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Native Andean Potato Varieties in Argentina: Conservation and Evaluation of an Endangered Genetic Resource

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ABSTRACT

The native potatoes (*Solanum tuberosum* ssp. *andigena* and *S. curtilobum*) constitute a valuable genetic resource, adapted to grow under extreme environmental conditions in the Andean highlands. These landraces are grown by subsistence farmers and possess resistance to pests and diseases and environmental stresses that affect the potato crop. Several causes are affecting the persistence of the landraces in the areas studied. We present the results of collection and conservation initiatives carried out in Argentina and the current germplasm holdings at the Genetic Resources Network of INTA. The landraces have been evaluated by the use of morphological, agronomic and molecular markers, and evaluated against environmental stresses and diseases. Ongoing research on the Andean landraces will allow us to identify the genetic variability present in farmer's fields and strengthen the activities carried out including farmers as well as public and private organizations.

Keywords: Andean landraces, genetic diversity, *ex situ*, molecular diversity
Abbreviations: CIP, International Potato Center; **INTA**, Instituto Nacional de Tecnología Agropecuaria; **MS**, Murashige and Skoog; **PVS2**, Plant Vitrification Solution 2

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INTRODUCTION

The tuber-bearing species included in section *Petota* Dumort. contain cultivated and wild species distributed from the south-western Unites States to central Chile (Hawkes 1990; Spooner and Hijmans 2001). *S. tuberosum* L. is a cultivated tetraploid species of the series *Tuberosa* that includes two subspecies: the world-wide distributed *tuberosum* and *andigena*, with a more restricted area of cultivation in the Andean highlands, at elevations of 2500-4300 m (Correll 1962; Hawkes and Hjerting 1969; Hawkes 1990; Ochoa 1990; Spooner and Hijmans 2001).

Solanum tuberosum subsp. *andigena* Juz. and Bukasov is grown in the Andes from Venezuela, through Colombia, Ecuador, Peru, Bolivia and Northwest Argentina (Hawkes 1990). In the highlands of Northwest Argentina, the potatoes are the most important crop as staple food, with different culinary properties as well as agronomic properties. Cultivars of this species are grown in the Provinces of Jujuy, Salta and Catamarca (Viirsoo 1954a, 1954b; Hawkes and Hjerting, 1969; Okada 1979; Clausen 1989; Clausen *et al.* 2005). These landraces are grown mainly in the high mountain valleys and *quebradas* of the Puna and Prepuna phytogeographical areas.

A considerable varietal heterogeneity in native potato fields has been detected along the wide distribution of the Andean potato varieties. This fact has had a considerable importance in the evolution of the crop where the mixture of genotypes is the rule and not the exception. This mixture of genotypes promotes the hybridization among clones (Brush *et al.* 1980) and has undoubtedly played an important role in creating new variants or genotypes.

Several causes contribute to the loss of native crop diversity, not only in the potato but other crops as well. Many problems have been detected in the farming systems of local communities in Argentina (Alcoba *et al.* 2006). For example the lack of access to enough water for irrigation; the recognition of the right of indigenous people to own their land according to the Constitutional Amendment in 1994 that has not yet resulted in the tenure of the land where the people has cultivated their crops for centuries. Other causes contributing to the loss of cultivated Andean

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potato diversity in Northwest Argentina are: the changes in agricultural practices, which result in some cases in the replacement by other crops, the migration of the inhabitants of Puna and Prepuna towards urban centres in search of a better way of life, the impossibility of obtaining clean potato seed of their local cultivars and the restricted access to markets that could pay for specific products. Local farmers are introducing virus free potato seed of Andean varieties from Bolivia into Argentina, and this fact is attempting against the maintenance of the local diversity, as local Argentinean varieties are replaced by the Bolivian germplasm (Clausen *et al*. 2005).

GERMPLASM COLLECTING AND CURRENT GERMPLASM HOLDINGS

The collection of both wild and cultivated potato germplasm has been carried out during several decades. An initiative was carried out by E. Viirsoo who collected potato germplasm in 1960 and 1961 for the Estación Agro-Industrial Obispo Colombres (Province of Tucumán) and the germplasm was maintained during several years at this experimental station (Viirsoo *et al*. 1974).

The Argentinean Potato Germplasm Bank at the Experimental Station of Balcarce, INTA (Instituto Nacional de Tecnología Agropecuaria), was created in the 1970's. Native potato varieties were collected during the expeditions of Okada (Okada 1976, 1979; Okada and Clausen 1984; Clausen 1989; Clausen *et al.* 2005). The collecting work carried out by INTA has revealed that the growing of landraces of cultivated potatoes has disappeared from some areas, compared to earlier collections (Clausen *et al*. 2005). The disappearance of local varieties and the substitution of the potato crop by other alternatives are related to several factors. In the low valleys close to the main roads and towns, current production is mainly based upon introduced crops, fruits, leafy vegetables, onion, garlic and flowers, which are sold on markets of major towns in the provinces. Other causes threatening potato cultivation in the Northwest of Argentina are the result of a better road infrastructure and communications that have improved in recent years and the emigration of people from the valleys down to larger towns, without improving the social condition of many of the former farmers. The actual rate of unemployment in towns and cities is increasing and inhabitants of the Andean valleys are suffering the social relegations and lack of opportunities in a different society. Recently, special projects that are arising could contribute to solve some of the permanent and emerging problems (Alcoba *et al*. 2006). Another situation has emerged as a result of the growth of tourism in northwest Argentina in recent years and this could affect crop production in the area and may promote the local culture and the cultivation of local crops for traditional dishes.

As a result of our collecting efforts, more that 40 Andean potato local cultivars or landraces, with a variable number of accessions in each case, from the provinces of Salta, Jujuy and Catamarca are conserved *ex situ* at the Argentinean Genebank of INTA, Estación Experimental Agropecuaria Balcarce (Active Genebank) and by the Base Genebank, located at the Instituto de Recursos Biológicos (INTA, Castelar). The current germplasm holdings are detailed in **Table 1**. Almost all the local varieties are tetraploid belonging to the subspecies *andigena* (**Fig. 1**). Hawkes and Hjerting (1969) reported the presence in Argentina of other cultivated species such as *S. juzepczukii* Bukasov (2n=3x =36) *S. chaucha* Juz. & Bukasov (2n=3x=36) and *S. curtilobum* Juz. & Bukasov (2n=5x=60). A few pentaploid accessions, identified as *S. curtilobum* ('Luqui') have been identified recently and are conserved in the genebank at Balcarce (Clausen *et al*. 2005).

