Genetic and morphological variability in populations of the wild diploid potato species *Solanum maglia* and *Solanum kurtzianum* from Argentina

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Abstract: *Solanum maglia* Schltdl., a wild potato species that has its widest geographical distribution in Chile, is restricted in Argentina to Quebrada La Cumbre (quebrada = gorge), Mendoza province, where no other potato species has been reported. During two collecting expeditions carried out in 2006 and 2007 in this gorge, tubers of two potato populations separated by 500 m (area 1 and 2) were sampled. The morphological and genetic diversity of these two newly sampled populations and of five accessions from the same gorge classified as *S. maglia*, a plant of *Solanum kurtzianum* collected outside this gorge, and a Chilean plant of *S. maglia* were examined by comparing 24 morphological characters and electrophoretic patterns for simple sequence repeat and amplified fragment length polymorphism markers, respectively. Based on the current taxonomic concept, the results support the classification of plants from area 1 as *S. kurtzianum* and those from area 2 as *S. maglia*, except for one plant from the former area, which shared electrophoretic bands from both species. This is the first report on the sympatry of populations of the two species in Quebrada La Cumbre. The importance of performing in situ population studies in the same sites over years is discussed.

Key words: amplified fragment length polymorphism, genetic resources, introgression, *Solanum kurtzianum*, *Solanum maglia*, simple sequence repeat.

Résumé : Le *Solanum maglia*, une espèce de pomme de terre sauvage ayant sa plus importante distribution géographique au Chili, est confiné en Argentine à la Quebrada La Cumbre (quebrada = gorge), de la province Mendoza, où aucune autre espèce de pomme de terre n'a été rapportée. Au cours de deux expéditions de récoltes en 2006 et 2007 dans cette gorge, les auteurs ont pu échantillonner des tubercules de deux populations séparées de 500 m (aire 1 et 2). Les auteurs ont examiné la morphologie et la diversité génétique de ces deux populations nouvellement récoltées, et de cinq accessions de la même gorge classifiées comme *S. maglia*, un plant du *Solanum kurtzianum* récolté en dehors de cette gorge, et une plante provenant du Chili, en comparant 24 caractères morphologiques ainsi que les patrons électrophorétiques pour les marqueurs répétition de séquences simples et polymorphisme des longueurs des fragments amplifiés respectivement. Sur la base des concepts taxonomiques actuels, les résultats supportent la classification des plantes de l'aire 1 comme *S. kurtzianum*, et ceux de l'aire 2 comme *S. maglia*, sauf pour une plante de la première aire, partageant des bandes électrophorétiques avec les deux espèces. Il s'agit du premier rapport sur la sympatrie des populations des deux espèces à Quebrada La Cumbre. Les auteurs discutent l'importance de réaliser des études de populations in situ sur les mêmes sites au cours des années.

Mots-clés : polymorphisme des longueurs des fragments amplifiés, ressources génétiques, introgression, *Solanum kurtzianum*, *Solanum maglia*, répétition de séquences simples.

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Introduction

Potatoes (*Solanum* L. sect. *Petota* Durmort, subsec. *Potatoe*) constitute a significant reservoir of genetic variability for potato improvement, including resistance/tolerance to biotic and abiotic stresses, as well as desirable industrial and culinary traits (Ross 1986; Huamán et al. 2000; Santini et al. 2000; Davies et al. 2002; Oltmans and Novy 2002; Jansky and Peloquin 2005). Modern potato cultivars were derived initially from a few clones taken to Europe from the New World (Grun 1970; Provan et al. 1999); thus, they possess a narrow genetic base that helps to explain the vulnerability of the crop to new pathogens or variants of existing ones, and limits the diversity of environments in which they can be successfully cultivated (Camadro and Mendiburu 1988). Given this narrow genetic base, the use of wild germplasm to increase genetic variability is a central objective of potato breeding programs around the world. Ross (1986) emphasized the importance of wild species in potato breeding and many cultivars developed in Europe and North America have wild germplasm in their genealogy that confers resistance/tolerance to many pathogens and pests.

