Euphytica (2009) 168:249–262
DOI 10.1007/s10681-009-9938-z

Persistence over time, overlapping distribution and molecular indications of interspecific hybridization in wild potato populations of Northwest Argentina

Luis E. Erazzú · Elsa L. Camadro ·
Andrea M. Clausen

Received: 6 November 2008 / Accepted: 7 April 2009 / Published online: 26 April 2009

Abstract *Solanum gourlayi* and *Solanum spegazzinii*, wild potatoes endemic to Argentina, possess desirable traits for breeding. In periodical regenerations of accessions, variability was detected for morphology and breeding barriers. The persistence of these populations in nature was evaluated after more than 20 years. Both species were observed in all visited sites, along with other wild and cultivated potatoes. Chromosome numbers coincided with the originally reported, except for one population of *Solanum gourlayi*, with diploid and tetraploid cytotypes. The accompanying flora and environmental conditions revealed important alterations as the result of road construction, excessive stocking rates and overgrazing. Principal coordinate and cluster analyses and an AMOVA using AFLP data of three original accessions and the corresponding new accessions revealed high molecular variability and extensive overlapping. Plant grouping of accessions occurred at a distance of 0.58 for *S. gourlayi*, 0.62 for *S. spegazzinii* and 0.67 for both species. The role of natural hybridization and sexual polyploidization in the evolution of sympatric populations of wild potatoes is discussed.

Keywords AFLP markers · Gene flow · Genetic resources · Sympatric species · Wild potato

Introduction

The common potato, *Solanum tuberosum* L. (tbr,[1] $2n = 4x = 48$, 4EBN) has around 200 related wild species that constitute an important genetic resource for crop improvement (Correll 1962; Hawkes and Hjerting 1969; Hawkes 1990). These species have numerous characteristics of agronomic interest such as resistance/tolerance to water and temperature stresses, nematodes, harmful insects, fungi, bacteria

This paper is a part of first author's Doctoral thesis.

L. E. Erazzú
Estación Experimental Agropecuaria (EEA) Famaillá,
Instituto Nacional de Tecnología Agropecuaria (INTA),
C.C. 9, 4132 Famaillá, Tucumán, Argentina

E. L. Camadro (✉) · A. M. Clausen
Estación Experimental Agropecuaria (EEA) Balcarce,
Instituto Nacional de Tecnología Agropecuaria (INTA),
C.C. 256, 7620 Balcarce, Buenos Aires, Argentina
e-mail: ecamadro@balcarce.inta.gov.ar

E. L. Camadro
Facultad de Ciencias Agrarias, Universidad Nacional
de Mar del Plata (UNMdP), Mar del Plata, Buenos Aires,
Argentina

E. L. Camadro
Consejo Nacional de Investigaciones Científicas
y Técnicas (CONICET), Buenos Aires, Argentina

---

[1] Abbreviation proposed by Simmonds (1963).

and viruses, as well as desirable culinary and industrial qualities like high dry matter content, low reducing sugar content and vitamin C (Huamán et al. 2000; Santini et al. 2000; Davies et al. 2002; Oltmans and Novy 2002; Jansky and Peloquin 2005).

Ross (1986) emphasized the importance of wild tuber-bearing *Solanum* species in the concrete contribution of genes of interest to potato breeding. Although many European and North American cultivars have resistance/tolerance to numerous pathogens and insects introgressed from these species, the necessity of making a greater use of the wild genetic pool for the improvement of the cultivated forms is established. A particular group that has potential in this respect includes, according to the previously mentioned author, the Argentinean species *Solanum spegazzinii* Bitt. and *Solanum gourlayi* Hawkes.

Solanum spegazzinii (spg, $2n = 2x = 24$, 2EBN) is an endemic species of the provinces of Salta, Catamarca and La Rioja, reaching the province of San Juan in its distribution (Hawkes and Hjerting 1969). It has been reported as a source of resistance to adverse biotic agents such as *Fusarium* spp. and *Synchytrium endobioticum,* a certain degree of resistance to *Phytophthora infestans*, *Verticillium alboatrum* and *Globodera* spp. (Ross 1986; Rousselle-Bourgeois and Mugniery 1995; Rouppe van der Voort et al. 1997). It has also been cited for its low reducing sugar content in tubers, which is a desirable trait for the industrial process.

In Argentina, *S. gourlayi* (grl, $2n = 2x =$ 2EBN and $2n = 4x =$ 4EBN) is found in the provinces of Salta and Jujuy (Clausen and Okada 1987; Spooner et al. 1998; Clausen et al. 2005). It is a source of resistance to *Globodera pallida* and *Fusarium* spp. (Lynch et al. 2003) and the tubers have low reducing sugar content, as those of spg (Bamberg et al. 1996; Hayes and Thill 2002). Santini et al. (1976), working with grl × tbr hybrids, detected desirable tuber characteristics like high yield and tuber quality. More recently, Santini et al. (2000) reported that hybrid families derived from crosses between tbr haploids and pooled pollen from spg, grl and *Solanum chacoense* (chc, $2n = 2x = 24$, 2EBN) showed a positive influence of the wild species on desirable tuber traits such as dry matter content (21.8 vs. 17–20% in tbr reported by Cacace et al. 1994), tuber number, size and shape, skin texture and colour, and eye depth.

Both, spg and grl, occupy inter-mountainous valleys in Northwest Argentina (Hawkes and Hjerting 1969; Hanneman 1989). The Potato and Forages Genebank of the Estación Experimental Agropecuaria (EEA) Balcarce, INTA, maintains many accessions of spg and both grl cytotypes collected over many years. Periodically, these accessions are regenerated and evaluated ex situ in the facilities of the Genebank. In a previous work, Ispizúa (1994) observed morphological variability in spg accessions from different departments of Salta Province and proposed to arrange them into three morphological groups. These groups comprised populations from (a) Quebrada de Escoipe (with narrow leaflets of reduced length, acuminate leaflet apex, purple corolla and low pubescence), (b) Quebrada Calchaquí (with wide leaflets, long, acute leaflet apex, light violet corolla with violet shades and white acumens, very pubescent) and (c) Quebrada de Belén (with wide leaflets, long, acute leaflet apex, light violet corolla, low pubescence). Morphological variability has also been observed in some grl accessions regenerated at the Genebank (Clausen, unpublished data). Moreover, Erazzú et al. (1999), in studying cross-compatibility relations between and within Ispizúa's (1994) morphological groups, detected pre-zygotic breeding barriers, both within two of the groups and between the three of them. They consisted in the arrest of pollen tube growth at four different sites: stigma, first third, middle and second third of the style. More recently, Erazzú and Camadro (2008) reported that, following intra- and inter-specific crosses of morphologically variable spg and $2x$ grl accessions, a reproductive behaviour occurred which was unexpected according to their taxonomic status. In fact, a high percentage of intra- and inter-specific genotypic combinations did not set seeds (77% in spg, 60% in grl $2x$ and 66% in spg × $2x$ grl and 84% in $2x$ grl × spg, respectively) due to pre-zygotic breeding barriers that acted at several sites (stigma, first third, middle and second third of the style) and, presumably, post-zygotic barriers, since a large number of pollen tubes (around 150) were observed growing among the ovules.

The exploration, collection, characterization and evaluation of genetic materials are required to make proper use of wild germplasm in breeding programs. Two complementary strategies can be used to preserve the collected germplasm: (a) ex situ conservation in genebanks, when the species are considered of strategic importance and when they are in danger of extinction, and (b) in situ conservation in the original natural habitats, where the species are exposed to natural selection processes (Maxted et al. 1997). Furthermore, the characterization of the environment where wild species grow and reproduce is of fundamental value to design conservation strategies as well as to select progenitors (or parental species) for genetic improvement with given purposes (e.g., drought tolerance).

