 2005; Spooner *et al.*, 2003; van Hintum *et al.*, 2007).

The regeneration process is one of the most critical steps and a major challenge in gene bank management, which involves the greatest risks to the genetic integrity of germplasm, due to selection, outcrossing or mechanical mixing (Dulloo *et al.*, 2010; Dullo *et al*., 2008). This study has found that in order to maintain the genetic integrity of accessions, standard procedures for regenerating wild potato species should be followed. Particularly the population size used during the regeneration procedure seems to be critical, as was also reported in other studies (Chebotar *et al*., 2003; Johnson *et al*., 2002). It is important to use a minimum number of plants for regeneration, in order to avoid loss of alleles. For potato, this number should not be below 20 because this study showed increased risk of genetic changes in regenerations with less than 20 plants used in the process. Chebotar *et al*. (2003) suggest that in the case of a serious decrease in the total number of individuals during the regeneration cycle, for whatever reason, the harvest should be omitted. Starting up a new regeneration procedure, and hence a repeated sowing, in the coming season should be favored instead.

To measure the impact that specific methods of conservation and regeneration of accessions have on genetic diversity of collections, details of such procedures must be well documented. These details can provide key information to correct errors or to remove limitations during seed management that may be the source of shifts in genetic variation between regenerations and hence a loss of the original genetic diversity.

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Summary

The wild relatives of potatoes (*Solanum* sect. *Petota*) form the genetic reservoir for the improvement of the cultivated potato. While wild potatoes can be found from the south-western United States to central Argentina and Chile, their centre of diversity is located in the Peruvian and Bolivian Andes. Bolivia harbours 39 wild taxa, 21 of which are endemic species. Because of the high importance of potato for human nutrition, vast efforts have been made to conserve these genetic resources, particularly *ex situ* in gene banks. In Bolivia, *in situ* conservation of wild potato has been incidental, rather than being the result of a planned policy. This study aimed to evaluate to what level the current *ex situ* and *in situ* management efforts have adequately conserved the genetic diversity of Bolivian wild potato species, and what recommendations can be formulated for improvement.

The study addressed the assessment of the status of wild potato species under *in situ* conditions for which complementary methods were used that included field observations and the use of geographic information tools (chapter 2, 3 and 4). Emphasis was put on endemic species, but during field trips observations of non-endemic species were also recorded. The effects of *ex situ* conservation efforts were analyzed through information obtained from databases of world gene banks of potato germplasm. The results allowed to estimate the representativeness of, and gaps in, the Bolivian *ex situ* potato collection (chapter 3). The genetic stability and diversity of material from different species under *ex situ* management practices was evaluated using molecular tools and compared with material re-collected *in situ* (chapter 5). The study provided recommendations for an integrated conservation approach based on our own results and in line with other studies (chapter 6).

In situ conservation status

The current conservation status of Bolivian endemic wild potato species was assessed using the globally accepted IUCN criteria and a methodology developed within the framework of the UNEP/GEF-Crop Wild Relative Project (CWR Project). Species classified with some degree of threat (Critically Endangered CR, Endangered EN and Vulnerable VU) according to the IUCN categories, were classified differently using the CWR Project criteria. Some of the CWR Project criteria should be revised so as to apply to

wild potato habitats. As an estimation of potential population decline, threat maps for natural ecosystems were used to understand the major factors that could affect the distribution and species richness of endemic wild potatoes. According to this assessment, five of the 14 endemic species placed in one of the IUCN threat categories (according to the IUCN criterion B) are of particular concern for protection because they are facing significant threats.

In Bolivia, the wild potato species are distributed in four of the ten biogeographic provinces existing in the country.

1. **Yungas**. Thirteen species occur in this province. Three of them (*S. bombicynum, S. flavoviridens* and *S. soestii*) have been preliminary categorized as Critically Endangered (CE) following the IUCN criteria. This category was assessed by the low number of collections. The analysis of the status of conservation, using additional information such as habitat quality, shows the conservation state of species occurring in this province is generally good.

2. **Bolivian Tucuman.** Twenty species occur in this province; the highest number of wild potato species in the country. Out of these, seven have received a preliminary threat category following the IUCN criteria. Additional analyses using threat maps for natural ecosystems confirm high threat levels in this province and data on populations and habitats also confirm the fragile situation of these species, mainly due to the effect of intense human activities (overgrazing, burning, agriculture and accessibility). There is no protection system (protected area) in this province and climate variability seems to be more severe in terms of increased temperatures and prolonged droughts.

3. **Puna Mesophytic.** Six species were reported in this province; five of them are widely dispersed, with only *S. achacachense* being a threatened species due to its restricted distribution and because it is seriously affected by the effect of overgrazing, agriculture and fires. This situation is confirmed by spatial analysis and field observations.

4. **Puna Xerophytic**. In this province six species were reported. The most common one is the frost-resistant *S. acaule*, the other five species are rare but more widespread in other biogeographic provinces with more suitable climatic conditions. espite the severe weather conditions in this province, the threat maps did not identify areas with high threat

levels and field observations showed that *S. acaule* forms abundant populations either in semi or heavily disturbed environments.

Spatial analysis allowed to distinguish eight priority areas for *in situ* conservation of the 21 Bolivian endemic wild potato species. These areas represent a high concentration of endemic species and have a relatively low level of threat, but only one of them has a conservation status. This is a first step to direct the conservation efforts for wild potato species.