Hawkes (1990) classified the tuber-bearing species of section *Petota* into 21 series. Series *Maglia* includes only one species, *Solanum maglia* Schltdl., which can be distinguished from others in the same section by its characteristic loose barrel-shaped anther column, with anthers and filaments not well differentiated from each other (Bitter 1912; Correll 1962; Brücher 1965; Hawkes and Hjerting 1969). In accessions of *S. maglia*, resistance to *Verticillium* wilt, aphids, potato viruses A (PVA), X (PVX), and Y (PVY), wart, nematodes, leaf hoppers, and flea beetles has been reported (Hawkes and Hjerting 1969; Contreras 1987). The first attempts to hybridize wild species with the cultivated potato *Solanum tuberosum* involved the use of different collections (accessions) of *S. maglia* and *Solanum commersonii* (Hawkes 1958).

The widest distribution of *S. maglia* occurs in Chile, where it can be found both on the coast and at about 35 km inland in the provinces of Santiago, Aconcagua, and Coquimbo, from 30°00′S to 33°20′S at sea level up to 700 m of altitude (Hawkes and Hjerting 1969; Contreras 1987; Spooner et al. 1991). In Argentina, Clausen and Masuelli (in Spooner and Clausen 1993), sampled tubers in five sites along a distance of 5 km in the Quebrada de Alvarado, Mendoza province, between 1630 and 1820 m of altitude. Tubers from each site were considered as one accession, classified as *S. maglia* for conservation purposes in germplasm banks, to preserve the biodiversity outside its natural habitat (*ex situ*). These are the only Argentinian records for this species, which has not been sampled in any other site of that province.

In studying the sexual crossability between plants derived from the originally collected tubers, Ispizúa et al. (1999) concluded that the five *S. maglia* accessions were a single clone. The collection site, registered as Quebrada de Alvarado, was actually Quebrada La Cumbre, also known as Quebrada El Chalet (from here on, referred to as Quebrada La Cumbre). No other species have been found growing in this gorge so far, even though it has been explored in different years.

In Chile, the species is in danger of extinction by housing developments near the Pacific coast, with the consequent elimination of cultivated areas (Contreras 1987). In Mendoza, the species is threatened by its restricted distribution in Quebrada La Cumbre and the drastic changes that are occurring in its ecosystem, as a result of cattle farming.

The main goal of germplasm collections is to preserve the genetic variability available in wild and cultivated forms, an essential component of all breeding programs. Studies designed to explore the genetic variability of wild populations need to be geographically extensive and intensive, to analyze the processes of biological evolution. In a recent report, Jacobs et al. (2008) reported the lack of phylogenetic structure in section *Petota*, based on the results of the analysis of 196 different taxa with amplified fragment length polymorphism (AFLP) markers. They proposed that this lack of phylogenetic structure represented the actual biological situation within the section, in which hybridization had possibly played an important role. In this regard, Masuelli et al. (2009) proposed that homoploid hybridization is the main mechanism involved in the origin and evolution of diploid potato species. The results of Bedogni and Camadro (2009) and Erazzú et al. (2009), who worked with wild diploid potato species from Argentina, give support to this assertion. The former authors analyzed various accessions from the Potato and Forages Germplasm Bank of Instituto Nacional de Tecnología Agropecuaria, Balcarce, Argentina (PFGB-INTA), classified as *Solanum kurtzianum*, that were chosen because they represented the distribution area of the species from the provinces of Catamarca to Mendoza, and accessions of the sympatric populations classified as *Solanum chacoense*, *Solanum spegazzinii,* and *S. maglia*, using morphological, biochemical, and molecular (simple sequence repeat (SSR)) markers. They concluded, from the results of phenetic analyses, that the wide morphological and genetic variability detected in the accessions classified as *S. kurtzianum* could be attributed to hybridization and introgression with the other diploid species in areas of sympatry. The results of Erazzú et al. (2009), who worked with accessions classified as *Solanum gourlayi* and *S. spegazzinii*, also supported the assertion of Masuelli et al. (2009). Hereafter, all accessions will be identified according to their current taxonomic status in germplasm banks.