Recently, Bamberg et al. (2003) reported numerous potato germplasm collection trips in the USA, and a dispersion area of *Solanum fendleri* and *Solanum jamesii* greater than the originally cited for these species. They were able to correct location data with modern instruments as well as to make genetic studies in the accessions. del Rio et al. (1997) detected changes in the genetic diversity with molecular markers in collections of *S. fendleri* and *S. jamesii* carried out in different times at the same geographic locations.

In Argentina, important environmental disturbances and degradation processes have been reported for the provinces of Salta and Jujuy by anthropic interventions, that are in the rise and have continuous negative impacts on the habitats (Bertonatti and Corcuera 2000; Vorano and Vargas Gil 2002). Thus, the aims of the present work were to: (a) evaluate the in situ persistence of morphologically variable wild populations of grl (2*x* and 4*x*) and spg in Andean valleys of Argentina, that had been sampled more than 20 years ago, (b) if populations persisted, to do a new sampling and to describe the natural habitats, and (c) to analyze the molecular variability among collection dates (original and recent), sites and populations, for the eventual proposal of conservation and ex situ regeneration strategies.

Materials and methods

The collection trip was planned for March 2005 (late summer) to increase the chances of finding plants with fruits. The Andean valleys were travelled across, searching for sites where variable grl and spg accessions—available from the Genebank at Balcarce (http://www.balcarce.inta.gov.ar/banco_germop/)—had been originally collected in 1973, 1974 and 1983 (Clausen and Castaño 1998) (Table 1).

Germplasm sampling

Altitude, latitude and longitude of each explored site were determined with global positioning system (GPS) and altimeter, and the original description notes of each of them were revised. Berries were collected considering the sampling strategy of genetic diversity proposed by Marshall and Brown (1975): 50 plants were taken as a suitable effective number per site and, if there were plenty of fruits, five to ten fruits were collected per plant. If the population was not bearing fruits or if the number of fruits was reduced, plantlets and tubers were collected (Frankel and Hawkes 1975).

Germplasm storage and regeneration

The collected material was prepared for ex situ conservation according to genebank guidelines [berry

Table 1 Collection sites of accessions of grl and spg in valleys of Northwest Argentina (Clausen and Castaño 1998)

Species	Accessions[a]	Collection sites				
		Province	Department	Quebrada–locality	Latitude–longitude–altitude (m.a.s.l.)	Collection date (dd/mm/yy)
4*x* grl	*Oka7588 A*	Jujuy	Tilcara	Jueya–Jueya	23°30′S–65°27′W–2,700	08/04/83
	Oka7547 A	Jujuy	Tumbaya	Lipán–Lipán	23°36′S–65°35′W–3,300	31/03/83
2*x* grl	*ORHL4841*	Salta	La Poma	Calchaquí–El Rodeo	24°33′S–66°12′W–3,900	21/03/73
	OL4858	Salta	Rosario de Lerma	del Toro–Las Cuevas	24°20′S–66°05′W–3,500	27/03/73
2*x* spg	*OL4911*	Salta	Chicoana	Escoipe–Cuesta del Obispo	25°10′S–65°52′W–3,340	04/04/73
	Oka5662	Salta	La Poma	Quebrada Calchaquí–Peña Agujereada	24°49′S–66°10′W–2,620	09/04/74

[a] Collectors: *Oka* K. A. Okada; *OL* K. A. Okada and O. Lucarini; *ORHL* K. A. Okada, K. Rahn, J. P. Hjerting and O. Lucarini

cleaning, germination test by ISTA (2003) regulations and seed drying with silica gel], and stored in sealed aluminum bags at 3°C. All the accessions were regenerated in the following season. Seeds of accessions that were used in further studies were kept at room temperature until sowing. The collected tubers and plantlets were cultivated in pots in the screenhouse; at flowering, they were sib-mated to generate seeds.

Soil sampling and analyses

Composed soil samples (~3 kg) were taken from the top 20 cm at each collection site. The samples were analyzed for physical and chemical composition in the Soil Laboratory of the EEA Balcarce, INTA. Only pH and texture will be reported.

Chromosome counts

Chromosome counts were performed in 10–18 plantlets per accession. Root tips of individual plantlets were treated with an 8-hydroxyquinoline solution (0.002 M) for 4 h, fixed in 3:1 (v/v absolute ethanol:glacial acetic acid) for 24 h, hydrolyzed in 1 N HCl at 60°C for 10 min, stained with leucobasic fuchsine (Coleman 1938) for 4 h in the dark, placed on a glass slide, squashed with a cover slip, and observed under a light microscope.

Description of habitats

Information on the habitats was compiled from Cabrera and Willink (1980), Cabrera (1994), Bianchi (1996), Bertonatti and Corcuera (2000) and Vorano and Vargas Gil (2002). For each sampled site, plants were herborized for identification of the potato species and accompanying flora. The area and landscape occupied by potatoes was documented with photographs, and records of temperature and precipitation in the nearby sites were obtained from Bianchi and Yañez (1992) and Vorano and Vargas Gil (2002). If not available, temperatures were estimated according to Bianchi (1996).

Molecular analyses

AFPL marker analyses were carried out in samples of three original accessions previously recorded as morphologically variable and in three new accessions sampled at the same sites. The objective was to study the distribution of genetic variability according to sites, collection dates and populations as follows: (a) Quebrada del Toro–Las Cuevas: 11 plants of the original OL 4858 accession (go), and eight plants of the new ClE 1575 accession (gn); (b) Quebrada de Escoipe–Cuesta del Obispo: 12 plants of the original OL 4911 accession (so) and eight plants of the new ClE 1581 accession (sn), and (c) Quebrada Calchaquí–Peña Agujereada: 10 plants of the original Oka 5662 accession (SO) and seven plants of the new ClE 1561 accession (SN). DNA was extracted from fresh leaves according to Dellaporta et al. (1983); four AFLP primers (*Eco*RI + ACA/*Mse*I + CTA; *Eco*RI + AGT/*Mse*I + CTA; *Eco*RI + ACA/*Mse*I + CAA and *Eco* RI + AGT/*Mse*I + CAA) were used to generate AFLP fragments following the procedure described by Vos et al (1995). Electrophoretic bands of each plant, classified as either present or absent, were transferred to a 1 (presence) and 0 (absence) matrix.

Principal coordinates analysis was performed, based on the similarity matrix and using the simple matching coefficient (Sokal and Michener 1958); a minimum spanning tree was established using the statistical INFOSTAT software package (2008). The proportion of the variability explained by each eigenvector and the corresponding representations in two dimensions were obtained. Cluster analysis based on the simple matching coefficient was also carried out using the UPGMA method. To analyze the distribution of genetic variation, an AMOVA was carried out with a hierarchical structure [collection sites, collection dates within sites, and accessions (plants) within collection dates and sites] using the Arlequin ver 3.0 software package (Excoffier et al. 2005). The variance components were tested for significance based on re-sampling, using 1,023 permutations of the data sets.

Results

Itinerary: The following provinces (italicized) and localities were visited: (a) *Tucumán*: Leales, (b) *Salta*: Valles Calchaquíes, Cafayate, Cachi, La Poma, Quebrada de Toro, Campo Quijano, Chicoana, Quebrada de Escoipe, Cuesta del Obispo, and (c) *Jujuy*: Tilcara, Quebrada de Jueya, Purmamarca, Quebrada de Lipán, Humahuaca, Coctaca, Hornillos and San Salvador de Jujuy.

Collection sites

The coordinates of previous collection trips (Clausen and Castaño 1998) were used to accurately identify the sites; records and field notes of those trips allowed us to register each site as either coincident with or close to the original. In this way, it was possible to localize all chosen sites, although some discrepancies in the geographic localization (i.e. coordinates, altitude) or ambiguities in the field notes were observed. In a few instances, close alternative sites were explored to determine the most likely original site.