Ex situ conservation

Representative samples of the wild potato species of Bolivia were maintained in the gene bank of the Center for Genetic Resources (CGN), the Netherlands, and in 2004, this collection was repatriated to the country of origin to be inserted in the National gene bank of Andean tubers and roots. The collection was subsequently supplemented with new collections during 2006 to 2009, making a total of 618 accessions of 30 wild species, of which 235 accessions correspond to 19 endemic species.

Despite efforts to have a balanced germplasm collection in terms of number of accessions per species in the potato gene bank, there are still two species lacking (*S. bombycinum* and *S.* × *litusinum*) while three more species (*S. neovavilovii, S. soestii* and *S. flavoviridens*) are poorly represented. Areas for additional collections of these wild potato species were identified by a gap analysis.

Genetic integrity and genetic differentiation

An important concern of *ex situ* conservation is to preserve the genetic integrity of the material originally collected. Thus, when the Bolivian gene bank repatriated the wild potato collection with most of the accessions being more than 40 years old, the immediate question was whether the material received still retained its genetic integrity and whether it was still representative of the diversity existing *in situ*. Microsatellite markers were used for this study.

The analysis was performed on accessions of seven taxa (*S. acaule* subsp. *acaule*, *S. avilesii, S. berthaultii, S. leptophyes, S. megistacrolobum* var.

toralapanum, S. neocardenasii and *S. okadae*) that went through a process of seed regeneration and multiplication during *ex situ* conservation. Genetic changes between different generations of *ex situ* germplasm were not observed for *S. leptophyes* and *S. megistacrolobum* var. *toralapanum*, but were detected for *S. neocardenasii* and *S. okadae*, and partially for *S. acaule* subsp. *acaule*, *S. avilesii* and *S. berthaultii*. Potential causes of these changes include genetic drift and contamination resulting from human error during regeneration.

To determine genetic differences, populations generated under *ex situ* conditions of accessions belonging to the taxa *S. acaule* subsp. *acaule, S. avilesii, S. berthaultii, S. leptophyes, S. megistacrolobum* var. *toralapanum* and *S. neocardenasii* were also compared with re-collected *in situ* populations from the same location or area as the original collection. The results showed highly significant differences in all cases. Potential causes for these differences are changes during *ex situ* maintenance, sampling effects during collecting and *in situ* genetic change over time.

Strong differences between *ex situ* and *in situ* material indicated the wide genetic variation in wild potatoes in time and space, and show that all variability cannot be captured in a single collection composed of a few samples. As such, it also stresses the importance of *in situ* conservation of wild potato species.

Recommendations for an integrated conservation strategy

The integrated conservation of Bolivian wild potatoes requires a combination of *in situ* and *ex situ* activities. The principle recommendation for the *in situ* conservation is to move from a passive to an active approach. For this three main steps are necessary: 1. Establish priorities: this study has provided indications to prioritize eight areas to conserve the 21 Bolivian endemic wild potato species; 2. esign a tailor-made conservation plan for wild potatoes according to the type of area identified i.e. protected area and agro-ecosystems; and 3. Involvement of local stakeholders for the conservation of potato wild relatives in order to construct partnerships and combine resources and work for the common purpose of strengthening the *in situ* conservation.

The underlying principle of the *ex situ* approach is the conservation of the maximum amount of genetic variation and the maintenance of the genetic integrity of the samples. Six steps are recommended for Bolivian potato wild relatives. This study used Species Distribution Modelling to identify the most appropriate areas and to guide targeted germplasm collecting trips (first step). The sampling strategy should ensure a sufficient coverage of the genetic diversity of a potato population, in general more than one visit to the collecting area is recommended and seed collecting should follow the FAO gene bank standards (second step). To make sure that *ex situ* material provides a good representation of the *in situ* genetic variability, regular re-collecting of species with few accessions (and therefore less variability), endangered *in situ*, and with known or potential favourable traits is necessary (third step). Collecting trips to obtain new acquisitions have to be made in accordance with relevant international and national regulations (fourth step). Gene bank management procedures should follow the FAO gene bank standards and this should be monitored by a national body responsible for genetic resources (fifth step). And finally, periodic monitoring of the genetic integrity should be implemented as part of good practices during regeneration procedures in order to detect possible changes and to help combat human errors (sixth step).

Resumen

Los parientes silvestres de papa (*Solanum* sect. *Petota*) constituyen la reserva genética para el mejoramiento de la papa cultivada. Las papas silvestres pueden encontrarse desde el sudoeste de Estados Unidos hasta el centro de Argentina y Chile, pero el centro de diversidad está en los Andes de Perú y Bolivia. En Bolivia se encuentran 39 taxas silvestres, 21 de las cuales son especies endémicas.   ebido a la gran importancia de la papa para el ser humano, amplios esfuerzos se han hecho para conservar estos recursos genéticos, particularmente *ex situ* en bancos de germoplasma. En Bolivia, la conservación *in situ* de las papas silvestres hasta ahora ha sido incidental, más que el resultado de una política planificada. En este sentido, este estudio estuvo orientado a evaluar si los actuales esfuerzos de manejo *ex situ* e *in situ* han conservado adecuadamente la diversidad genética de las especies silvestres bolivianas de papa, y qué recomendaciones pueden ser formuladas para mejorar su situación.