An important collection of native potatoes are held at the International Potato Center (CIP), which includes germplasm from Argentina. Recently, several clones from this country, including different varieties, have been returned and are now maintained in the Balcarce genebank.

Fig. 1 Diversity in Argentine Andean Potato landraces.

EX SITU CONSERVATION

The Convention on Biological Diversity in 1992, define the *ex situ* strategy as the conservation of the components of biological diversity outside their natural habitat (UNCED 1992). This means that the target biological components have to be collected, transferred and stored away from were they were encountered, for example in botanical gardens, seeds genebanks, field genebanks, *in vitro*, DNA and pollen storage (Engelmann and Engels 2002).

A genebank network, integrated by active genebanks and a Base genebank, form the INTA's Genetic Resources Network. In one of these, the active genebank of Balcarce, accessions of wild and native potatoes are preserved. The potato seeds are considered to be orthodox (Holle 1988), this means that seeds can be dried to low moisture contents and stored at cold temperature without damage, for long periods of time (Roberts and Ellis 1984). At the local active genebank the true potato seeds are conserved at 4-9% moisture content and 4°C for the short-term storage. At the Base Genebank, the seeds are stored at 3-7% moisture content and -20°C for the long-term preservation. The methodology employed in both genebanks for the management of stored seeds was described by Ellis *et al.* (1985).

Conventional seed storage is not possible for the landraces, because they produce highly heterozygous seeds and clonal maintenance is required for the conservation of specific genotypes (Ashmore 1997). Therefore, *in vitro* conservation has been implemented at the genebank. This method involves techniques for the growth of tissue culture under sterile conditions and is more secure than field conservation method, in which there are risks of loosing the genetic resources because of attacks by pests and pathogens, and/or the occurrences of climatic adversities (Oka 1988; Engelmann 1997).

As a first step for the *in vitro* conservation, a surface sterilization procedure is applied to tuber sprouts that will be introduced into medium culture, checking any possible contamination that could appear (Pierik 1988). For the short term conservation, *in vitro* plantlets are grown in a medium composed by Murashige and Skoog (MS) salt mixture (Murashige and Skoog 1962), vitamins, sucrose and phytagel. After 2-4 months, plantlets need to be transferred to fresh medium. The plantlets are kept in culture rooms at 20°C light intensity of 45 µmol m^{-2} sec^{-2} and a 16-h photoperiod (Golmirzaie and Toledo 1997/1998)

For medium-term conservation, around 8 months to 2 years, the accessions are preserved in a slow-growth medium containing MS salt mixture, agar, sucrose and sorbitol. The culture conditions in this case are modified: low light intensity, 21 µmol m^{-2} sec^{-2} at 7°C (Golmirzaie and Toledo 1997, 1998).

For the maintenance of the complete *in vitro* collection,

Table 1 Andean potato varieties collected and conserved. Locality data (Province/Department/Locality), morphotypes/number of genotypes identified and morphological tuber description