In reciprocal, artificial, interspecific crosses between *S. kurtzianum* and *S. maglia*, seeds were formed regardless of female or male parent in approximately 50% of the genotypic combinations (Ispizúa et al. 1999).This is an indication that reproductive isolation between the two species is incomplete, and therefore, that gene exchange between them is possible. However, evidence of natural hybridizations or introgression between these species has not been provided.

Introgression is a means by which the genetic variability of endangered species can become available in the genetic background of other species with adaptation to specific habitats. Thus, an understanding of the extent and distribution of the genetic variation present within and among wild potato populations is essential for devising sampling and conservation strategies of these genetic resources.

The hypothesis of the present work was that populations of *S. maglia* can naturally hybridize with *S. kurtzianum* if the populations are sympatric. To test this hypothesis, we

extensively and intensively explored the gorge Quebrada La Cumbre, in Mendoza province, the only location in Argentina for which *S. maglia* had been reported. We report the results of (*i*) two collecting expeditions in 2006 and 2007 to that gorge and (*ii*) the morphological and genetic diversity for 24 morphological characters and SSR and AFLP markers of two newly sampled populations and of accessions from the same gorge, classified as *S. maglia,* provided by a germplasm bank.

Material and methods

Plant material

The genetic materials were (*i*) five clones collected in 1991 in the gorge Quebrada La Cumbre that were provided by the PFGB-INTA, each one classified as *S. maglia* and registered as an accession, (see Spooner and Clausen 1993); (*ii*) two populations constituted from 5 and 10 plants sampled as tubers by R.W. Masuelli, C.F. Marfil, and N.B. Pigni in 2006 and 2007 in two separate sites of the same gorge; (*iii*) one genotype of *S. kurtzianum*, collected in Estancia La Cumbre, outside the gorge (see Spooner and Clausen 1993), and one Chilean genotype of *S. maglia*, also provided by the PFGB-INTA (Table 1 and Fig. 1). Location and altitude of collection points were taken with the help of a global positioning device. For comparison purposes, a commercial tetraploid clone of *S. tuberosum* (cv. Spunta) and the accession ClAlo 943 of the wild Argentinian diploid species *S. chacoense*, provided by the PFGB-INTA, were included in the analyses. Plants from *ii* were assigned two numbers, the first for the collection area and the second for the plant.

Morphological analyses

All genotypes were cloned by means of tubers. Plants were grown under uniform conditions in an insect-proof screen house in Mendoza, Argentina. Twenty-four characters (Table 2) were measured at bloom in three plants (clones) of each genotype, which were grown in the greenhouse in a randomized arrangement. Measurements were performed on the fifth true leaf from the base of the plant, and in the first inflorescence. The Gower general similarity coefficient (Gower 1971) was used and clusters were generated from the matrices with the Unweighted Pair Group Method Arithmetic mean (UPGMA) method of the Sequential Agglomerative Hierarchical Nested (SAHN) using the NTSYS-pc version 2.10t program (Rohlf 1992).

Molecular analyses

DNA was extracted from leaves according to Dellaporta et al. (1983). After spectrophotometric measurement of DNA concentration (GeneQuant RNA/DNA Calculator, Pharmacia Biotech), DNA was diluted in 1× TE buffer to 100 ng/μL for use in the PCR analysis. DNA amplification was performed for six nuclear simple sequence repeats (nSSRs) and for three primer combinations of AFLP markers.

The following six nuclear SSR primers were tested: StI001, StI004, and StI005, described by Feingold et al. (2005), and POT 45/46, ST 17/18, and ST 63/64, described by Ashkenazi et al. (2001). Polymerase chain reactions (PCRs) were performed in 20 μL volumes containing approximately 30 ng template DNA in 1.5 mmol/L $MgCl_2$, 1× PCR buffer (50 mmol/L KCl; 10 mmol/L Tris-HCl, pH 8.3), 0.2 mmol/L dNTP mix, 0.12 μmol/L of forward and reverse primers, and 1 U of *Taq* polymerase. Amplified fragments were separated with denaturing polyacrylamide gel electrophoresis and visualized with the silver stain method as described by Bassam et al. (1991).