El estudio encaró la evaluación del estado de las especies silvestres de papa bajo condiciones *in situ* para lo cual se utilizaron métodos complementarios que incluyeron observaciones de campo y el uso de sistemas de información geográfica (capítulos 2, 3 y 4). Se puso énfasis en especies endémicas, pero durante los viajes de campo, observaciones de especies no endémicas también fueron registradas. Los efectos de los esfuerzos de conservación *ex situ* fueron analizados a través de información obtenida de bases de datos de colecciones mundiales de germoplasma de papa. Los resultados permitieron estimar la representatividad y los vacíos existentes en la colección *ex situ* de papa boliviana (capítulo 3). La estabilidad y diversidad genética del material de diferentes especies conservadas *ex situ* fueron evaluadas utilizando herramientas moleculares, y se compararon con material re-colectado *in situ* (capítulo 5). El estudio concluye con recomendaciones para una conservación integral basada en nuestros propios resultados y en sintonía con otros estudios (capítulo 6).

Estado de conservación *in situ*

El estado actual de conservación de las especies silvestres de papa endémicas de Bolivia fue evaluado utilizando los criterios de la UICN globalmente aceptados y una metodología desarrollada dentro del marco del proyecto

UNEP/GEF de Parientes Silvestres de Cultivo (Proyecto PSC). Las especies clasificadas con algún grado de amenaza (Críticamente amenazados CR, Amenazados EN, y vulnerables VU) de acuerdo a las categorías de la UICN tuvieron una clasificación diferente utilizando los criterios del Proyecto PSC. Sin embargo, algunos de estos criterios deberían ser revisados y ajustados para aplicarse al contexto de las papas silvestres para siguientes evaluaciones. Para estimar la declinación potencial de las poblaciones silvestres, mapas de amenazas de ecosistemas naturales fueron utilizados para entender los mayores factores que pueden afectar la distribución y la riqueza de especies silvestres endémicas de papa. e acuerdo a esta evaluación, cinco de 14 especies endémicas clasificadas en una de las categorías de amenaza de la UICN (de acuerdo al criterio B) merecen particular atención para protección porque están enfrentando amenazas significativas.

En Bolivia, las especies silvestres de papa están distribuidas en cuatro de las diez provincias biogeográficas que existen en el país.

1. **Yungas**. Trece especies ocurren en esta provincia. Tres de ellas *(S. bombicynum, S. flavoviridens y S. soestii*) han sido categorizadas preliminarmente como Críticamente amenazadas (CE) siguiendo los criterios de la UICN. Esta categoría fue asignada en base a un bajo número de colectas. El análisis del estado de conservación utilizando información adicional como calidad del hábitat, muestra un estado general bueno de conservación para todas las especies que ocurren en esta provincia.

2. **Boliviano Tucumano.** Veinte especies ocurren en esta provincia, que es el más alto número de especies silvestres de papa en relación a otras provincias. e éstas, siete han recibido una categoría de amenaza preliminar siguiendo los criterios de la UICN. Análisis adicional utilizando mapas de amenaza para ecosistemas naturales confirman el alto nivel de amenaza en esta provincia y datos de población y hábitat también confirman la situación frágil de estas especies, principalmente debido al efecto de intensas actividades humanas (sobrepastoreo, quema, agricultura y facilidad de acceso). No existe ningún sistema de protección (área protegida) en esta provincia y la variabilidad climática parece ser más severa en términos de incrementos de temperatura y sequía prolongadas.

3. **Puna Mesofítica.** Seis especies fueron reportadas en esta provincia; cinco de ellas tienen una amplia distribución, y solo *S. achacachense* ha sido determinada una especie amenazada debido a su distribución restringida y porque está seriamente afectada por efecto del sobrepastoreo, la agricultura y el chaqueo (quemas). Esta situación es confirmada por análisis espacial y observaciones de campo.

4. **Puna Xerofítica**. En esta provincia seis especies fueron reportadas. La más común es *S. acaule*, resistente a heladas, las otras cinco especies son raras en esta provincia pero están ampliamente distribuidas en otras provincias biogeográficas con mejores condiciones climáticas. A pesar de las condiciones climáticas severas en esta provincia, los mapas de amenaza no identificaron áreas con altos niveles de amenaza y observaciones de campo evidenciaron que *S. acaule* forma abundantes poblaciones tanto en ambientes poco o altamente disturbados.

Los análisis espaciales permitieron distinguir ocho áreas prioritarias de conservación *in situ* para las 21 especies silvestres endémicas de Bolivia. Estas áreas representan una alta concentración de especies endémicas y tienen relativamente bajo nivel de amenaza, pero sólo una de ellas está dentro de un sistema formal de protección (área protegida). Este es un primer paso para dirigir los esfuerzos de conservación de especies silvestres bolivianas de papa.

Conservación *ex situ*

Muestras representativas de especies silvestres de papa de Bolivia son mantenidas en el banco de germoplasma del Centro de Recursos Genéticos (CGN, por sus siglas en inglés) en Holanda, y en 2004, esta colección fue repatriada al país de origen para ser insertada al Banco Nacional de Germoplasma de Tubérculos y Raíces Andinas. Esta colección fue luego suplementada con nuevas colectas realizadas en el período 2006 al 2009, haciendo un total de 618 accesiones de 30 especies silvestres, de las cuales 235 corresponden a 19 especies endémicas.

A pesar de los esfuerzos de tener una colección *ex situ* de germoplasma de papa balanceada en términos de número de accesiones por especie, aún dos especies (*S. bombycinum* y *S. × litusinum*) están ausentes en la colección y otras tres (*S. neovavilovii*, *S. soestii* y *S. flavoviridens*) están pobremente representadas. Mediante el análisis de vacíos, se identificaron áreas para realizar colectas adicionales de estas especies silvestres de papa en Bolivia.