Cultivars*	Province/Department/Locality	Morphotypes/ No. of genotypes	Morphological tuber description
'Airampía'	Jujuy/Valle Grande/Santa Ana.	1/2	Tubers oblong to ovate with red skin with brownish irregular areas. Eye depth medium, evenly distributed. Yellow flesh.
'Astilla'	Jujuy/Tumbaya/El Angosto.	1/1	Tubers long-oblong, with pink skin and yellow eyes and eyebrows. Eye depth shallow, evenly distributed. Yellow flesh.
'Allo'	Salta/Iruya/Campo Carreras; Santa Victoria/Poscaya	1**	Tubers elliptic with brownish skin. Eye depth shallow, evenly distributed. Yellow flesh with violet vascular ring and medulla.
'Azul'	Jujuy/Humahuaca/Coctaca, Varas, Ocumazo, Palca de Aparzo, Patacal; Tilcara/Huella; Cochinoca/Agua de Castilla.	1/1	Tubers oblong with blue skin and yellow eyes and eyebrows. Eye depth shallow, evenly distributed. Cream flesh.
'Blanca'	Jujuy/General Belgrano/Papachacra; Humahuaca/Huachichocoana, Ocumazo, Patacal; Santa Catalina/Cabrería, Morco Esquina; Tumbaya/Cieneguillas, El Angosto, El Moreno; Valle Grande/Santa Ana; Salta/Santa Victoria/La Huerta, Rodeopampa.	1/3	Tubers elliptic to oblong with yellow skin. Eye depth deep, evenly distributed. Cream flesh.
'Blanca redonda'	Jujuy/Valle Grande/Santa Ana, Valle Colorado; Santa Catalina/Cabrería; Susques/Lapao; Tumbaya/El Angosto.	2/ 2	Tubers round with yellow skin or yellow with purple irregular areas. Eye depth deep, apically distributed. Yellow -cream flesh or yellow cream with purple vascular ring.
'Bolincha'	Catamarca/Belén/Villa Vil, Laguna Blanca.	1**	Tubers round, with white-cream flesh. Eye depth deep, evenly distributed. White flesh.
'Chacarera'	Jujuy/Tilcara/ Casa Colourada; Humahuaca/Ocumazo, Coctaca, Aparzo; Cochinoca/Cochinoca, Quebraleña; General Belgrano/ Cuevas, Papachacra; Tumbaya/ Cieneguillas, Cárcel, Tumbaya; Santa Catalina/ Santa Catalina, Cabrería, Oratorio.	1/2	Tubers round to ovate with yellow rough skin. Eye depth deep, predominantly apically distributed. Yellow flesh sometimes with purple vascular ring.
'Chaqueña'	Jujuy/General Belgrano/Papachacra; Humahuaca/Patacal.	1/1	Tubers elliptic-oblong, slightly flattened, with brownish, netted skin. Eye depth medium, predominantly apically distributed. White flesh.
'Collareja' ('Chorcoyeña')	Jujuy/Tilcara/Maimará,Alfarcito, Juella; Casa Colourada; Humahuaca/Uquía, Pucará, Aparzo, Palca de Aparzo, Varas, Molloj, Cholcán, Ocumazo, Huachichocana, Hornaditas, Hornillos,Yacoraite, Cianzo; Santa Catalina/Casira; Cochinoca/Agua Caliente, Doncella, Rachaite; Tumbaya/, Cárcel, Patacal; General Belgrano/Cuevas; Valle Grande/Santa Ana; Salta/Iruya/Valle de Iruya; Santa Victoria/Abra Colorada, Arpero, Chorro, Hornillos, La Huerta, Lizoite, Nazareno, Poscaya, Rodeopampa, Trancas, Trigohuaico; Catamarca/San Antonio.	2/3	Tubers ovate, brownish skin with violet areas, predominantly apically distributed. Eye depth deep medium with apical or even distribution. Cream flesh.
'Collareja redonda'	Jujuy/ Humahuaca/Coctaca, Chaupi Rodero, Ocumazo; Cochinoca /Agua Caliente, Doncella, Rachaite, Quebraleña; Tumbaya/El Moreno; Valle Grande/Santa Ana; Salta/Iruya/Pie de la Cuesta.	1/1	Tubers round, brownish skin with violet areas predominantly apically distributed. Eye depth medium, evenly distributed. Cream flesh.
'Colorada'	Jujuy/Humahuaca/Chaupi Rodero, Coctaca, Hornaditas, Ocumazo, Varas; Tumbaya/Patacal, El Angosto; Cochinoca/Quebraleña; Salta/Iruya/Campo Carreras, Colanzulí, Pueblo Viejo, Valle de Iruya; Santa Victoria/Chorro, Poscaya Trigohuaico.	4/4	Tubers round with pink to red skin. Eye depth medium, distributed predominantly apically in the majority of the genotypes. White, cream or yellow flesh, in some cases the tubers with red skin with a red vascular ring and pith.
'Condorilla'	Jujuy/Humahuaca/Varas;/Santa Catalina/Cabrería; Salta/Santa Victoria/Chorro; Yavi/Yavi.	1/1	Tubers ovate with yellow skin and violet areas distributed predominantly in the apical area. Eye depth superficial, distributed regularly. Yellow flesh.
'Corbatilla'	Jujuy/Tumbaya/Patacal.	1**	Tubers oblong to long oblong, with yellow skin and violet areas distributed predominantly in the apical area. Eye depth superficial, distributed regularly. Yellow flesh
'Cuarentilla' 'Colorada'/'Tonca'/ 'Cuarentona Colorada'	Jujuy/Valle Grande/Santa Ana, Valle Colorado.	2/3	Tubers round with red skin or bicoloured with red and yellow skin. Eye depth deep, distributed predominantly apically. Yellow flesh.
'Cuarentona'	Jujuy/General Belgrano/Cuevas, Papachacra; Tilcara/Alfarcito, Casa Colourada; Tumbaya/El Angosto; El Moreno; Humahuaca/Cianzo; Susques/Agua Chica; Yavi/Yavi Viejo; Salta/Iruya/Colanzulí; La Poma/La Quesera	1/4	Tubers ovate to oblong with purplish- red skin. Eye depth deep, distributed predominantly apically.
'Cuella'	Jujuy/General Belgrano/Cuevas, Papachacra	1/1	Tubers round slightly flattened with yellow skin and violet areas distributed around the eyes. Eye depth deep, distributed regularly. Cream flesh.

Table 1 (Cont.)