AFLP analysis was performed as described by Vos et al. (1995), using *Eco*RI- and *Mse*I-digested DNA to generate AFLP data. Three primer combinations with different specific 3 bp overhangs were used to amplify AFLP bands. The primer combinations were E-ACA/M-CAA, E-ACA/M-CAT, and E-AAG/M-CAC. The amplification products were electrophoresed on 6% polyacrylamide gels and silver stained.

Data analysis

For both SSR and AFLP procedures, two independent amplifications were performed for each sample. Only stable and repeatable patterns were used for analysis. Amplification products observed in the polyacrylamide gel were scored as either present (1) or absent (0). Two binary matrices were obtained, one for SSR and the other for AFLP. Pairwise comparisons were used to generate a similarity matrix based on the Dice coefficient (Sneath and Sokal 1973). Clusters were generated from the matrices with the UPGMA method of the SAHN module in NTSYS pc 2.10t (Rohlf 1992). For bootstrapping analysis, the WinBoot program was used (Yap and Nelson 1996) (1000 bootstraps involving random fragment sampling with replacement).

Results

Field observations and morphological analysis

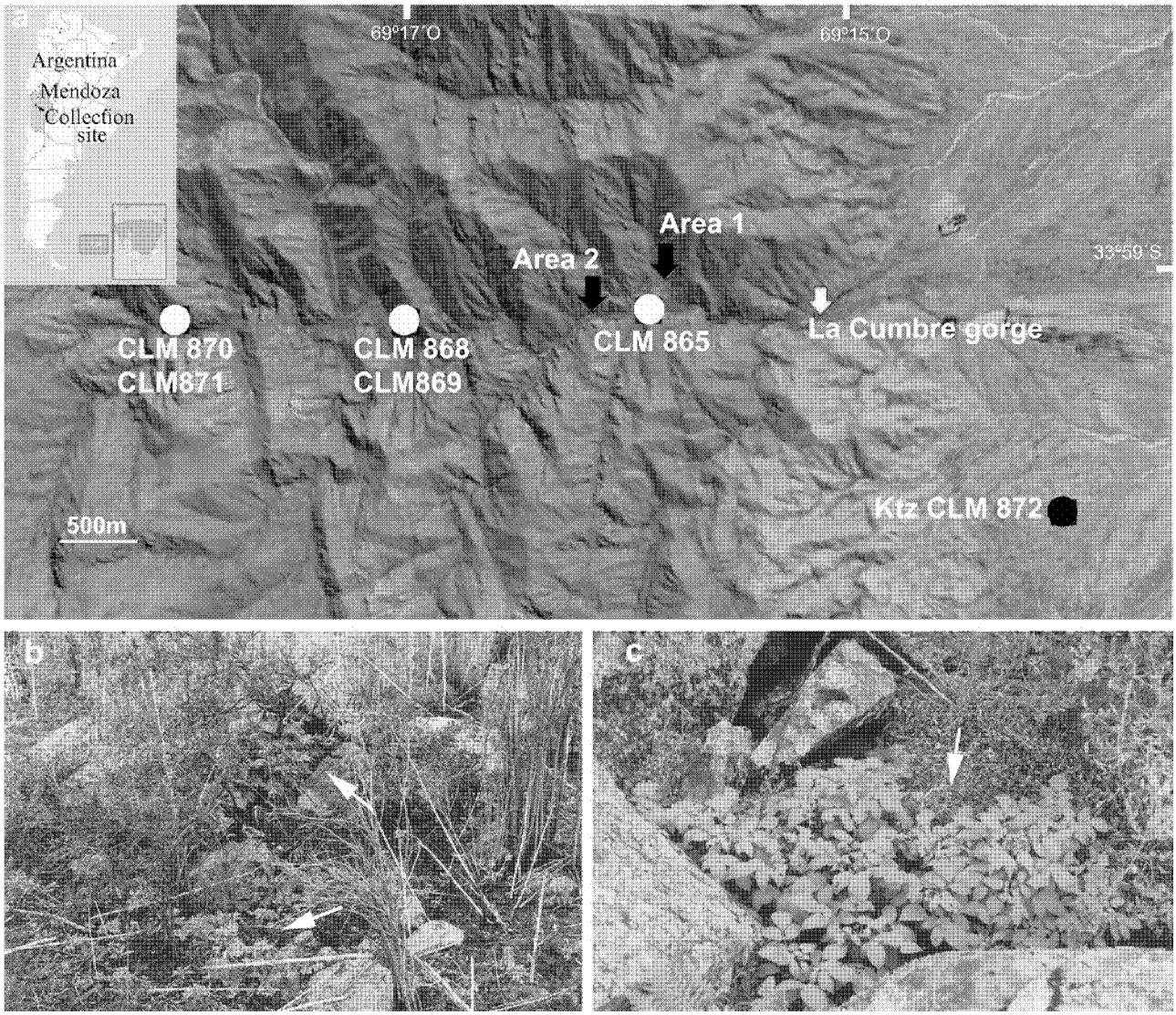
In two collecting explorations in Quebrada La Cumbre, carried out in 2006 and 2007, only two populations of wild potatoes were found, separated by a distance of 500 m (Fig. 1). These populations consisted of approximately 10–15 plants each. The first collection in that area was carried out in 1991 (Spooner and Clausen 1993); since then, the habitat in the gorge has been highly disturbed. A fire in December of 2005 affected the north slope of the gorge; the bushes, mostly *Larrea* spp., in area 1 were destroyed by fire (see Fig. 1). Plants collected in area 1 were growing in dry soil, without protection of bushes, shrubs, or trees. In the 2006 exploration trip, plants with fruits were observed in area 1. Area 2 had not been affected by the fire and plants were found growing in a damp, shady environment, among stones and trees (*Maitenus* ssp.) near a stream.