Integridad y diferenciación genética

Una preocupación importante de la conservación *ex situ* es preservar la integridad genética del material originalmente colectado. Entonces, cuando el banco boliviano repatrió la colección de papa silvestre con la mayoría de las accesiones que tenían más de 40 años de haberse colectado, la pregunta inmediata fue si el material recibido aún mantenía su integridad genética y si era aún representativo de la diversidad existente *in situ*. Marcadores microsatélites fueron utilizados en este estudio.

El análisis fue realizado en accesiones de siete taxa (*S. acaule* subsp. *acaule*, *S. avilesii*, *S. berthaultii*, *S. leptophyes*, *S. megistacrolobum* var. *toralapanum*, *S. neocardenasii* y *S. okadae*) que pasaron por un proceso de regeneración y multiplicación de semilla durante la conservación *ex situ*. No se detectaron cambios genéticos entre diferentes regeneraciones del germoplasma *ex situ* de *S. leptophyes* y *S. megistacrolobum* var. *toralapanum*, pero sí hubo cambios genéticos en *S. neocardenasii* y *S. okadae*, y parcialmente en *S. acaule* subsp. *acaule*, *S. avilesii* y *S. berthaultii*. Causas potenciales de estos cambios incluyen deriva y contaminación genética resultado de errores humanos durante el proceso de regeneración.

Para determinar diferencias genéticas, poblaciones generadas bajo condiciones *ex situ* de accesiones pertenecientes a *S. acaule* subsp. *acaule*, *S. avilesii*, *S. berthaultii*, *S. leptophyes*, *S. megistacrolobum* var. *toralapanum* y *S. neocardenasii* fueron comparadas con poblaciones recolectadas *in situ* en la misma localidad o área de la colección original. Los resultados mostraron diferencias altamente significativas en todos los casos. Causas potenciales para estas diferencias son cambios durante el mantenimiento *ex situ*, efectos de muestreo durante las colectas y cambios genéticos *in situ* en el tiempo.

Las fuertes diferencias entre los materiales *ex situ* e *in situ* evidenciaron la amplia variación genética existente en las papas silvestres en el tiempo y en el espacio, y muestran que toda la variabilidad no puede ser capturada en una sola colección compuesta por pocas muestras. Esto enfatiza la importancia de la conservación *in situ* de las especies silvestres de papa.

Recomendaciones para una estrategia integral de conservación

La conservación integral de las papas silvestres bolivianas requiere una combinación de acciones *in situ* y *ex situ*. El principio fundamental de un enfoque *in situ* es mover de una conservación pasiva hacia una conservación activa. Para esto, tres pasos principales son necesarios: 1. Establecer prioridades: este estudio provee indicaciones para priorizar ocho áreas para conservar las 21 especies silvestres endémicas de Bolivia; 2. Diseñar planes de conservación a medida para papas silvestres de acuerdo al tipo de área identificada, es decir para áreas protegidas y para agroecosistemas; y 3. Involucrar actores locales para la conservación de los parientes silvestres de la papa, esto para construir alianzas y combinar recursos y trabajo con el propósito común de fortalecer la conservación *in situ*.

El principio base de un enfoque *ex situ* es la conservación de la máxima cantidad de variación genética y el mantenimiento de la integridad genética. Seis pasos son recomendados para las papas silvestres bolivianas. Este estudio utilizó un análisis de modelamiento de distribución de especies para identificar las áreas más apropiadas y para guiar las colectas de germoplasma futuras (primer paso). La estrategia de muestreo debería asegurar una suficiente cobertura de la diversidad genética de una población de papa, en general se recomienda más de una visita al área de colecta y se deberían seguir los estándares de manejo de bancos de la FAO para la toma de muestras semilla (segundo paso). Para asegurar que el material *ex situ* provee una buena representación de la variabilidad genética *in situ*, son necesarias re-colecciones regulares de especies con pocas accesiones (y por lo tanto menos variabilidad), de poblaciones amenazadas *in situ*, y de materiales con caracteres conocidos o potencialmente favorables (tercer paso). Los viajes de colecta para obtener nuevas adquisiciones tienen que ser realizados siguiendo la normativa relevante internacional y nacional (cuarto paso). Los procedimientos de manejo de bancos de germoplasma deberían seguir los estándares de la FAO y éstos deberían ser monitoreados por la autoridad nacional competente de recursos genéticos (quinto paso). Y finalmente, un monitoreo periódico de la integridad genética debería ser implementado como parte de las buenas prácticas durante el proceso de regeneración con el fin de detectar posibles cambios y ayudar a combatir errores humanos (sexto paso).