Cultivars*	Province/Department/Locality	Morphotypes/ No. of genotypes	Morphological tuber description
'Huareña'	Jujuy/Humahuaca/Palca de Aparzo; Salta/Iruya/Colanzulí.	1**	Tubers round, with dark purple skin, with yellow areas scattered around the eyes. Eye depth deep, distributed predominantly apically. Yellow flesh.
'Imilla'	Jujuy/General Belgrano/Cuevas; Humahuaca/Huachichocana; Santa Catalina/Casira; Yavi/La Quiaca Vieja.	1/3	Tubers round with purple skin, with yellow scattered areas irregularly distributed. Eye depth deep, distributed apically. Cream flesh.
'Luqui'	Jujuy/Humahuaca/Cholcán, Molloj.	1/1	Tubers ovate, flattened with yellow skin. Protruding eyes, distributed regularly. White flesh.
'Malgacha'	Salta/Rosario de Lerma/ El Gólgota, Quebrada del Toro; Jujuy/Santa Catalina/Casira; Susques/Lapao.	1/3	Tubers oblong with yellow skin and. Shallow eyes, distributed regularly. Yellow-cream flesh.
'Moradita'	Jujuy/Tilcara,Alfarcito, Casa Colourada/Patacal; Doncella, Rachaite, Tambillos; Santa Catalina/Cabrería; Catamarca/San Antonio/Agronomía.	4/4	Tubers round, obovate or fusiform with deep violet skin or dark purple with purple and yellow eyes and eyebrows. Eye depth deep, distributed regularly or apically. Yellow flesh or yellow with purple vascular ring.
'Navecilla'	Jujuy/Valle Grande/Santa Ana.	1/1	Tubers round with yellow skin, with purple irregularly scattered areas. Eye depth medium. Cream flesh.
'Negra Ojosa'	Jujuy/Yavi/Yavi, La Quiaca Vieja; Salta/Santa Victoria/Hornillos, Nazareno, Trigohuaico.	2/4	Tubers round with dark purple skin or yellow with dark purple skin distributed around the eyes and eyebrows. Eye depth deep, apically distributed. White flesh.
'Negra redonda'	Jujuy/Valle Grande/Santa Ana.	1/1	Tubers round with dark purple skin. Eye depth deep, predominantly apical. Cream flesh.
'Negra sayama', 'Sayama'	Jujuy/Valle Grande/Santa Ana.	1/4	Tubers round, with dark purple skin. Eye depth deep. Cream flesh. White flesh.
'Ojos colorados', 'Señorita'	Jujuy/Tilcara/Alfarcito, Casa Colorada; Humahuaca/Aparzo, Palca de Aparzo, Varas, Molloj, Ocumazo; Tumbaya/Cárcel, Patacal; Tilcara/Juella.	1/1	Tubers elliptic to oblong with yellow skin and red irregular areas, as well as spectacled. Eye depth shallow, predominantly apical. Cream flesh
'Ojosa'	Jujuy/Rinconada/Rinconada; Santa Catalina/Casira; Salta/Iruya/Pueblo Viejo.	1/4	Tubers round, flattened, with dark purple skin. Eye depth deep, apically distributed. Cream flesh.
'Overa'	Jujuy/Humahuaca/Chaupi Rodero, Palca de Aparzo; Tumbaya/El Moreno;Valle Grande/Santa Ana; Yavi/Yavi; Salta/Santa victoria/Chorro, Lizoite, Poscaya, Trigohuaico.	1/2	Tubers round with yellow skin and dark purple areas distributed mainly around the eyes and eyebrows. Eye depth medium, apically distributed. Yellow flesh.
'Papa Baya', 'Runa rosada'	Salta/Santa Victoria/Hornillos, Nazareno.	1**	Tubers round, often compressed, with pink skin. Eye depth deep, apically distributed. Cream flesh.
'Papa oca'	Jujuy/Humahuaca/Palca de Aparzo, Varas; Salta/Santa Victoria/Chorro, Poscaya.	1/1	Tubers ovate to oblong with yellow skin. Eye depth shallow evenly distributed. Yellow flesh.
'Rosada'	Jujuy/Tilcara/Maimará; Cochinoca/Agua Caliente, Rachaite, Tambillos; General Belgrano/Cuevas, Papachacra; Humahuaca/Cianzo, Palca de Aparzo, Patacal; Rinconada/Rinconada; Santa Catalina/Casira; Susques/Corral Blanco; Tumbaya/El Angosto, El Moreno; Valle Grande/Valle Grande; Salta/Santa Victoria/Chorro, Rodeopampa, Trigohuaico.	2/5	Tubers round and oblong, with pink to purple skin. Eye depth medium or deep. Cream or yellow flesh.
'Runa'	Jujuy/Humahuaca/Chaupi Rodero, Coctaca, Ocumazo; Rinconada/Rinconada; Santa Catalina/Casira; Susques/Lapao; Tumbaya/Tumbaya, Patacal: Salta/Iruya/Colazulí; Santa Victoria/Arpero, Chorro, La Huerta, Lizoite, Nazareno, Poscaya, Rodeopampa, Trigohuaico.	1/3	Tubers ovate to long oblong, with brownish skin. Eye depth medium, evenly distributed. White-cream flesh.
'Sani', 'Sisa'	Jujuy/Yavi/Yavi; Santa Catalina/Casira;. Salta/Santa Victoria/Chorro, Trigohuaico.	1/1	Tubers round, slightly compressed, skin brownish, with purple areas around eyes and eyebrows. Eye depth deep, evenly distributed. Cream flesh.
'Santa María'	Jujuy/Yavi/Yavi.	1/1	Tubers fusiform with red skin. Eye depth medium, evenly distributed. Flesh red.
'Tigrera'	Santa Catalina/Cieneguillas.	1**	Tubers elongate. Skin purple with yellow areas in the apical region of the tuber. Eye depth shallow, evenly distributed. Yellow flesh.
'Tuni blanca'	Jujuy/Humahuaca/ Palca de Aparzo, Uquía, Pucará, Jujuy/Cochinoca/Ojo de Agua, Yacoraite; Tumbaya/Patacal; Valle Grande/Santa Ana.	1/3	Tubers ovate-oblong, flattened with white – cream skin. Eye depth shallow, evenly distributed. White flesh.
'Tuni morada'	Jujuy/Humahuaca/Patacal, Yacoraite; Tumbaya/Cieneguillas; Salta/Iruya/Campo Carreras; Santa Victoria/Lizoite.	1/1	Tubers ovate-oblong flattened with purple skin and. Shallow eyes, evenly distributed. White flesh.
'Tuni rosada'/'Rosilla'	Jujuy/Cochinoca/Ojo de Agua; Valle Grande/Santa Ana.	1/1	Tubers ovate-oblong flattened with yellow skin, tinged with purple. Eye depth shallow, evenly distributed. White flesh.
'Yaguana'	Jujuy/Susques/Sala.	1/1	Tubers ovate-oblong with brownish skin and purple irregular areas, especially around the eyes and eyebrows. Eye depth deep, evenly distributed. Cream flesh.
'Yuruma'	Jujuy/Santa Catalina/Casira.	1**	Tubers oblong with pink brownish skin. Eye depth shallow, evenly distributed. Yellow flesh.

* The only pentaploid variety is 'Luqui' (*S. curtilobum)*; **no molecular data available

first the accessions are introduced into the propagation medium, once 3-6 plantlets from each accession are obtained they are immediately introduced into the conservation medium for 15 days, after that they are transferred to the conservation chamber. A total of 364 accessions are maintained under slow growth conditions, 10 replicates of each introduction are kept at the Active Genebank while others are sent to the Base Genebank to safeguard the collection. The *in vitro* preservation is time consuming, because plantlets need to be replicated, which implies high maintenance costs (Epperson *et al.* 1997). Other disadvantages of this method are the possible losses of germplasm due to human errors, contaminations, tissue hyperhydricity and somaclonal variation which alters the genetic integrity of the accessions maintained (Whiters 1984; Bajaj 1987; Niino *et al.* 2000). To avoid these problems, cryopreservation is the only technique that exits today for the long-term conservation of germplasm (Engelmann 1997). It consists in introducing explants into ultra low temperature, generally -196°C the temperature of liquid nitrogen, to preserve the tissue. Mazur (1984) stated that at such temperatures all the metabolic activities in the cell and all the cellular divisions are stopped, and it means that no genetic variation will occur. At present, many protocols for the cryopreservation of *Solanum tuberosum* are used (Keller *et al.* 2008); the droplet method with aluminium foil and 10% dimethylsulphoxide is routinely applied to the potato collection in Germany (Schäfer-Menuhr *et al.* 1997), droplet/vitrification on aluminium foil with plant vitrification solution 2 (PVS2) (Sakai *et al.* 1990) was investigated by Panta *et al.* (2006) for the long-term conservation for potato landraces at CIP in Peru.