Collected accessions

Potato populations were sampled and, according to their morphological phenotypes, identified as belonging to one of the following species: *S. gourlayi*, *S. spegazzinii*, *Solanum okadae* Hawkes et Hjerting (oka), *Solanum acaule* Bitter (acl), *S. chacoense* Bitter, *S. infundibuliforme* Phil. (ifd), *Solanum vernei* Bitter et Wittm. (vrn) and *S. tuberosum* spp. *andigena* (Juz. et Buk.) Hawkes (adg) (Table 2).

Details are given only for accessions collected at the original sampled sites for which the original samples had been registered as morphologically highly variable (Ispizúa 1994; Clausen, unpublished data). Average monthly temperature and average monthly rainfall of each Quebradas are presented below, along with soil pH values and texture, and accompanying flora of each site.

Salta Province

Quebrada Calchaquí

CIE 1561[2] *S. spegazzinii*, latitude 24°49′69S, longitude 66°09′84W, 2,762 m.a.s.l. La Poma Department, probable collection site of Oka 5662, 1 km North of Peña Agujereada, near the Calchaquí River, on both sides of National Route No. 40 and 800 m away from km 1,248. Populations of about 500 plants grew below thorny bushes and columnar cacti, protected from grazing animals, in an area of 5 × 20 m, and distributed in a typical cone of dragging soil with a 20% slope. The soil had scarce development, influenced by the nature of the local landscape and the erosive action of water. Plants were at the vegetative stage, with four to six leaves. Thirty tubers were sampled on both sides of the route. The phenotype corresponded to the pubescent type described by Ispizúa (1994). The accompanying species were: *Larrea cuneifolia* Cav., *Eupatorium* spp., *Prosopis ferox* Griseb. *Cercidium praecox* (Ruiz & Pav.), *Trichocereus* spp. and other cacti. Soil pH = 8.3, texture = loamy sand. Average annual rainfall is 139 mm, of which 94% occurs between December and March; average monthly temperature in the potato growing season is 14°C, with contrasting day and night average temperatures of 16°C.

ClE 1572 S. gourlayi, longitude 24°31′972S, latitude 66°11′279W, 3,672 m.a.s.l, La Poma Department, probable collection site of Oka 4841, National Route No. 40 km 1,289, 1.5 km away from El Rodeo, between La Quesera and El Rodeo, on both sides of the road, very common. The gorge in this site is transformed into a narrow place with high slopes on both sides framing the Calchaquí River that runs parallel to the route. The population consisted of about 500 plants. Berries of five plants and tubers of 60 plants, growing on both sides of the river, were collected. The accompanying species were: (muña-muña) *Satureja parvifolia* (Phil.) Epling, *Baccharis grisebachii* Hieron. *Cortaderia rudiuscula* Stapf, *Mutisia kurtzii* R.E.Fr. var. *anomala* (Lillo) Cabrera, *Stipa* spp., *Festuca* spp., *Baccharis ulicina* Hook. & Arn. The environment is more humid than in the surrounding area because the steep slopes determine a shaded area. The soil is about 20 cm deep by input of alluvional sediments. Soil pH = 8.2, texture = loamy sand. Average annual rainfall is 130 mm, of which 92% occurs between December and March; average monthly temperatures in the potato growing season is 13.8°C, with contrasting day and night average temperatures of 16.8°C.

Quebrada del Toro

ClE 1575 S. gourlayi, latitude 24°20′74S, longitude 66°03′64W, 3,500 m.a.s.l. Rosario de Lerma Department, probable collection site of Oka 4858, 6 km northwest of Las Cuevas, right side of National Route

---

[2] Collectors: A. M. Clausen, L. E. Erazzú

Table 2 New accessions with their geographical localization

Accession	Species	Department–locality	Latitude–longitude–altitude (m.a.s.l.)
ClE 1566	*S. gourlayi*	La Poma–La Quesera	24°32′S–66°11′W–3,645
ClE 1567	*S. acaule*	La Poma–La Quesera	24°32′S–66°11′W–3,645
ClE 1568	*S. gourlayi*	La Poma–La Quesera	24°32′569S–66°11′388W–3,602
ClE 1569	*S. tuberosum* ssp. andigena	La Poma–La Quesera	24°32′S–66°11′W–3,645
ClE 1570	*S. tuberosum* ssp. andigena	La Poma–La Quesera	24°32′S–66°11′W–3,645
ClE 1571	*S. tuberosum* ssp. andigena	La Poma–La Quesera	24°32′S–66°11′W–3,645
ClE 1578	*S. spegazzinii*	Chicoana	25°11′162S–65°51′20W–3,272
ClE 1579	*S. vernei*	Chicoana	25°10′95S–65°50′647W–3,070
ClE 1582	*S. spegazzinii*	Chicoana–Quebrada El Sunchal	25°09′81S–65°50′389W–2,864
ClE 1583	*S. okadae*	Chicoana	25°10′258S–65°49′75W–2,798
ClE 1584	*S. spegazzinii*	Chicoana	25°11′306S–65°48′170W–2,354
ClE 1585	*S. chacoense*	Chicoana	25°10′819S–65°46′595W–2,139
ClE 1586	*S. spegazzinii*	Chicoana	25°10′68S–65°45′W–2,030
ClE 1588	*S. infundibuliforme*	Tilcara–Quebrada de Jueya	23°29′22S–65°26′651W–2,990
ClE 1590	*S. gourlayi*	Tilcara–Quebrada de Jueya	23°29′46S–65°26′397W–2,831
ClE 1593	*S. gourlayi*	Tumbaya–Quebrada de Lipán	23°39′314S–65°34′472W–3,076
ClE 1594	*S. tuberosum* ssp. *andigena*	Tumbaya–Quebrada de Lipán	23°39′314S–65°34′472W–3,076
ClE 1595	*S. tuberosum* ssp. *andigena*	Tumbaya–Quebrada de Lipán	23°39′314S–65°34′472W–3,076
ClE 1596	*S. tuberosum* ssp. *andigena*	Tumbaya–Quebrada de Lipán	23°39′314S–65°34′472W–3,076
ClE 1598	*S. tuberosum* ssp. *andigena*	Humahuaca–Coctaca	23°08′63S–65°17′85W–3,227
ClE 1599	*S. tuberosum* ssp. *andigena*	Humahuaca–Coctaca	23°08′63S–65°17′85W–3,227
ClE 1600	*S. tuberosum* ssp. *andigena*	Humahuaca–Coctaca	23°08′63S–65°17′85W–3,227
ClE 1601	*S. tuberosum* ssp. *andigena*	Humahuaca–Coctaca	23°08′63S–65°17′85W–3,227
ClE 1602	*S. tuberosum* ssp. *andigena*	Humahuaca–Coctaca	23°08′63S–65°17′85W–3,227
ClE 1603	*S. tuberosum* ssp. andigena	Humahuaca–Coctaca	23°08′95S–65°17′747W–3,216
ClE 1604	*S. tuberosum* ssp. *andigena*	Humahuaca–Coctaca	23°08′95S–65°17′747W–3,216
ClE 1605	*S. tuberosum* ssp. *andigena*	Humahuaca–Coctaca	23°08′95S–65°17′747W–3,216
ClE 1606	*S. tuberosum* ssp. *andigena*	Humahuaca–Coctaca	23°08′95S–65°17′747W–3,216
ClE 1607	*S. tuberosum* ssp. *andigena*	Humahuaca–Coctaca	23°08′95S–65°17′747W–3,216

No. 51 on the way to San Antonio de los Cobres, 4.5 m away from the road. Tubers were collected from the 11 plants that formed this population, some of them had been chewed by livestock; in the rest, leaflets were glaucous and fleshy. They were accompanied by *Senecio* spp. and dry vegetation that could not be taxonomically identified. The landscape is framed in a steep slope of loose stones; the larger ones form receptacles containing the soil where potato plants grow; the soil has scarce evolution. The area occupied by potato plants is 10 m high above the road and 7 m wide, approximately; the slope was 60%, so that soil infiltration and runoff processes are dominant and determine a low accumulation of moisture. The original environment was apparently modified by the construction and maintenance of a road. Soil pH = 7.0, texture = sandy. Average annual rainfall is 120 mm, of which 95% occurs between December and March; average monthly temperatures in the potato growing season is 14°C, with contrasting day and night average temperatures of 17°C.