Samenvatting

e wilde verwanten van de aardappel (*Solanum* sect. *Petota*) vormen een genetisch reservoir voor de verbetering van de cultuuraardappel. Terwijl wilde aardappelsoorten voorkomen van het zuid-westen van de Verenigde Staten tot aan centraal Argentinië en Chili, ligt hun centrum van diversiteit in de Peruaanse en Boliviaanse Andes. Bolivia herbergt 39 wilde taxa, waaronder 21 endemische soorten. Wegens het grote belang van de aardappel voor de menselijke voeding, heeft men veel moeite gedaan deze genetische hulpbronnen te conserveren, vooral *ex situ* in genenbanken. In Bolivia heeft *in situ* conservering van wilde aardappels tot nog toe incidenteel plaatsgevonden, en niet als resultaat van gerichte planning. Deze studie had als doel te evalueren in hoeverre de huidige inspanningen gericht op *ex situ* en *in situ* management geleid hebben tot een adequate conservering van de genetische diversiteit van Boliviaanse wilde aardappelsoorten, en welke aanbevelingen ter verbetering er kunnen worden geformuleerd.

e studie richtte zich op het vaststellen van de toestand van wilde aardappelsoorten onder *in situ* omstandigheden, waarbij complementaire methoden werden gebruikt waaronder veld observaties en het verwerken van geografische informatie (hoofdstukken 2, 3 en 4). e nadruk lag op endemische soorten maar tijdens het veldwerk werden er ook waarnemingen van niet-endemische soorten gedaan. e effecten van *ex situ* conserveringsinspanningen werden geanalyseerd met behulp van informatie uit de databanken van mondiale genenbanken met aardappel genen materiaal. e resultaten maakten het mogelijk in te schatten hoe representatief de Boliviaanse *ex situ* aardappel collectie is en welke lacunes ze bevat (hoofdstuk 3). e genetische stabiliteit en diversiteit van materiaal van verschillende soorten onder *ex situ* management werden geevalueerd met behulp van moleculaire methoden, en vergeleken met herverzameld *in situ* materiaal (hoofdstuk 5). e studie leverde aanbevelingen op voor een geintegreerde conserveringsbenadering gebaseerd op eigen resultaten en in lijn met andere studies (hoofdstuk 6).

De status van *in situ* conservering

e huidige conserveringsstatus van Boliviaanse endemische wilde aardappelsoorten werd bepaald met behulp van de breed geaccepteerde IUCN criteria en een methodologie ontwikkeld binnen het UNEP/GEF-Crop Wild Relative project. Soorten die volgens de IUCN categorieën als in een zekere mate bedreigd (Critcally Endangered, Endangered, en Vulnerable) werden beschouwd, werden anders ingedeeld met de CWR project criteria. Sommige van de CWR project criteria zouden moeten worden herzien om ze van toepassing te laten zijn op de habitats van wilde aardappelen. Als een inschatting van de potentiële teruggang van populaties, werden bedreigingskaarten voor natuurlijke ecosystemen gebruikt om de voornaamste factoren te begrijpen die de verspreiding en soortenrijkdom van endemische wilde aardappelen kunnen beinvloeden. Volgens deze inschatting, zijn vijf van de veertien endemische soorten die in één van de IUCN bedreigingscategorieën zijn geplaatst (volgens IUCN criterium B) onderwerp van bijzondere zorg met betrekking tot bescherming, omdat zij significant bedreigd worden.

In Bolivia zijn wilde aardappel soorten verspreid in vier van de tien biogeografische provincies die in het land aanwezig zijn.

1. **Yungas**. ertien soorten komen in deze provincie voor. rie daarvan (*S. bombicynum*, *S. flavoviridens* en *S. soestii)* zijn voorlopig ingedeeld als Critically Endangered volgens de IUCN criteria. Ze werden aan deze categorie toegeschreven door het lage aantal verzamelingen. e analyse van hun conserveringsstatus, met gebruikmaking van aanvullende informatie als habitat kwaliteit, toont dat die status van de soorten in deze provincie in het algemeen goed is.

2. **Boliviaans Tucuman**. Twintig soorten komen in deze provincie voor; dit is het hoogste aantal wilde aardappelsoorten in het land. Hiervan hebben zeven een voorlopige bedreigingscategorie gekregen volgens de IUCN criteria. Verdere analyses met gebruikmaking van bedreigingskaarten voor natuurlijke ecosystemen bevestigen de hoge mate van bedreiging in deze provincie en gegevens over populaties en habitats wijzen ook op de risicovolle situatie van deze soorten, vooral toe te schrijven aan het effect van intensieve menselijke activiteiten (te intensieve begrazing, brand, landbouw en betreding). Er is geen

beschermd gebied in deze provincie en de klimaat omstandigheden lijken extremer met hogere temperaturen en langdurige droogte.

3. **Puna Mosophytic**. Zes soorten zijn in deze provincie waargenomen. Vijf daarvan zijn wijd verpsreid en alleen *S. achacachense* is een bedreigde soort door een beperkte verspreiding en omdat deze soort ernstig wordt beinvloed door intensieve begrazing, landbouw en branden. Deze situatie werd bevestigd door ruimtelijke analyse en veld observaties.

4. **Puna Xerophytic**. In deze provincie zijn zes soorten aanwezig. e meest algemene soort is de vorst tolerante *S. acaule*, de overige vijf zijn zeldzaam maar hebben een wijdere verspreiding in andere biogeografische provincies met geschiktere klimatologische omstandigheden. Ondanks de invloed van het weer gaven bedreigings kaarten geen gebieden met een hoge mate van bedreiging aan en uit veldobservaties bleek dat *S. acaule* overvloedig populaties vormt, zowel in gedeeltelijk als in ernstig verstoorde groeiplaatsen.

Ruimtelijke analyses leidden tot het onderscheiden van acht gebieden met prioriteit voor *in situ* conservering van de 21 Boliviaanse endemische wilde aardappelsoorten. eze gebieden vertegenwoordigen een hoge concentratie van endemische soorten en hebben een relatief laag bedreigingsniveau, maar slechts één er van heeft een status als beschermd gebied.