Procedures of cryopreservation are being studied at the Balcarce active genebank. These are based on the vitrification technique; in which water in biological tissue is solidified without crystallization, forming a metastable glassy system. Prior the introduction of the tissue into liquid nitrogen, the cells must be dehydrated to increase the concentration of solutes (Benson 2008). The first approach assayed at the active genebank was developed by Steponkus *et al.* (1992). Besides, this method was applied at CIP's collections (Golmirzaie and Panta 2000). This technique consists of preculturing the potatoes shoot tips in sucrose enriched medium, then transferred them into a dehydration solution (50% ethylene glycol, 15% sorbitol and 6% bovine serum albumin) for several minutes into a propylene straw and immediately introduced into liquid nitrogen. After 24 hrs, they were recovered from liquid nitrogen and then transferred to normal medium in dark conditions, for 3 days, and then transferred to normal growth conditions (20°C, light intensity of 45 µmol $m^{-2}.sec^{-2}$ and a 16-h photoperiod). Many parameters were tested: type of explant, exposure time to dehydration solution, dehydration solution concentration and type of rewarming solution. The variable measured was regeneration %, i.e. percentage of shoot tips that regenerate a normal plant after cryopreservation over the total number of shoot tips cryopreserved. The results obtained were genotype dependent ranging from 4.5 and 27.3% of success. The landraces that showed the best performance were 'Chacarera', 'Tuni' and 'Chaqueña' (Digilio 2004, 2007). Another experiment was performed with the droplet/vitrification method. Potato shoot tips where precultured on a loading solution (sucrose 0.4 M and glycerol 2 M) for 20 min, then transferred to PVS2 (30% glycerol, 15% ethylene glycol, 15% dimethyl sulfoxide and 0.4 M sucrose) for 50 and 60 min. Finally each shoot tip was transferred to a single droplet of PVS2 on an aluminium foil strip, following direct immersion in liquid nitrogen. The regeneration percentages for 'Chaqueña' genotype were 25 and 50%, for each dehydration time, respectively. Further experiments needs to be performed before a cryopreservation protocol can be implemented for the long term conservation of the Andean potatoes at the genebank in Balcarce.

ON-FARM CONSERVATION OF POTATO DIVERSITY

The value of on farm conservation of plant genetic resources has been underestimated during decades, favored by the great efforts employed in the *ex situ* conservation worldwide, which has resulted in the establishment of international and national genebanks (FAO 1996). Many farmers, especially in areas of crop origin and diversity, have persisted in cultivating, managing and developing their plant material, generating crop plant diversity (Brush *et al*. 1980; Altieri and Merrick 1987; Brush 2000). The maintenance of different crops under traditional farming systems is relevant as the varieties continue to evolve and adapt under different pressures, generating novel diversity or a continuum of new variants that can persist under specific growing conditions.

The *in situ* conservation of crops is designed to maintain an ecologically dynamic agro-ecosystem where the farmers continue generating new genetic resources; it means that this system is changing and this is the state in which it has to be preserved. In the case of the Andean potato production in our country, several causes of changes can be pointed out: the introduction of new potato varieties from neighboring countries, adoption of new crops which can easily be commercialized for a specific market, the exchange of varieties between different communities or localities, migration of farmers to urban areas, different cultural practices and gene flow involving wild potato species (Clausen *et al*. 2005).

Another important reason for promoting the *in situ* conservation of local potato varieties is that we have detected genetic variability within varieties and within morphotypes (Ispizúa 2004; Ispizúa *et al*. 2007); this fact is not unexpected as we are dealing with local varieties where mixtures within varieties is a common phenomenon. As our work concerned with the study of the genetic variability present in Andean potato landraces is pursued, we will undoubtedly identify many different genotypes; we do not yet know the magnitude of the genetic variability present in the local varieties. Another fact is that it is likely that not all the diversity may have been captured and new genetic variation may have arisen since the collecting work was carried out.

The relationship of the *ex situ* conservation carried out by official institutions and the *in situ* conservation on farms will be strengthened in the future; activities involving farmers, public organizations, non-governmental organizations as well as private activities are under way. Potato tuber seed accumulate viruses over time and this process is known as degeneration. According to the farmers, the yield of many of the varieties is decreasing and when we analyzed an important number of varieties, we found that the majority of the varieties were heavily virus infected. When the farmers reported that a specific variety was bought in Bolivia and planted in the Province of Jujuy, we found that it was virus-free (Clausen *et al.* 2005) as a result of a local seed system organized in Bolivia that is providing the farmers with clean potato seed (Iriarte *et al*. 2000). As a result of different techniques (meristem culture and thermotherapy) 20 virus-free varieties are available and have been distributed to farmers; furthermore the farmers were trained in their own fields by agronomists and extension officers on different topics related to cultural practices, clean potato seeds production and management of greenhouses made of adobe bricks (Sossa Valdéz *et al.* 2006).

Participatory plant breeding is another strategy that will contribute to the *in situ* conservation of the local potato varieties. In this case the selection is directed by the farmer in response to local needs and specific conditions. A specific participatory project, including a local community from the locality of Hornaditas (Province of Jujuy), is in progress and has resulted in the selection of several promising *andigena* clones on the basis of morphological and agronomic evaluations (Andrade *et al*. 2008).

DIVERSITY IN ANDEAN POTATOES

Local varieties

The landraces of the subspecies *andigena* present numerous local varieties that differ in their growth habit, flower colour and tuber characteristics such as shape, skin and flesh colour, distribution and depth of the eyes (**Fig. 1**).

According to Gomez (1946, cited by Albeck 1993), a total of 75 varieties were found in the 1940's in the Quebrada de Humahuaca (Province of Jujuy). Viirsoo (1967) collected 21 varieties in NW Argentina (Jujuy, Salta) and Hawkes and Hjerting (1969) cited 25 varieties for the country although they stated that is was likely that this was not the total number of local varieties grown. Clausen (1989) and Clausen *et al*. (2005) listed more than 40 varieties from each of the Provinces of Salta and Jujuy. As a result of the collecting and description work we are carrying out, we have identified the local landraces present in the country and the identification of the genotypes is in progress. The provenances of these varieties are the provinces of Jujuy, Salta and Catamarca (**Table 1**).

Although potato diversity is declining in our Andean valleys, almost in all the communities visited potatoes were cultivated, but generally the number of different varieties held by each farmer was low. Among the cultivars that are widespread and common in several Departments and/or Provinces, we can cite 'Collareja', 'Churqueña bola' or 'Collareja redonda', 'Chacarera', 'Colorada', 'Rosada', 'Runa', 'Blanca', 'Morada', 'Overa', 'Negra overa', 'Tuni blanca', 'Tuni morada'. In many cases the farmers ask for specific cultivars they used to grow which have disappeared in their area, indicating that there is still a need for certain cultivars for specific uses. In other cases, a specific cultivar is only grown by a few families in a more restricted area; that is the case of 'Chaqueña', 'Azul' and 'Bolincha'.