Quebrada de Escoipe

ClE 1581 S. spegazzinii, latitude 25°10′244S, longitude 65°50′823W, 3,005 m.a.s.l. Chicoana Department, probable collection site of Oka 4911, km 56 of Provincial Route No. 33, on the right side of the road to Cuesta del Obispo. The population had around 100 plants with very large stolons (up to 6 m long) and no flowers; berries and tubers were collected from 20 plants that, as the berries, had been chewed by goats and sheep. The accompanying species were: *Aloysia citriodora* Palau, *Senecio* spp., *Salvia* spp., *Festuca* spp., *Bidens andicola* Kuntze, *Tagetes minuta* L., *Acacia* spp., *Grindelia* spp. Soil pH = 8.3, texture = sandy loam. Average annual rainfall is 260 mm, of which 92% occurs between December and March; average monthly temperatures in the potato growing season is 15.2°C, with contrasting day and night average temperatures of 14°C.

Jujuy Province

Quebrada de Jueya

ClE 1587 S. gourlayi, latitude 23°29′22S, longitude 65°26′651W, 2,990 m.a.s.l. Tilcara Department, probable collection site of Oka 7588A, 10 km west of National Route No. 9, by the Quebrada de Jueya, on the north side of the gorge. The population had around 500–600 plants (that had been chewed by cattle), with no flowers or berries, growing among loose stones in an extension of 20 × 20 m on the north, more shaded slope. Tubers of 60 plants were collected. The accompanying species were *S. parvifolia*, *Nicotiana glauca*, *Grindelia* spp., *A. citriodora* and *Senecio* spp. Soil pH = 8.3, texture = loamy sand. Average annual rainfall is 118 mm, of which 94% occurs between December and March; average monthly temperatures in the potato growing season is 14.3°C, with contrasting day and night average temperatures of 18°C.

Quebrada de Lipán

ClE 1597 S. gourlayi, latitude 23°38′489S, longitude 65°34′292W, 3,188 m.a.s.l. Tumbaya Department, probable collection site of Oka 7547A, 2 h away on foot towards the northern part of the gorge by its eastern side. Population of about 100 plants at the vegetative stage, with two or three leaves; were growing by the river side. Tubers and 50 plantlets were sampled. The soil had been altered by human actions and was eroded by water. The accompanying species were: *C. rudiuscula*, *A. citriodora*, *S. parvifolia*, *Medicago sativa* L. and *Trichocereus* spp. Soil pH = 7.1, texture = sandy loam. Average annual rainfall is 129 mm, of which 94% occurs between December and March; average monthly temperature in the potato growing season is 14°C, with contrasting day and night average temperatures of 18.2°C.

Chromosome counts

The results of chromosomes counts of accessions identified as spg and grl and their collection sites are presented in Table 3.

Molecular analyses

The primer combinations produced 230 polymorphic bands. The results of the principal coordinate analysis are presented in Figs. 1, 2 and 3 in which the 1–2, 1–3 and 2–3 axes are displayed. It can be observed that (a) the three first eigenvectors explain an accumulated proportion of 44.5% of the total variability, (b) there is high variability given by the spatial plant dispersion; in fact, the plant data from each spg and grl accession do not present points of coincidence and plants are highly dispersed (see Figs. 1, 2, 3, as examples, SO7, SO1, so7, so14, sn8, sn13, go1, go13, gn1 and gn10); (c) there is an apparent separation among groups and an extensive overlapping of plants belonging to accessions from different sites and collection dates (see in Figs. 1, 2, 3, as examples, sn11, SO1, SN4, gn5 and go11). The cluster analysis (Fig. 4) had a cophenetic correlation coefficient of 0.924; plants of the original and new grl accessions were closely grouped, in contrast to plants of the spg accessions that exhibit extensive overlapping. Notwithstanding, at a distance of 0.67, the separation between the grl and spg accessions becomes apparent.

The results of the AMOVA are presented in Table 4. The largest percentage of the molecular variance, 49.58%, is due to variation within accessions, statistically significant at the 0.5% level. Smaller values with similar percentages of variation were observed among collection sites (23.58) and between collection dates within each site (26.84).

Discussion

Some discrepancies were observed between the coordinates registered for the 1973, 1974 and 1983 collection trips (Clausen and Castaño 1998; field

Table 3 Chromosome numbers in samples of accessions of grl and spg and their collection sites

Species	Collection sites	Accessions	No. of sampled plants	Chromosome number
spg	Peña Agujereada	ClE 1561	10	$2n = 2x = 24$
grl	La Quesera	ClE 1566b[a]	11	$2n = 4x = 48$
grl	La Quesera	ClE 1566a[a]	6	$2n = 2x = 24$
grl	La Quesera	ClE 1568	9	$2n = 2x = 24$
grl	El Rodeo	ClE 1572	14	$2n = 2x = 24$
grl	Las Cuevas	ClE 1575	7	$2n = 2x = 24$
spg	Cuesta del Obispo	ClE 1581	18	$2n = 2x = 24$
spg	Cuesta del Obispo	ClE 1584	10	$2n = 2x = 24$
spg	Cuesta del Obispo	ClE 1586	11	$2n = 2x = 24$
grl	Jueya	ClE 1587	9	$2n = 4x = 48$
grl	Jueya	ClE 1590	11	$2n = 4x = 48$
grl	Lipán	ClE 1597	11	$2n = 4x = 48$

[a] Samples from both sides of the road

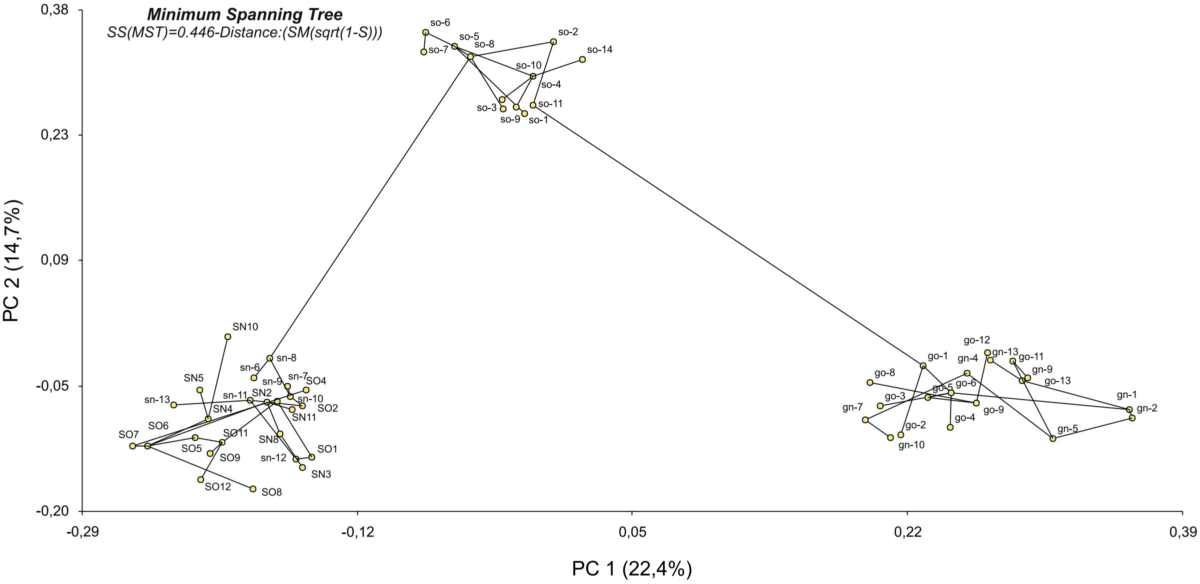

Fig. 1 Principal coordinates analysis, 1–2 axes of plants of the original and new spg and grl accessions based in AFLP markers with so = OL 4911, sn = ClE 1581, SO = Oka 5662, SN = ClE 1561, go = OL 4858, gn = ClE 1575

notes of the Genebank of the EEA Balcarce, INTA) and those of the present collections. This probably results from the fact that the former were calculated by manual approximations whereas the latter were registered with digital precision instruments. There is a low probability that the sites of the new collections may not actually correspond to the original sites because precautions were taken to minimize errors. Discrepancies of this sort were also reported by Hijmans et al. (2000) when generating a data base for the Bolivian Potato Genebank—which led these authors to propose new coordinates for already available accessions—and by Bamberg et al. (2003) for accessions of wild potatoes from Southwestern USA. Also, Hijmans et al. (1999) and Hijmans and Spooner (2001) reported important discrepancies in records from genebanks in Central America, with errors as extreme as indicating with precision instruments that some of the recorded sites were actually situated in the ocean. In comparison with these