In the UPGMA analysis, plants of area 1 were morphologically distinct from plants of area 2. In the phenogram, two clusters were differentiated: one of them grouped plants of area 1 plus the genotype of *S. kurtzianum*, while the other grouped plants of area 2 with plants of *S. maglia* provided by the PFGB-INTA (Fig. 2). Although intrapopulation variability was detected in both clusters, plants from area 1 were morphologically more diverse than plants from area 2. In addition, plant 1.1 was grouped apart from the other plants from the same area. Accessions A1.2, A2.1, and A2.4 were missing from the analysis because morphological characters

Table 1. Plant material used in morphological and molecular analyses.

Collection	Origin	Accession No.	Location	Altitude (metres above sea level)	No. of plants	Morphological analysis	Molecular analyses	Taxon
INTA Balcarce (1991)	Quebrada "La Cumbre"	ClM 865	S 33°59′ W 69°15′	1628	5	Yes	Yes	*S. maglia*
		ClM 868	S 33°59′ W 69°17′	1770		Yes	Yes	
		ClM 869	S 33°59′ W 69°17′	1780		Yes	Yes	
		ClM 870	S 33°59′ W 69°18′	1820		Yes	Yes	
		ClM 871	S 33° 59′ W 69°18′	1789		Yes	Yes	
Masuelli–Marfil–Pigni (2006)	Quebrada "La Cumbre"	Area 1	S 33°59′8″ W69°15′52″	1561	4			?
		1.1				Yes	Yes	
		1.2				No	Yes	
		1.3				Yes	Yes	
		1.4				Yes	Yes	
		Area 2	S 33°59′15″ W69°16′11″	1594	5			?
		2.1				No	Yes	
		2.3				Yes	Yes	
		2.4				No	Yes	
		2.5				Yes	Yes	
		2.6				Yes	Yes	
Masuelli–Marfil–Pigni (2007)	Quebrada "La Cumbre"	Area 1	S 33°59′8″ W69°15′52″	1561	6			?
		1.5				Yes	Yes	
		1.6				Yes	Yes	
		1.7				Yes	Yes	
		1.8				Yes	Yes	
		1.9				Yes	Yes	
		1.10				Yes	Yes	
INTA Balcarce (1991)	Estancia "La Cumbre"	ClM 872-Ktz	S 34°00′ W 69°14′	1440	1	Yes	Yes	*S. kurtzianum*
INTA Balcarce	Chile	Contreras without No. (Mgl-CHILE)	—	—	1	No	Yes	*S. maglia*
INTA Balcarce	San Luis	ClAlo 943-Chc	S 32°48′ W 65°01′	1000	1	Yes	Yes	*S. chacoense*
FCA U.N.Cuyo	—	Commercial cultivar (TBR)	—	—	1	No	Yes	*S. tuberosum* var. Spunta