The diversity of varieties varies from farmer to farmer. Usually 3-6 varieties are reported by each farmer but up to 10-12 varieties can be held by one farmer in a few cases. The diversity on the farms in the highlands is not very high: a few other crops such as oca (*Oxalis tuberosa* Mol.), ullucus (*Ullucus tuberosus* Caldas), mashua (*Tropaeolum tuberosum* Ruiz & Pav.), fava beans (*Vicia faba* L.), barley (*Hordeum vulgare* L.), maize (*Zea mays* L.) and quinoa (*Chenopodium quinoa* Willd.) and a few herbs for medicinal or aromatic purposes are found.

Farmer classification

The Andean potato varieties have been locally identified and named by farmers according to their tuber characteristics, cultivation, edibility, processing and frost resistence (Quirós *et al*. 1990). As a result, one variety may receive different names according to the different districts or communities where it is found. Furthermore, the same name may be applied to different varieties when they share morphological similarities. Quechua and Aymará languages are used for the identification of varieties, although in Northwestern Argentina the peasant population is not exclusively native as in neighbouring countries such as Perú and Bolivia, and Spanish-Quechua combinations are frequently used (Hawkes 1947).

A discrepancy in naming the landraces was found in many cases. The term 'Overa' is now being used for bicoloured tubers instead of the term 'Condorilla'; the Quechua word 'Runa' was replaced in some cases by the Spanish name 'Blanca rosada'. The term 'Rosada' is replacing in some valleys the name 'Astilla', which is a distinct landrace with pink skin, yellow eyes and eyebrows. Another example is 'Rosada' also known as 'Cuella' characterized by bicoloured tuber. In north-western Argentina we have observed that the knowledge concerning the naming of potato varieties as well as their different uses is held generally by elderly people, mainly women. The young potato growers are replacing many old vernacular names in native languages by Spanish names, which describe tuber skin or flesh colour leading to an unspecific way of identification each landrace (Ispizúa *et al*. 2007).

Fig. 2 Andean potato field in Coctaca, Quebrada de Humahuaca, Province of Jujuy.

Molecular and morphological diversity

The morphological characterization of the potato varieties were carried out in a mountainous area in the province of Tucumán and Jujuy (**Fig. 2**). An internationally accepted descriptor list has been utilized (Huamán *et al*. 1977; Huamán and Gómez 1994) as well as other descriptors we considered to be important for the description of the material. The descriptions of each variety or morphotype included stem characters (growth habit, number of stems, colour of stem, diameter, presence of wings, plant height); leaf characters (leaf length, number of lateral leaflets, number of interjected leaflets, number of secondary lateral leaflets, leaflet margin, length and width of terminal leaflet, terminal leaflet base, length and width of primary lateral leaflet, length and width of second lateral leaflet, apex, etc.); floral characters (length of peduncle, number of peduncle forks, number of flowers per inflorescence, length of pedicel, length of calyx acumen, length of calyx lobe, radius of corolla, length of anther, length of style, length of style exsertion from apex of anther to apex of stigma, stigma form, colour of adaxial corolla interpetolar tissue, colour of adaxial corolla ray, colour of abaxial corolla interpetolar tissue, colour of abaxial corolla ray, degree of flowering, pollen production, duration of flowering, fruit colour, shape, seed set); agronomic characters (weeks to flowering, weeks to harvest, number and tuber weight, dry matter content); tuber characters (tuber size, predominant tuber skin colour, secondary tuber skin colour, distribution of secondary tuber colour, tuber skin type, tuber shape, tuber flesh colour, secondary tuber flesh colour, distribution of secondary tuber flesh colour, depth of tuber eyes, distribution and number of eyes, predominant sprout colour, secondary sprout colour); reaction to pathogens and abiotic stresses.

As a result of the morphological characterization, morphotypes were identified within varieties (**Table 1**). Huarte *et al*. (1991), found a considerable morphological variability in local varieties of wide distribution, and were able to differentiate by growth habit, flower, vascular tissue and tuber skin colour, morphotypes of the same variety (for example Colorada). Rovaretti (1992) detected by means of storage proteins, different electrophoretic phenotypes between varieties and within morphotypes of the same variety. In this study correlation was observed between the results obtained from electrophoretic and morphological data.

Ispizúa *et al*. (2007) carried out a study to analyse the genetic diversity within and among potato landraces from different sites and departments of Jujuy Province, using four SSRs markers. In this study 155 accessions were grouped in 72 genotypes and more than one genotype was detected in the majority of the landraces. Analysis of molecular variance revealed that most of the genetic variation occurred among sites within departments and among local varieties. All the accessions studied revealing different morphotypes presented different genotypes. However, landraces with similar tuber characteristic were genetically different in some cases. Pissard *et al*. (2008) reported the lack of perfect congruence between morphological and molecular data from the analysis of the intra-morphotype genetic diversity of *Oxalis tuberosa*. These authors concluded that the morphological characters could be used as an accurate tool for the identification of morphotypes by grouping the accessions according to their similarities and the molecular markers subsequently used to characterize more precisely the accessions when identifying duplicates of a collection.

Native agriculture, in relation to the genetic diversity planted by the farms are resulting in: (a) the maintenance of numerous genotypes over space and time, (b) the wide distribution of particular genotypes, and (c) the generation of new genotypes (Brush *et al*. 1980). The crop evolution of the cultivated potato is closely linked to the mixture of species and genotypes which promotes hybridization and natural hybrids are unintentionally incorporated into the cultivated potato gene pool (Ugent 1970; Brush *et al*. 1980; Johns and Keen 1986; Zimmerer and Douches 1991). The fields are commonly invaded by large populations of wild potato species, which tend to become established in and between the rows of potato plants, as well as along marginal thickets of the fields (Ugent 1970). Several diploid and tetraploid species as *S. gourlayi* Hawkes, *S. megistracrolobum* Bitt., *S. acaule* Bitt. and *S. infundibuliforme* Phil were found growing as weeds in farmers' fields of NW Argentina providing an appropriate environment for hybridisation with the crop and establishment of new genotypes (Clausen *et al.* 2005). Intra- and interploidy crosses between species of the *Solanum* complex are facilitated by the occurrence of both n and 2n gametes, and presents opportunities for introgression throughout the sympatric species (den Nijs and Peloquin 1977; Camadro and Peloquin 1980).