Ex situ conservering

Representatieve monsters van wilde aardappelsoorten van Bolivia werden bewaard in de genenbank van het Centrum voor Genetische hulpbronnen, Nederland (CGN), en in 2004 werd deze collectie gerepatrieerd naar het land van oorsprong om ingevoegd te worden in de nationale genenbank voor knollen en wortels van de Andes (*Banco Nacional de Germoplasma de Tubérculos y Raíces Andinas)*. De collectie werd vervolgens aangevuld met nieuwe verzamelingen in de periode van 2006 tot 2009, leidend tot een totaal van 618 accessies van 30 wilde soorten, waarvan 235 accessies 19 endemische soorten betreffen.

Ondanks het streven naar een evenwichtige collectie in termen van aantallen accessies per soort in de aardappel genenebank, ontbreken er toch twee soorten

(*S. bombycinum* en *S.* × *litusinum*), terwijl nog drie soorten (*S. neovavilovii*, *S. soestii* en *S. flavoviridens*) slecht vertegenwoordigd zijn. Gebieden waar aanvullende verzamelingen van deze wilde aardappelsoorten zouden moeten worden gedaan werden geidentificeerd door 'gap analysis'.

Genetische integriteit en genetische differentiatie

Bij *ex situ* conservering is het van veel belang de genetische integriteit van het oorspronkelijk verzamelde materiaal te bewaren. Toen de Boliviaanse genenbank de verzameling wilde aardappels repatrieerde, waarvan het merendeel van de accessies meer dan 40 jaar oud was, was de onmiddelijke vraag of het ontvangen materiaal nog zijn genetische integriteit had bewaard en of het nog representatief was voor de *in situ* aanwezige diversiteit. Microsatelliet merkers werden voor dit onderdeel van de studie gebruikt.

e analyse werd toegepast op materiaal van zeven taxa (*S. acaule* subsp. *acaule*, *S. avilesii*, *S. berthaultii*, *S. leptophyes*, *S. megistacrolobum* var. *toralapanum*, *S. neocardenasii and S. okadae*) dat door een proces van zaad regenratie en vermeerdering was gegaan tijdens de *ex situ* conservering. Genetische veranderingen tussen de verschillende *ex situ* generaties werden niet waargenomen voor *S. leptophyes* en *S. megistacrolobum* var. *toralapanum*, maar wel voor *S. neocardenasii* en *S. okadae*, en geddeltelijk voor *S. acaule* subsp. *acaule*, *S. avilesii* en *S. berthaultii*. Mogelijke oorzaken voor deze veranderingen zijn genetic drift and vervuiling van monsters door menselijke fouten tijdens de regeneratie.

Om genetische verschillen vast te stellen werden populaties ontstaan onder *ex situ* omstandigheden behorend tot de taxa *S. acaule* subsp. *acaule*, *S. avilesii*, *S. berthaultii*, *S. leptophyes*, *S. megistacrolobum* var. *toralapanum* en *S. neocardenasii* ook vergeleken met herverzamelde *in situ* populaties afkomstig van dezelfde locatie of hetzelfde gebied als de oorspronkelijke verzameling. e resultaten lieten zeer significante verschillen zien in alle gevallen. Mogelijke oorzaken voor deze verschillen zijn veranderingen tijdens de *ex situ* instandhouding, steekproef-effecten tijdens het verzamelen en *in situ* genetische verandering in de loop van de tijd.

e grote verschillen tussen *ex situ* en *in situ* materiaal wijzen op de brede genetische variatie in wilde aardappelen in de tijd en de ruimte, en tonen aan dat niet alle variatie in één enkele verzameling bestaande uit slechts nekele monsters gevangen kan worden. it benadrukt ook het belang van in situ conservering van wilde aardappelsoorten.

Aanbevelingen voor een geintegreerde conserveringsstrategie

De geintegreerde conservering van Bolivaanse wilde aardappelen vereist een combinatie van *in situ* en *ex situ* activiteiten. e voornaamste aanbeveling voor *in situ* conservering is om van een passieve naar een actieve benadering te gaan. Hiervoor zijn drie belangrijke stappen nodig: 1. Prioriteiten vaststellen: deze studie heeft aanwijzingen opgeleverd om acht gebieden prioriteit te verlenen om de 21 Boliviaanse endemische wilde aardappelsoorten te conserveren; 2. Een toegesneden conserveringsplan ontwerpen voor wilde aardappels per type geidentificeerd gebied, te weten beschermd gebied en agro-systeem; en 3. Locale partijen betrekken bij het conserveren van de wilde verwanten van aardappel om samenwerkingsverbanden op te zetten en de hulpbronnen en activiteiten te combineren gericht op het versterken van *in situ* conservering.