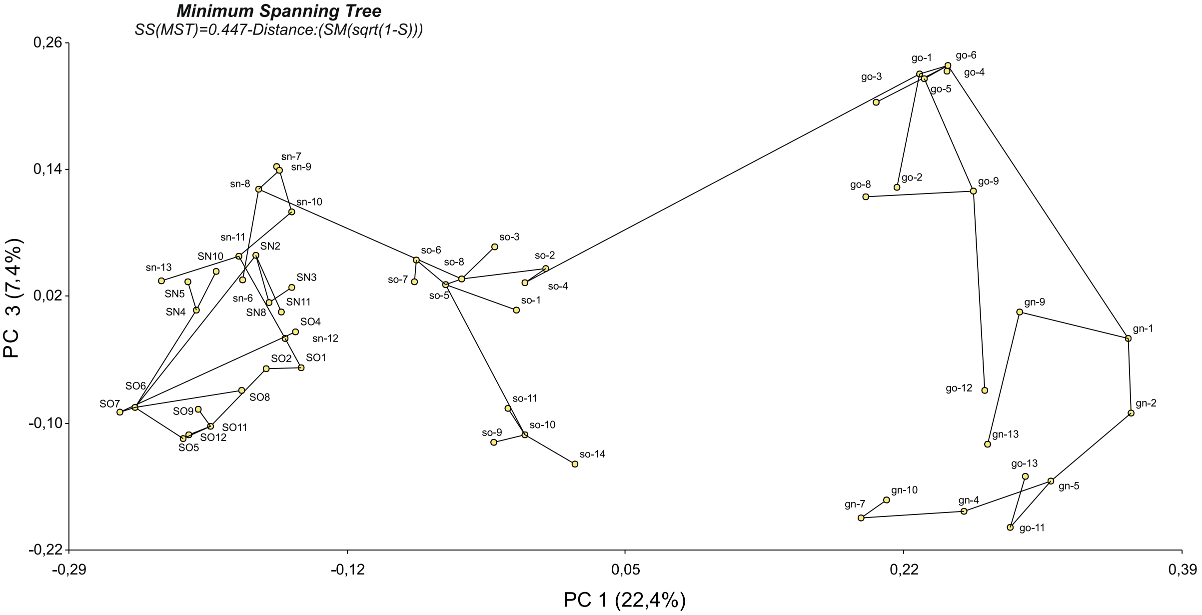

Fig. 2 Principal coordinates analysis, 1–3 axes of plants of the original and new spg and grl accessions based in AFLP markers with so = OL 4911, sn = ClE 1581, SO = Oka 5662, SN = ClE 1561, go = OL 4858, gn = ClE 1575

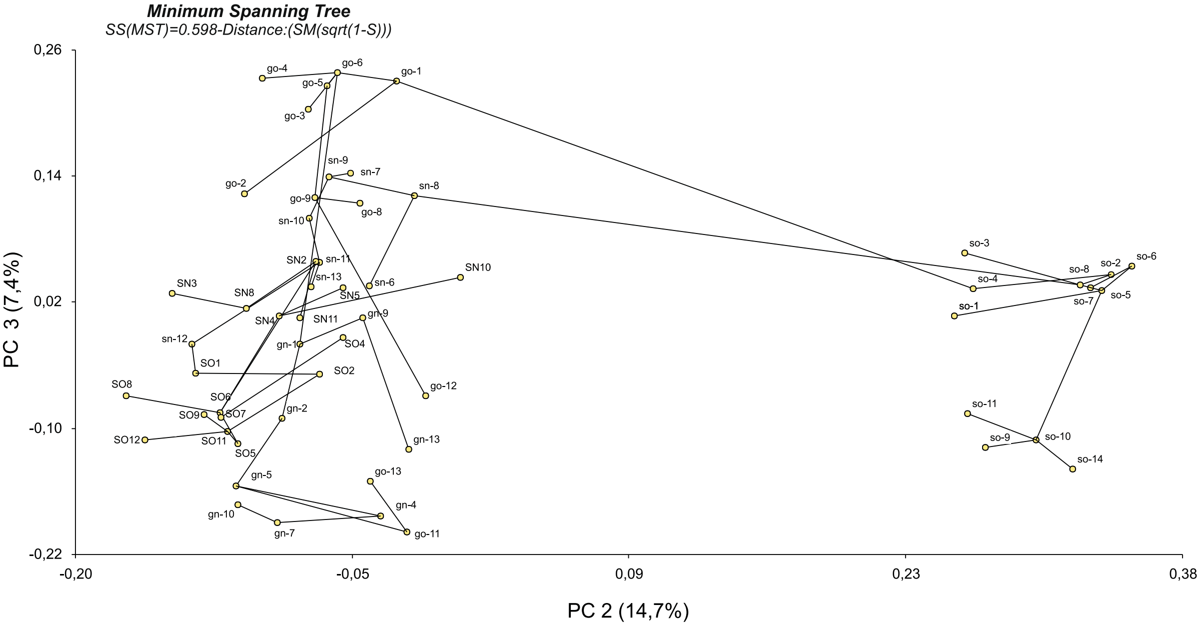

Fig. 3 Principal coordinates analysis, 2–3 axes of plants of the original and new spg and grl accessions based in AFLP markers with so = OL 4911, sn = ClE 1581, SO = Oka 5662, SN = ClE 1561, go = OL 4858, gn = ClE 1575

reports, the discrepancies observed in the new collections sites of grl and spg accessions were minor.

The results of the present collection trip provide evidence of in situ persistence of 2*x* and 4*x* grl and spg populations in intermontaneus valleys of Salta and Jujuy after more than 20 years from previous collections. This situation is similar to the one reported by Spooner et al. (1998) for wild potato species in valleys of Guatemala and Perú and by Bamberg et al. (2003) for accessions of wild potatoes