Fig. 1. Geographical distribution of wild potato populations in the gorge Quebrada La Cumbre. (*a*) Map location and satellite image of the gorge. Circles, material provided by the PFGB-INTA. White circles, sites of tuber collection of *Solanum maglia* in 1991. Black circle, collecting site of the *Solanum kurtzianum* genotype. Black arrows, sites of tuber population sampling (areas 1 and 2) in 2006 and 2007. White arrow, gorge entrance. (*b*) Plants collected in area 1, growing in an open field affected by fire in 2005. (*c*) Plants collected in area 2, growing among stones.



could not be measured in three plants (clones) of each genotype.

Molecular analyses

Out of six SSR primers assayed in the samples studied, four gave satisfactory amplification products. Amplifications with primers ST 63/64 and StI004 were successful only in *S. tuberosum*, which was used as a positive control because the assayed primers had been developed in this species.

The number of different alleles per locus ranged from 1 to 8, and a total of 24 distinct alleles were identified across all loci. The SSR patterns were monomorphic in all plants collected in area 1. Identical results were obtained by analyzing plants collected in area 2, which in addition, shared 100% of the SSR markers with plants of *S. maglia* provided by the PGB-INTA.

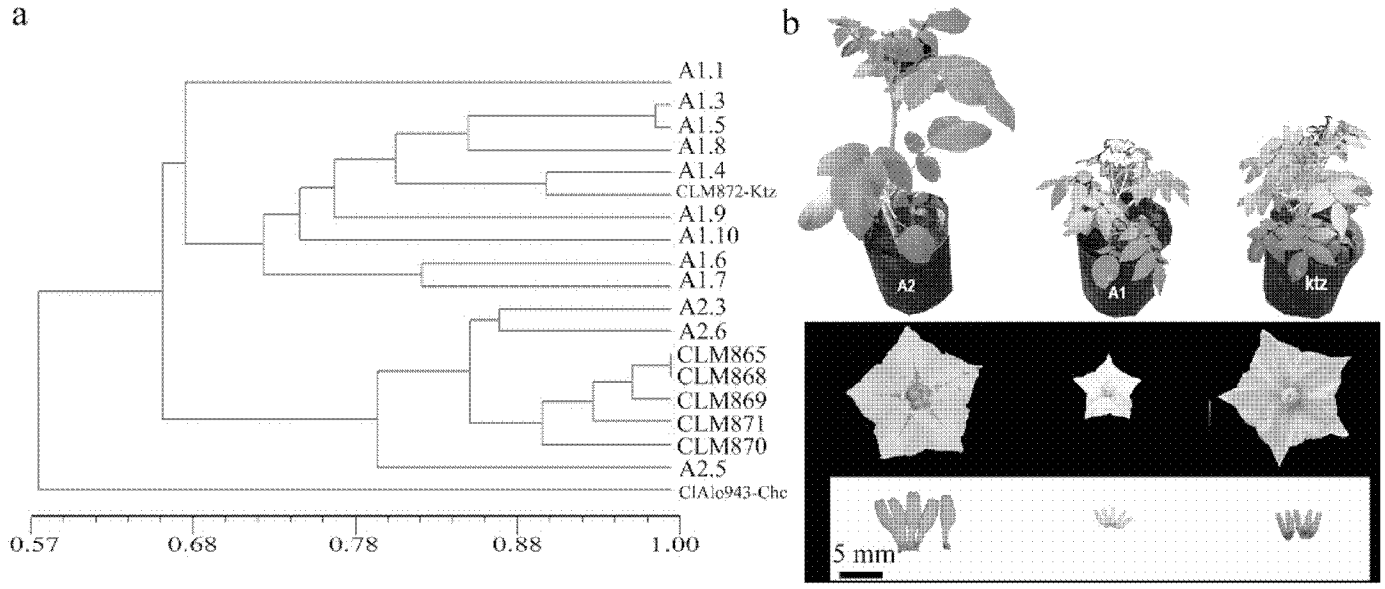
The UPGMA dendrogram clustered the plants from area 1 with the genotype of *S. kurtzianum* at 75% similarity, with a 91.6% bootstrap support (Fig. 3). Plants from area 2 and *S. maglia* were grouped with the genotype of *S. maglia* from Chile at 88% similarity, with 100% bootstrap support (Fig. 3). The species *S. chacoense* and *S. tuberosum* formed a separate group, which was closer to the area 1–*S. kurtzianum* cluster than the area 2–*S. maglia* cluster.