Het onderliggende principe van de *ex situ* benadering is het bewaren van de maximale hoeveelheid genetische variatie en het in stand houden van de genetische integriteit van de monsters. Zes stappen worden aanbevolen voor de wilde verwanten van de Boliviaanse aardappels. Deze studie gebruikte het modelleren van soortsverspreidingen (Species Distribution Modelling) om de meest geschikte gebieden te identificeren en om gerichte verzamelreizen op te zetten (eerste stap). De verzamel-strategie zou er voor moeten zorgen dat voldoende van de genetische diversiteit van een aardappel populatie gedekt wordt, in het algemeen wordt meer dan één bezoek aan een gebied aangeraden, en het verzamelen van zaden zou de FAO genenbank standaarden moeten volgen (tweede stap). Om er zeker van te zijn dat *ex situ* materiaal de *in situ* genetische variatie goed vertegenwoordigt, is het nodig regelmatig soorten met weinig accessies (en daardoor minder variatie) die *in situ* bedreigd worden en bekende of potentiële gunstige eigenschappen bezitten opnieuw te verzamelen (derde stap). Verzamelreizen om nieuw materiaal te verkrijgen moeten zich houden aan de relevante internationale en nationale regels (vierde stap). e management procedures van genenbanken moeten de FAO genenbank standaarden volgen en dit zou door een nationale instantie verantwoordelijk voor genetische hulpbronnen moeten worden gecontroleerd (vijfde stap). En, tenslotte, de genetische integriteit moet van tijd tot tijd worden gecontroleerd als onderdeel van een juiste handelwijze tijdens het regeneratie proces, om eventuele veranderingen te ontdekken en om te helpen menselijke fouten de bestrijden (zesde stap).

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The research presented in this book is the result of contributions from many colleagues who have directly or indirectly participated in different stages throughout my work done in the field, laboratory and desktop, both in Bolivia and the Netherlands. To all of them I owe my eternal gratitude for contributing to my professional development. A huge list of names comes to my mind, and surely I will make the mistake of not being able to mention all, so I apologize if I miss a name. I want to start with my (co) promoters: Prof. dr. Marc S.M. Sosef, Dr. Ronald G. van den Berg and Dr. Robbert van Treuren to whom I am very grateful for their valuable support, for the profound criticisms they provided, making it a constant challenge to find answers, I thank them for their constructive suggestions and insights. I thank r. Loek van Soest for originally motivating the research and get me in touch with the Biosystematics group and the Centre for Genetic Resources, the Netherlands (CGN) at Wageningen to start the project of my thesis. I also thank Roel Hoekstra from CGN with whom I had fruitful professional interactions and whose kindness in providing information on CGN gene bank was critical to feed the discussions of the thesis. I am indebted to the Fundación PROINPA, to all my colleagues and friends of the technical and administrative staff, for the continuous support I have enjoyed. I am especially grateful to Antonio Gandarillas, general manager, from whom I have received constant support to finish my thesis. During the fieldwork (prospecting trips and collecting wild potatoes) the contribution of my colleagues Fernando Patiño and Franz Terrazas from PROINPA, and Alberto Salas from CIP was decisive. Thanks to Alberto and Ronald van den Berg (who accompanied me on a trip) for master classes on taxonomy of wild potatoes in the field. In the laboratory of molecular biology PROINPA, I had the valuable support of Jorge Rojas at the beginning of my study, and Silene Veramendi to the end. I could also count on the timely and gentle support of Nynke Groendijk-Wilders from the Biosystematic group with the regeneration of plants in the Netherlands. The introduction to the world of GIS I got from experts of Bioversity International, thanks for your valuable teachings Maarten van Zonneveld, Xavier Scheldeman and Nora Castañeda. Also thanks to André van Proosdij from the Biosystematics group whose assistance was decisive to untangle some methodological aspects of this research. I also thank Marielos Peña with whom I had a short but very productive meeting where she gave an impulse to this work. I am grateful to NUFFIC for providing a PhD fellowship to make this research possible. I am also grateful to the whole Biosystematics group for their warm welcome during my stays in the Netherlands, and to my "Wageningen friends" who made me feel at home.

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My parents and my brother and sisters were always with me, *aunque mi papito no está presente siento que su fuerza y espíritu nunca me abandonó, sólo lamento no haber podido poner en tus manos el resultado final de este esfuerzo antes que abandonaras este mundo*. Last but definitely not least, I want to thank my husband Iván del Callejo and my children Elías and Andrés for their love and continuous support. *Iván eres mi equilibrio, el alimento a mi espíritu y mi alma, a ti te debo la construcción de nuestro más grande reto que es el proyecto de nuestras vidas. Gracias por estar siempre a mi lado.*

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Curriculum vitae

Name: Cadima Ximena

Place of birth: Cochabamba, Bolivia

Date of birth: February, 18, 1970

Nationality: Bolivian

Undergraduate studies:

Universidad Mayor de San Simón, Facultad de Ciencias Agrícolas y Pecuarias "Martín Cárdenas" – Bolivia. Agricultural Engineer, 1993.

Graduate studies:

Wageningen University, the Netherlands. M.Sc. in biotechnology 1999.

Career:

2001 – Present. Coordinator for the Agrobiodiversity and Genetic Resources Area in Fundación PROINPA (Promotion and Research of Andean Products) in Bolivia. Leader of more than 10 research projects from 2005 to date.

2002-2010 Responsible Subsystem Andean tubers and roots under the National System of Genetic Resources for Food and Agriculture (SINARGEAA by its Spanish acronym). Gene bank curator of Andean roots and tubers.

2000-2001 Consultant in genetic resources and biodiversity. Regional Biodiversity Strategy for the Tropical Andean countries. Andean Community, Consortium GTZ / FUNDECO / Ecology Institute / Fundación PROINPA.

1999-2000 Consultant in Genetic Resources. Bolivian National Strategy for Biodiversity Conservation. Ministry of Sustainable evelopment and Planning, Department of Biodiversity / Fundación PROINPA.