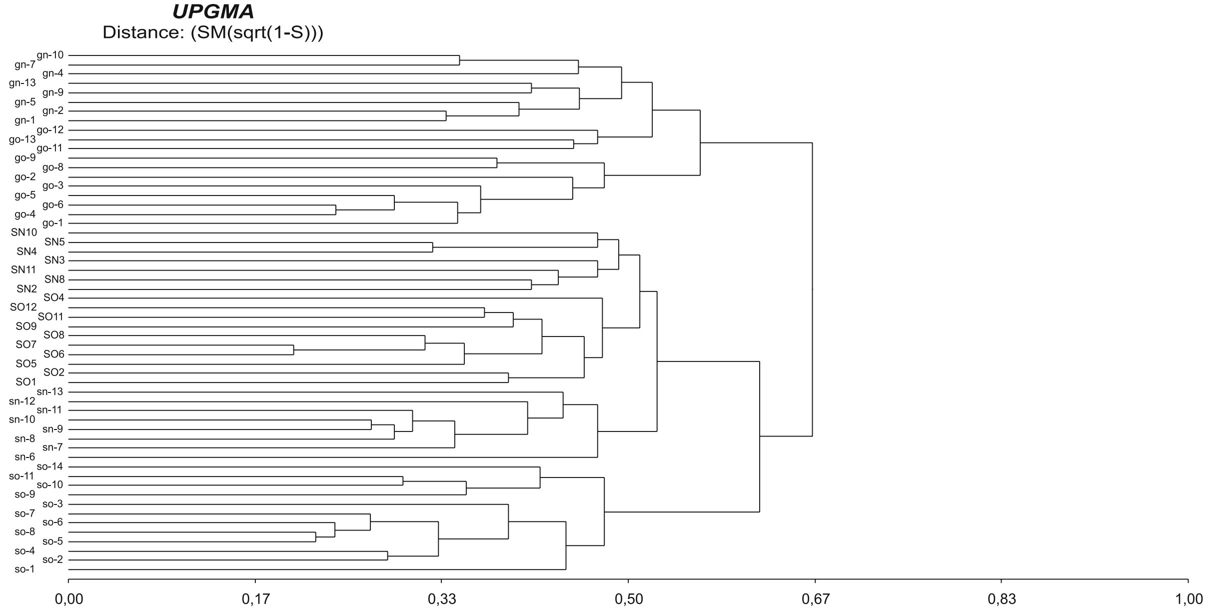

Fig. 4 UPGMA phenogram (simple matching distance coefficient) of the original and new spg and grl accessions plants based in the AFLP markers

Table 4 AMOVA based in the spg and grl accessions

Source of variation	*df*	SSD	Variance components	% Variation	*P*
Among sites	2	377.559	5.96166	23.58	ns
Between dates within sites	3	221.453	6.78438	26.84	ns
Within accessions	50	626.774	12.53549	49.58	0.048
Total	55	1,225.786	25.28152		

from Southwest USA. In spite of the fact that strong environmental disturbances were detected in some of the sites that had been previously sampled, most populations contained a large number of plants (50–100), except for one 2*x* grl population (ClE 1575) from Las Cuevas, Quebrada del Toro, that had <15 plants. This low number of plants could be an indication of genetic erosion, because important land movements carried out recently for a road construction had strongly modified the landscape.

The persistence of wild potato populations in the sites sampled in previous trips can be attributed to their capacity to reproduce both sexually and asexually. The combination of these two types of reproduction proves advantageous in disturbed environments (Hawkes 1990). In fact, genetic variability can be created in each generation by sexual reproduction whereas adapted genotypes can be maintained over time by asexual means.

Overlapping potato populations were registered in various sites of the distribution area of the species under study. As examples, accession ClE 1581 of spg from Quebrada de Escoipe, Salta, was sympatric with accessions ClE 1579 of vrn, ClE 1585 of chc and ClE 1583 of oka; similarly, accession ClE 1566 of 2*x* grl from La Poma grew in the same site as accession ClE 1567 of acl and closely to a cultivated potato field; in the collection site of accession ClE 1587 of 4*x* grl in Quebrada de Jueya, a population of 2*x* ifd (ClE 1588) was found growing 800 m apart; and in Quebrada de Lipán, accession ClE 1597 of 4*x* grl was recorded growing with Andean potato varieties.

The overlapping of wild potato species is frequently due to anthropic actions that in direct and indirect ways (see Vorano and Vargas Gil 2002; Brown et al. 2005) can modify ecological and geographic isolation barriers, creating numerous hybrid environments (Clausen et al. 2005). This overlapping is not an event restricted in time and space because a similar observation of overlapping in distribution areas and the proximity of various wild and cultivated potato species was recorded by the Genebank in different collection years. Thus, in the distribution area described by Okada in 1973 (field notes of the Genebank of the EEA Balcarce, INTA), accession OL 4911 of spg was found growing with accession OL 4907 of chc and OL 4908 of oka. In La Poma, accession OL 4841 of 2*x* grl was found growing with accession OL 4842 of adg. Likewise, in the collection trip of 1983 (field notes of the Genebank of the EEA Balcarce, INTA), a population of 4*x* grl (Oka 7547A) was found in Quebrada de Lipán growing with adg (Oka 7547C) and *S. microdontum* (mcd, Oka 7547B); and in Quebrada de Jueya, accession Oka 7588A of 4*x* grl was sympatric with accession 7588B of adg and accession Oka 7588C of *Solanum oplocense* (opl). A long list of sympatric wild potato species was described by Hawkes and Hjerting (1969); in particular, populations of chc, *Solanum tarijense* (tar), spg, grl, mcd and vrn were described along with probable interspecific hybrids along the Cuesta del Obispo. These authors also recorded species that overlapped in their distribution area, like grl and ifd in the proximities of Tilcara, in typically dry quebradas. The incomplete internal barriers to hybridization that can be acting among many species (Camadro et al. 2004) would allow the formation of interspecific hybrids, introgression and gene flow as suggested by Clausen et al. (2005) in Palca de Aparzo or demonstrated for *Solanum xrechei* (Clausen and Spooner 1998).

Closely related potato species do not present important genomic differentiation (Matsubayashi 1991) and, thus, natural fertile hybrids can be formed (Rabinowitz et al. 1990; Camadro et al. 2004). In fact, it has been demonstrated that various potato species are of hybrid origin: *S. xrechei* (Clausen and Spooner 1998) and *Solanum xruiz-lealii* Brücher (Raimondi et al. 2005) and high morphological variability has been reported for others (Spooner and van den Berg 1992; Raimondi et al. 2005; Bedogni and Camadro 2007). Moreover, unexpected reproductive dysfunctions upon crossing within and among accessions of a given species have been reported, such as low seed set and several pollen–pistil incompatibility sites (Erazzú et al. 1999; Raimondi et al. 2003; Erazzú and Camadro 2008) and endosperm collapse (Masuelli and Camadro 1997). In controlled reciprocal crosses among plants of the same spg and 2*x* grl accessions used in the present study, some genotypic combinations produced hybrid seeds (34% and 16%, respectively) although many of them exhibited incompatibility reactions (Erazzú and Camadro 2008). These evidences have led Masuelli et al. (2009) to the speculation, based on numerous literature reports of over 50 years, that hybridization, specifically interspecific hybridization, can induce genetic and epigenetic changes that can act as creative evolutionary forces in wild potatoes. In this regard, two Argentinean species, chc and spg, are of particular interest, since both of them present a wide range of distribution as well as affinity with a number of species, many of them sympatric. The high morphological variability observed in populations of the two species in modified habitats (Hawkes and Hjerting 1969) could be the result of interspecific hybridization and introgression and (or) epigenetic effects (Marfil et al. 2006). Furthermore, the presence of meiotic mutants is widespread among potato species (den Nijs and Peloquin 1977; Camadro and Peloquin 1980; Leue and Peloquin 1980, Watanabe and Peloquin 1989; 1991). They can give rise to gametes and gametophytes with unreduced (sporophytic) chromosome numbers (2*n* gametes) that are functional in fertilization; in this way, polyploids can be formed by the sexual process (Mok and Peloquin 1975). Sexual polyploidization coupled with haploidization, as in *Dichantium* (de Wet 1968), can provide the opportunity for gene flow and introgression within and between ploidy levels (Camadro et al. 2004). Support for the preceding speculations is given by the results of chromosome counts carried out in populations of grl from La Poma, reported in the present work. Both 2*x* (accession ClE 1566a) and 4*x* grl (accession ClE 1566b) cytotypes, that had not been recorded in the 1973 collection trip, were found growing at the same site. In addition, plants of both cytotypes of grl and plants of 4*x* acl were found at the same site. As reported by Hawkes and Hjerting (1969), 4*x* acl can form hybrids with grl, making it sound to extend the previous speculations to interspecific crosses as well.

The conclusion of Ugent (1970) that “hybridization and subsequent gene flow within and between ploidy levels often result in exceedingly complicated patterns of variation” is reinforced by these observations, giving support to the idea that the biological species concept is not appropriate for the tuber-bearing *Solanum* (Camadro et al. 2004).