Another practice that allows the introduction of sexual recombinants into the landrace gene pool is a traditional rotation system. The farmers often plant another tuber crop, such as oca (*Oxalis tuberosa*) in fields that have been cultivated with native potatoes during the previous year. This rotation practice allows to harvest together other root and tuber crops as well as potato tubers developed from botanical seed, which are generally small. It is likely that they will be kept for planting as 'seed tubers' the following year rather than be eaten (Jackson *et al*. 1980; Johns and Keen 1986; Zimmerer and Douches 1991). Therefore the farmers occasionally use true botanical seeds to renovate degenerated tubers because of viral infection (Quiros *et al*. 1990).

Sukhotu *et al*. (2005), considered that the present genetic diversity in Andigena was modified and attenuated through sexual polyploidization and intervarietal and/or introgressive hybridisation after Andigena arose and by long-distance dispersal of seed tubers by humans. In the traditional potato-growing areas of northwestern Argentina, an intense exchange of 'seed tubers' occurs between farmers or through local markets where the farmer arrives from isolated valleys, leading to widespread distributions of the particular genotype. This situation becomes evident with 'Collareja'; this variety is a common landrace in NW Argentina, where one genotype has been sampled in six departments of Jujuy and in one department of Salta province (**Fig. 3**). A dynamic system therefore requires that seed is transferred on a regular basis from place to place. Where seed is lost then the variety can be restored by obtaining seed from another farmer (Terrazas and Valdivia 1998), contributing to the expansion of successful genotypes.

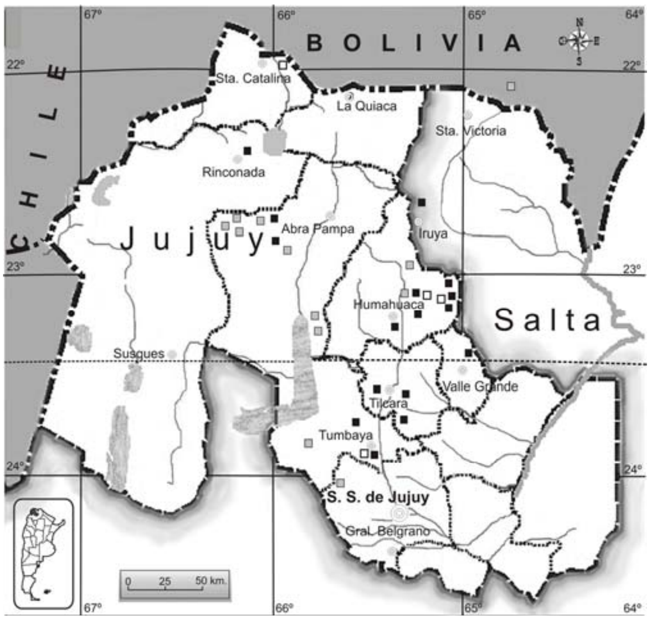

Fig. 3 Distribution of genotypes of 'Collareja' in the provinces of Jujuy and Salta.

The spatial structure of the genetic diversity is usually considered as an important parameter for the efficiency of the collecting trip (Pissard *et al*. 2008). According to these authors the genetic structure seems to be influenced by the collection site. In one Andean potato field, we sampled many potatoes of only one variety ('Collareja') from a newly harvested seed lot, and detected different morphotypes based upon vegetative and reproductive characters, that correlated with different genotypes (Atencio *et al.* 2008). Furthermore, some of the genotypes found in this farmers field, had not been detected before in the sampling carried out in our former collecting trips. It is likely that when dealing with Andean potato tubers, not only the sampling in several sites within departments may result in maximizing the genetic diversity. Also, a large sample within the farmer's field will permit to detect variants that would have been overlooked.

Resistance to biotic and abiotic stresses

The potato crop is affected by many pathogens and is susceptible to abiotic stresses that cause severe reductions in yields as well as in tuber quality. Numerous resistance genes have been discovered in both wild and cultivated potato species (Hanneman 1989; Ortiz 2001; Jansky 2000; Razdan and Mattoo 2005; Simko *et al*. 2007) although relatively few species have been used in the breeding of successful modern cultivars (Ross 1986). The Andean potato varieties are important sources against pests and diseases and possess variability for many valuable agronomic traits such as chipping quality, high content of solids and frost resistance. Andean landraces have been evaluated for resistance to fungi, bacteria, viruses, nematodes, insects and environmental stresses that affect the potato crop (Richardson and Estrada Ramos 1971; Huamán 1983; Hanneman 1989) and resistance have been detected for the majority of the

Table 2 Resistance to biotic and abiotic stresses in *S. tuberosum* ssp. *andigena* and *S. curtilobum*.