Educational activities:

Supervisor and tribunal reviewer of master's thesis (10) and undergraduate (5) in the period 2002-2011

Instructor in the short course "Plant Genetic Resources, Strategic richness for the country's development." Instituto Nacional de Innovación Agropecuaria y Forestal (INIAF), Bioversity International and Fundación PROINPA. April 20 to June 5, 2009. Cochabamba, Bolivia

Instructor in the e-learning course "Plant Genetic Resources". Inwent Internacional and Fundación PROINPA. May 27 to 29, 2009. Cochabamba, Bolivia

Teacher invited to the subject "Ex situ conservation" in Masters Program in Management and Conservation of Genetic Resources and Applied Plant Biotechnology. Faculty of Agriculture, Livestock, Forestry and Veterinary. Universidad Mayor de San Simon. August, 01-02, 2007.

Awards:

American Agricultural Award for Young Professionals. IICA. 2005

National Agricultural Award for Young Professionals. IICA - Bolivia. 2005

The Graduate School EXPERIMENTAL PLANT SCIENCES

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1) Start-up phase	date
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Comparative assessment of genetic diversity conservation of ex situ and in situ Bolivian wild potatoes	Nov 20, 2007
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Designing a strategy for a complementary ex situ and in situ conservation of genetic diversity of Bolivian wild potatoes	Nov 2007-Feb 2008
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Subtotal Start-up Phase — *7.5 credits**

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EPS PhD student day, Wageningen University, Wageningen, NL	Sep 13, 2007
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Theme 4 'Genome Biology', Wageningen University	Dec 04, 2014
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Invited Seminar Anja Geitmann: From molecule to morphology - Biomechanical principles governing cell morphogenesis	Sep 21, 2007
Plant Sciences Seminars by Harro Bouwmeester: The interaction of plants with their environment, and by Ton Bisseling: From epigenetics to novelle organelles and organs	Sep 8, 2009
Plant Sciences Seminars by Olaf van Kooten: Does plant physiology relate to consumer satisfaction?, and by Jack Leunissen: Bioinformatics	Oct 13, 2009
The 5th Annual International Symposium of Fontagro projects, Cali, Colombia	Jun 02-04, 2010
WEES Seminar Bas Haring: The value of Biodiversity	Sep 16, 2010
Plant Science Seminar: 'Creating the tools for plant health' . Seminars by Piet Boonekamp and Robert Czajkowski: Ecology and control of blackleg causing biovar 3 Dickeya sp. on potato	Oct 12, 2010
The 5th Annual International Symposium of Fontagro projects, Cali, Colombia	Jun 02-04, 2010
The 6th Annual International Symposium of Fontagro projects, Cochabamba, Bolivia	Jun 15-17, 2011
The 7th Annual International Symposium of Fontagro projects, Monteria, Colombia	Jul 10-12, 2012
The 8th Annual International Symposium of Fontagro projects, Montevideo, Uruguay	Jul 23-25, 2013
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5th International Symposium on the Taxonomy of Cultivated Plants	Oct 15-19, 2007
SIRGEALC, 2007. Mexico (International Symposium on Genetic Resources)	Nov 13-16, 2007
SIRGEALC, 2011. Ecuador (International Symposium on Genetic Resources)	Nov 21-23, 2011
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Poster: SIRGEALC 2007	Nov 13-16, 2007
Poster: Congreso Boliviano de la Papa 2008	Jun 04 -06, 2008
Oral: National Meeting of Agricultural and Forestry Technology Innovation	Jun 29-30, 2009
Oral: Special Seminar on Climate Change at FAO-CGRFA-13 session	Jul 16, 2011
Oral: SIRGEALC 2011	Nov 21-23, 2011
Oral: II Congreso Boliviano de Botánica	Oct 11-13, 2012
Oral: Bolivian meeting of Innovation in Agriculture and Forestry	Aug 22-23, 2013
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Subtotal Scientific Exposure — *16.2 credits**

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Postgraduate course "Molecular phylogenics:reconstruction and interpretation' (EPS)	Oct 19-23, 2009
Postgraduate course 'Scenario development:understanding and applying multi-scale and participatory concepts' (PE&RC)	Oct 22-26, 2007
International course 'Plant genetic resources and seeds: strengthening community resilience', CDI, Wageningen, The Netherlands	Oct 29-Nov 16, 2012
ZĀĀ6PUROBMĀDMVC	
participant of the Biosystematic literature discussion group	2010
ZĀĀ5OEJVJEUBMĀSFTFBRDIĀTRBJOJOH	
Training in GIS analysis in Cali, Colombia (4 days)	Dec 07-10, 2010

Subtotal In-Depth Studies — *9.2 credits**

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Endnote advanced	Oct 04, 2007
ExPectationS (EPS Career day), Wageningen, NL	Nov 19, 2010
ZĀ :R HBOJTBUJPOĀPGĀ;ISTUEFOTĀEBYĀDPVRSFĀPSĀDPOGFRFODF	
Organisation of two genetic resources courses in Bolivia	2009
ZĀĀ8FNCFRSIJQĀPGĀ/PBRE#Ā0PNNJUUFFĀPRĀ;I1ĀDPUODJMĀ	
Coordinator of Genetic Resources and Agrobiodiversity Area of PROINPA	2002-2012

Subtotal Personal Development — *4.9 credits**

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Herewith the Graduate School declares that the PhD candidate has complied with the educational requirements set by the Educational Committee of EPS which comprises of a minimum total of 30 ECTS credits

* *A credit represents a normative study load of 28 hours of study.*

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