The AFLP markers, almost always dominant, can mask recessive allele and differences tend to be exaggerated. The principal coordinates analyses did not separate the spg accessions; in fact, plants of distant sites and collected in different years appeared intermixed. These results may be questioned due to the relatively low percentage of the total variability explained by the first three axes. However, in the cluster analysis using the AFLP markers data, plants of the spg accessions from different sites and collection dates grouped intermixed, in disagreement with the expected; moreover, all of them grouped together at distance of 0.62. Grouping of plants of the grl accessions was, in contrast, consistent with the expected. It has to be pointed out that grl and spg accessions grouped at a distance of 0.67, very close to the spg accessions grouping. Similarly, a lack of consistency for RAPD markers was reported by del Rio et al. (1997) for populations of *S. jamesii* and *S. fendleri* sampled at the same collection sites in different years. Moreover, van den Berg et al. (2002), Giannattasio and Spooner (1994), and Jacobs et al. (2008), using various molecular markers, reported a weak separation or a lack of phylogenetic structure among the species studied. The results of the AMOVA revealed that a high percentage of the total variation was present in plants of different and distant sites and different collection dates, in contrast to the other two variation sources (sites and collection dates within sites).

Although the phenomenon of natural hybridization in potato species has been well known for many years (see Hawkes and Hjerting 1969; Okada and Clausen 1982), most accessions in genebanks have been classified as pure species. This is also the case with the accessions of the collection trip reported in this work. However, the results of this investigation point to the need of ascertaining if the morphological variability encountered in the grl and spg accessions analyzed, as well as in other tuber-bearing *Solanum*, is the expected for a given taxon or the result of hybridization and introgression events. This information is of importance for the efficient conservation, ex situ multiplication and utilization of the wild gene pool.

Acknowledgments This research was financially supported by the Instituto Nacional de Tecnología Agropecuaria (INTA), National Project No. 744, and the Universidad Nacional de Mar del Plata (UNMdP).

References

Bamberg JB, Martin MW, Schartner JJ, Spooner DM (1996) Inventory of tuber-bearing *Solanum* species. Catalog of Potato Germplasm-1996. NRSP-6, Sturgeon Bay, WI

Bamberg JB, del Río AH, Huamán Z, Vega S, Martin M, Salas A, Pavek J, Kiru S, Fernández C, Spooner DM (2003) A decade of collecting and research on wild potatoes of the Southwest USA. Am J Potato Res 80:159–172

Bedogni MC, Camadro EL (2007) Caracterización molecular de la especie silvestre diploide de papa *Solanum kurtzianum* Bitt. et Wittm mediante microsatélites. XXXVI Congreso Argentino de Genética, Pergamino, 23–26 de septiembre de 2007

Bertonatti C, Corcuera J (2000) Situación ambiental Argentina 2000. Fundación Vida Silvestre Argentina, Buenos Aires

Bianchi A (1996) Temperaturas medias estimadas para la región del Noroeste de Argentina. SAGPyA-INTA EEA Salta, Argentina

Bianchi R, Yañez E (1992) Las precipitaciones en el Noroeste Argentino, 2da. Edición. INTA EEA Salta, Argentina

Brown A, Martínez Ortiz U, Acerbi M, Corcuera J (2005) La situación ambiental Argentina 2005. Fundación Vida Silvestre Argentina, Argentina

Cabrera AL (1994) Enciclopedia Argentina de agricultura y jardinería: Regiones fitogeográficas Argentinas. Tomo II, Argentina

Cabrera AL, Willink A (1980) Biogeografía de América Latina: Monografía 13, Serie Biología, OEA

Cacace JE, Huarte MA, Monti MC (1994) Evaluation of potato cooking quality in Argentina. Am Potato J 71:145–153. doi:10.1007/BF02849049

Camadro EL, Peloquin SJ (1980) The occurrence and frequency of 2*n* pollen in three diploid *Solanums* from Northwest Argentina. Theor Appl Genet 56:11–15. doi:10.1007/BF00264421

Camadro EL, Carputo D, Peloquin SJ (2004) Substitutes for genome differentiation in tuber-bearing *Solanum*: interspecific pollen–pistil incompatibility, nuclear-cytoplasmic male sterility, and endosperm. Theor Appl Genet 109:1369–1376. doi:10.1007/s00122-004-1753-2

Clausen AM, Castaño MC (1998) Catálogo del material del herbario BAL. *Solanum* sect. Petota. Argentina

Clausen AM, Okada KA (1987) The subspecies of *Solanum gourlayi* Hawkes. Phytologia 62:165–170

Clausen AM, Spooner DM (1998) Molecular support for the hybrid origin of the wild potato species *Solanum* × *rechei* (*Solanum* sect. *Petota*). Crop Sci 38:858–865

Clausen AM, Colovita M, Butzonitch I, Carranza AV (2005) A potato collecting expedition in the province of Jujuy, Argentina and disease indexing of virus and fungus pathogens in Andean cultivars. Genet Resour Crop Evol 52:1099–1109. doi:10.1007/s10722-004-6131-z

Coleman LC (1938) Preparation of leucobasic fuchsin for use in the feulgen reaction. Stain Technol 13:123–124

Correll D (1962) The potato and its wild relatives. Texas Res Found, Renner, USA

Davies CS, Ottman MJ, Peloquin SJ (2002) Can germplasm resources be used to increase the ascorbic acid content of stored potatoes? Am J Potato Res 79:295–299

De Wet JMJ (1968) Diploid–tetraploid–haploid cycles and the origin of variability in *Dichantium* agamospecies. Evol Int J Org Evol 22:394–397. doi:10.2307/2406537

del Rio AH, Bamberg JB, Huaman Z, Salas A, Vega SE (1997) Assessing changes in the genetic diversity of potato gene banks. 2. In situ vs ex situ. Theor Appl Genet 95:199–204. doi:10.1007/s001220050548

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: version II. Plant Mol Biol 1:19–21. doi:10.1007/BF02712670

den Nijs TPM, Peloquin SJ (1977) 2*n* gametes in potato species and their function in sexual polyploidization. Euphytica 26:585–600. doi:10.1007/BF00021684

Erazzú LE, Camadro EL (2008) Barreras a la hibridación intra-e inter-específica en las especies de papa *Solanum spegazzinii* bitt., *S. gourlayi* hawkes y *S. tuberosum* ssp. *Tuberosum*. XXXVII Congreso Argentino de Genética, Tandil, 21–24 de setiembre de 2008. BAG, pp 165–166

Erazzú LE, Camadro EL, Clausen AM (1999) Pollen-style compatibility relations in natural populations of the wild diploid potato species *Solanum spegazzinii* Bitt. Euphytica 105:219–227. doi:10.1023/A:1003484010718

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50

Frankel OH, Hawkes JG (1975) Crop Genetic Resources for Today and Tomorrow, vol 2. Cambridge University Press, Cambridge

Giannattasio RB, Spooner DM (1994) A reexamination of species boundaries and hypotheses of hybridization concerning *Solanum megistacrolobum* and *S. toralapanum* (*Solanum* sect. *Petota*, series *Megistacroloba*): molecular data. Syst Bot 19:106–115. doi:10.2307/2419715

Hanneman RE Jr (1989) The potato germplasm resource. Am Potato J 66:655–667. doi:10.1007/BF02853985

Hawkes JG (1990) The potato. Evolution, biodiversity and genetic resources. Belhaven Press (Printer Publisher), London

Hawkes JG, Hjerting JP (1969) The potatoes of Argentina, Brazil, Paraguay and Uruguay. A biosystematic study. Oxford University Press, London

Hayes RJ, Thill CA (2002) Introgression of cold (4°C) chipping from 2*x* (2 Endosperm Balance Number) potato species into 4*x* (4EBN) cultivated potato using sexual polyploidization. Am J Potato Res 79:421–431

Hijmans RJ, Spooner DM (2001) Geographic distribution of wild potato species. Am J Bot 88:2101–2112. doi:10.2307/3558435