Source of resistance	Biotic/abiotic stresses	References
Virus		
Adg*	*Potato leaf roll virus* (PLRV)	Ross 1986; Bamberg *et al*. 1994; Mihovilovich *et al*. 2007; Velásquez *et al*. 2007
Adg	*Potato virus Y* (PVY)	Ross 1986; Huamán 1987; Fernández-Northcote 1991; Bamberg *et al*. 1994; Sing *et al*. 1994; Swiezynski 1994; Dalla Rizza *et al*. 2006; Ritter *et al*. 2008
Adg/Cur**	*Potato virus X* (PVX)	Ross 1986; Huamán 1987; Fernández-Northcote 1991; Bamberg *et al*. 1994; Swiezynski 1994; Querci *et al*. 1995; Huamán *et al*. 2000; Bradshaw and Ramsay 2005; Ritter *et al*. 2008
Adg	*Potato virus A* (PVA)	Bamberg *et al*. 1994; Ritter *et al*. 2008
Adg	*Potato virus M* (PVM)	Bamberg *et al*. 1994; Ritter *et al*. 2008
Adg	*Potato virus S* (PVS)	Fernández-Northcote 1991; Swiezynski 1994; Ritter *et al*. 2008
Oomycete		
Adg/Cur	Late blight (*Phytophthora infestans)*	Viirsoo *et al*. 1974; Hawkes and Hjerting 1989; Hawkes 1990; Wastie 1991; Bamberg *et al*. 1994; Ritter *et al*. 2008
Fungi		
Adg	Dry root - *Fusarium* wilt (*Fusarium* spp.)	Huamán 1987; Bamberg *et al*. 1994
Adg	Wart (*Synchrytium endobioticum*)	Huamán 1987; Hawkes 1990; Bamberg *et al*. 1994; Bradshaw and Ramsay 2005
Adg	Early blight (*Alternaria solani*)	Bamberg *et al*. 1994
Adg	Early dying disease- *Verticillium* Wilt (*Verticillium* ssp.)	Hanneman 1989; Bamberg *et al*. 1994
Bacteria		
Adg	Wilt, soft rot, black leg (*Pectobacterium* spp.)	Lojkowska and Kelman 1989; Hawkes 1990; Bamberg *et al*. 1994; Ritter *et al*. 2008
Adg	Bacterial wilt (*Ralstonia solanacearum*)	Huamán 1987
Adg	Powdery scab (*Streptomyces scabies*)	Huamán 1987
Nematodes		
Adg	*Globodera pallida/ Globodera rostochiensis*	Huamán 1987; Huamán *et al*. 2000; Bradshaw and Ramsay 2005; Kirú *et al*. 2005; Ritter *et al*. 2008
Adg	False root-knot nematode (*Nacobbus aberrans*)	Inserra *et al*. 1985; Ortuño *et al*. 2003; Suárez *et al*. 2009
Adg	Root-knot nematode (*Meloidogyne incognita*)	Huamán 1987; Hawkes 1990
Insects		
Adg	Andean potato wevil (*Premnotrypes* sp.)	Huamán *et al*. 2000
Adg	Tuber moth (*Phthorimaea operculella*)	Huamán 1987
Cur	Andean potato wevil (*Premnotrypes* sp.)	Hawkes and Hjerting 1989
Adg - Cur	Potato aphid (*Macrosiphum euphorbiae*)	Radcliffe *et al*. 1981
Adg - Cur	Powdery scab (*Spongospora subterranea*)	Hawkes and Hjerting 1989
Cur	Frost tolerance	Ross and Rowe 1965; Huamán 1987; Iriarte *et al*. 2000; Ortiz 2001
Adg/Cur	Drought tolerance	Bansal *et al*. 1991; Schafleitner *et al*. 2007
Adg	Salt tolerance	Velásquez *et al*. 2005

*Adg: *S. tuberosum* ssp. *andigena*; **Cur: *S. curtilobum*

abiotic and biotic stresses that affect the potato. Examples of the proven and potential utility of *S. tuberosum* ssp. *andigena* and *S. curtilobum* are summarized in **Table 2**. As can be observed, the tetraploid Andean cultivars possess resistance to the main virus, bacteria, fungus, nematodes and insects that affect the potato crop. Salt and drought tolerance has been also reported for this species. Bansal *et al.* (1991) evaluated 28 genotypes measuring growth reduction of leaf discs subjected to treatments of PEG (polyethylene glycol) and detected an excellent performance of *S. curtilobum* and *S. tuberosum* ssp. *andigena*; Iriarte *et al.* (2000) reported several drought-tolerant varieties from Bolivia; Schafleitner *et al*. (2007) exposed two drought-tolerant potato clones to water stress under field conditions and detected drought stress responses such as increased root growth, reduced canopy and relative leave water content, modifications of carbohydrate metabolism, among other traits.

Of the varieties cited for Argentina, Suárez *et al*. (2009) detected resistance to false root-knot nematodes in var. ‘Azul’. Velásquez *et al*. (2005) screened 12 potato varieties for salt tolerance with concentrations of 0.80 and 120 mM ClNa and reported that ‘Blanca’, ‘Airampia’, ‘Sisa’ and ‘Cuarentilla’ were less affected by salinity.

In *S. curtilobum* resistance has been reported for PVX, late blight, powdery scab and potato aphid (**Table 2**). Ross and Rowe (1965) reported field evaluations of several potato species and one of the most resistant species was *S. curtilobum*, tolerating -2.8°C. Huamán (1987) also reported frost tolerance in this species; Iriarte *et al.* (2000) reported one frost tolerant variety (‘Yurak Luk’i’) from Bolivia and Ortiz (2001) reported frost resistance as well as bitterness in this species.

As a result of the presence of a broad range of resistance to many pests and diseases in the Andean potato gene pool, this germplasm has been used in plant breeding programmes (Plaisted and Hoopes 1989; Ortiz 2001; Bradshaw and Ramsay 2005). Examples of potato varieties developed utilizing *andigena* varieties have been reported by Ross (1986); Brown (1993), Pavek and Corsini (2001). As ssp. *andigena* is adapted to tubering in short days, initiatives carried out in different programmes have demonstrated that mass selection of ssp. *andigena* under northern latitudes resulted in Neotuberosum (long-day ssp. *andigena*) which produced parents for direct incorporation into potato-breeding programmes (Bradshaw and Mackay 1994). Despite these promising results, only few clones have been used in the breeding of successful cultivars as a result of lack or regularity of tuber shape (Bradshaw 2007) and needs further improvement.

The use of the germplasm collections of Andean varieties will benefit by the increasing use of molecular approaches in the identification of the genetic diversity together with the greater understanding of gene structure and functions in the near future. As these tools permits to work on larger scales and becomes more efficient, an increased use of the germplasm collections will be pursued.

CONCLUDING REMARKS

The tetraploid and pentaploid Andean potatoes cultivated in Argentina are maintained by the farmers mainly for subsistence. The landraces are grown under environmental conditions where the modern cultivars usually do not perform due to the harsh environmental conditions. The diversity present in the Andean gene pool is the result of evolutionary processes and has resulted in phenotypic and genetic variability between and within varieties as well as resistance to biotic and abiotic stresses as well as specific culinary and nutritional properties. Our knowledge concerning the genetic variation within and between potato native varieties, as well as the geographic patterns of variation, are providing valuable information on practical issues concerning *ex situ* and *in situ* germplasm conservation and management. We have found unique genotypes and detected redundancy in the collection, as well as resistance to diseases. According to these results it is clear that a careful sampling has to be carried out when genotypes are selected for pathogen eradication to produce healthy accessions for re-distribution to Andean farmers. The conservation should be done based on genotypes within each variety with the aim of preserving the variability still present in the farmers' fields.

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