Hijmans RJ, Schreuder M, De La Cruz J, Guarino L (1999) Using GIS to check co-ordinates of germplasm accessions. Genet Resour Crop Evol 46:291–296. doi:10.1023/A:1008628005016

Hijmans RJ, Garrett KA, Huamán Z, Zhang DP, Schreuder M, Bonierbale M (2000) Assessing the geographic representativeness of genebank collections: the case of Bolivian wild potatoes. Conserv Biol 14:1755–1765. doi:10.1046/j.1523-1739.2000.98543.x

Huamán Z, Hoekstra R, Bamberg JB (2000) The inter-genebank potato database and the dimensions of available wild potato germplasm. Am J Potato Res 77:353–362

InfoStat (2008) InfoStat ver. 2008. (Online) Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. Available from http://www.infostat.com.ar. Accessed Dec 2008

Ispizúa VN (1994) *Solanum spegazzinii* Bitt: variabilidad intraespecífica y su relación con especies afines. Trabajo de Graduación Ing. Agr., FCA, UNMdP, Balcarce, Argentina

ISTA (International Seed Testing Association) (2003) International rules for seed testing. Basserdof, CH-Switzerland

Jacobs MMJ, van den Berg R, Vleeshouwers VGAA, Visser M, Mank R, Sengers M, Hoekstra R, Vosman B (2008) AFLP analysis reveals a lack of phylogenetic structure within *Solanum* section *Petota*. BMC Evol Biol 8:145. doi:10.1186/1471-2148-8-145

Jansky SH, Peloquin SJ (2005) Advantages of wild diploid *Solanum* species over cultivated diploid relatives in potato breeding programs. Genet Resour Crop Evol 56:669–674

Leue EF, Peloquin SJ (1980) Selection for 2*n* gametes and tuberization in *Solanum chacoense*. Am Potato J 57:189–195. doi:10.1007/BF02854230

Lynch DR, Kawchuk LM, Chen Q, Kokko M (2003) Resistance to *Fusarium sambucinum* in wild and cultivated *Solanum* species. Am J Potato Res 66:353–358

Marfil CF, Masuelli RW, Davison J, Comai L (2006) Genomic instability in *Solanum tuberosum* × *Solanum kurtzianum* interspecific hybrids. Genome 49:104–113

Marshall DR, Brown AHD (1975) Optimum sampling strategies in genetic conservation. In: Frankel OH, Hawkes JG (eds) Genetic resources for today and tomorrow. Cambridge University Press, pp 53–80

Masuelli RW, Camadro EL (1997) Crossability relationship among wild potato species with different ploidies and Endosperm Balance Numbers (EBN). Euphytica 94:227–235. doi:10.1023/A:1002996131315

Masuelli RW, Camadro EL, Erazzú LE, Bedogni C, Marfil C (2009) Homoploid hybridization in the origin and evolution of wild diploid potato species. Plant Syst Evol 277:143–151

Matsubayashi M (1991) Phylogenetic relationships in the potato and its related species. In: Tsuchiya T, Gupta PK (eds) Chromosome engineering in plants. Genetics, breeding, evolution, Part B. Elsevier Science Publishers BV, Amsterdam, pp 93–118

Maxted N, Ford-Lloyd BV, Hawkes JG (1997) Complementary conservation strategies. In: Maxted N, Ford-Lloyd BV, Hawkes JC (eds) Plant genetics resources conservation. Chapman & Hall, London, pp 15–39

Mok DWS, Peloquin SJ (1975) Breeding value of 2*n* pollen (diplandroids) in tetraploid × diploid crosses in potato. Theor Appl Genet 46:307–314

Okada KA, Clausen AM (1982) Natural hybridization between *Solanum acaule* Bitt. and *S. megistacrolobum* Bitt. in the province of Jujuy, Argentina. Euphytica 31:817–835. doi:10.1007/BF00039223

Oltmans SM, Novy RG (2002) Identification of potato (*Solanum tuberosum* L.) haploid × wild species hybrids with the capacity to cold-chip. Am J Potato Res 79:263–268

Rabinowitz D, Linder CR, Ortega R, Begazo D, Murguia H, Douches DS, Quirós CF (1990) High levels of interspecific hybridization between *Solanum sparsipillum* and *S. stenotomum* in experimental plots in the Andes. Am Potato J 67:73–81. doi:10.1007/BF02990957

Raimondi JP, Sala RG, Camadro EL (2003) Crossability relationships among the wild diploid potato species *Solanum kurtzianum, S. chacoense* and *S. ruiz-lealii* from Argentina. Euphytica 132:287–295. doi:10.1023/A:1025031425002

Raimondi JP, Peralta IE, Masuelli RW, Feingold S, Camadro EL (2005) Examination of the hybrid origin of the wild potato *Solanum ruiz-lealii* Brücher. Plant Syst Evol 253:33–51. doi:10.1007/s00606-005-0299-3

Ross H (1986) Potato breeding—problems and perspectives. Advances in plant breeding, supplement 13. Verlag Paul Parey, Berlin, Hamburg

Rouppe van der Voort J, Wolters P, Folkertsma R, Hutten R, van Zandvoort P, Vinke H, Kanyuka K, Bendahmane A, Jacobsen E, Janssen R, Bakker J (1997) Mapping of the cyst nematode resistance locus *Gpa2* in potato using a strategy based on comigrating AFLP markers. Theor Appl Genet 95:874–880. doi:10.1007/s001220050638

Rousselle-Bourgeois F, Mugniery D (1995) Screening tuber-bearing *Solanum* spp. for resistance to *Globodera rostochiensis* Ro1 Woll. and *G. pallida* Pa2/3 stone. Potato Res 38:241–249. doi:10.1007/BF02359906

Santini VE, Mendiburu AO, Okada KA, Monti MC (1976) Crossability of Solanum progeny. Am Potato J 53:371–372

Santini M, Camadro EL, Marcellán ON, Erazzú LE (2000) Agronomic characterization of diploid hybrid families derived from crosses between haploids of the common potato and three wild Argentinian tuber-bearing species. Am J Potato Res 77:211–218

Simmonds NW (1963) Abbreviations of potato names. Eur Potato J. 6:186–190. doi:10.1007/BF02365786

Sokal RR, Michener CD (1958) A statistical method for evaluating systematic relationship. Univ Kans Sci Bull 38:1409–1438

Spooner DM, van den Berg RG (1992) An analysis of recent taxonomic concepts in wild potatoes (*Solanum* sect. Petota). Genet Resour Crop Evol 39:23–37. doi:10.1007/BF00052651

Spooner DM, Hoekstra R, van den Berg RG, Martinez V (1998) *Solanum* sect. Petota in Guatemala: taxonomy and genetic resources. Am J Potato Res 75:3–17

Ugent D (1970) The potato. What is the botanical origin of this important crop plant, and how did it first become domesticated? Science 170:1161–1166. doi:10.1126/science.170.3963.1161

van den Berg RG, Bryan GJ, del Río A (2002) Reduction of species in the wild potato Solanum section Petota series Longipedicellata: AFLP, RAPD and chloroplast SSR data. Theor Appl Genet 105:1109–1114. doi:10.1007/s00122-002-1054-6

Vorano AE, Vargas Gil J (2002) Evaluación de la situación actual de los procesos de desertificación de la Puna Salto-Jujeña. Soluciones alternativas. INTA-EEA Salta

Vos P, Hoger R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acid Res 23:4407–4414. doi:10.1093/nar/23.21.4407

Watanabe K, Peloquin SJ (1989) Occurrence of 2*n* pollen and ps gene frequencies in cultivated groups and their related wild species in tuber-bearing *Solanums*. Theor Appl Genet 78:329–336. doi:10.1007/BF00265292

Watanabe K, Peloquin SJ (1991) The occurrence and frequency of 2*n* pollen in 2*x*, 4*x*, and 6*x* wild, tuber-bearing *Solanum* species from Mexico, and Central and South America. Theor Appl Genet 82:621–662. doi:10.1007/BF00226800