The AFLP analysis was conducted on 10 plants collected in area 1, 5 plants collected in area 2, 5 plants (1991 collection) of *S. maglia* from Quebrada La Cumbre, 1 plant of *S. maglia* from Chile, and 1 plant of *S. kurtzianum* (1991 collection). Using three pairs of primers, 412 amplified fragments were analyzed. The cluster analysis revealed two principal groups: one grouping plants from area 1 with *S. kurtzianum* and the other constituting plants from area 2

Table 2. Morphological characters evaluated in the accessions of *Solanum maglia* and *Solanum kurtzianum* (collected in Quebrada La Cumbre) and *Solanum chacoense* (provided by the PFGB-INTA).

Character (units or scale)
1. Stem pubescence ((1) abundant, (2) moderate, (3) absent)
2. Stem pigmentation ((1) green, (2) green with purple spots, (3) purple with green spots, (4) purple)
3. Leaf length (cm)
4. Leaf width (cm)
5. Number of lateral leaflets (no. of pairs)
6. Lateral leaflet length (cm)
7. Lateral leaflet width (cm)
8. Terminal leaflet length (cm)
9. Terminal leaflet width (cm)
10. Lateral leaflet base shape ((1) cuneate, (2) truncate, (3) cordate, (4) auriculate, (5) asymmetric)
11. Lateral leaflet apex shape ((1) obtuse, (2) acute, (3) acuminate)
12. Number of intercalary leaflets
13. Leaf pubescence ((1) abundant, (2) moderate, (3) absent)
14. Length of acumen of calyx lobule (mm)
15. Calyx pubescence ((1) abundant, (2) moderate, (3) absent)
16. Corolla diameter (mm)
17. Anther column shape ((1) with loose barrel-shaped, (2) without loose barrel-shaped)
18. Distance from corolla center to lobule apex (mm)
19. Distance from corolla center to lobule base (mm)
20. Corolla shape: ratio (character 18 / character 19)
21. Corolla pigmentation ((1) white, (2) white with purple acumens, (3) white with light purple star, (4) white with dark purple star)
22. Style length (mm)
23. Anther length (mm)
24. Tuber color ((1) white or beige, (2) purple)

Fig. 2. Morphological analysis of wild populations collected in the gorge Quebrada La Cumbre. (*a*) UPGMA phenogram based on 24 morphological characters. Labels A1 and A2, plants collected in areas 1 and 2, respectively; CLM865 to CLM871, plants of *Solanum maglia* provided by the PFGB-INTA; CLM872-Ktz, *Solanum kurtzianum;* ClAlo 943-Chc, *Solanum chacoense*. (*b*) Representative morphology of plants from area 2 (left), area 1 (center), and *S. kurtzianum* (right).



with *S. maglia* (Fig. 4). The UPGMA dendrogram clustered nine plants from area 1 with the genotype of *S. kurtzianum* at 88% similarity, with 100 bootstrap value. Similar to the morphological analysis, plant 1.1 was grouped apart from the other plants from the same area.

The genetic similarity was higher in plants from area 2 than in plants from area 1. Dice coefficients in the comparison of plants from area 1 varied from 0.72 to 1.00, while in area 2 they varied from 0.96 to 1.00. Analyzing the AFLP patterns in plants from area 1 and plants from area 2 sepa-

Fig. 3. Cluster analysis based on SSR markers. Labels A1 and A2, plants collected in areas 1 and 2, respectively; CLM865 to CLM871, plants of *Solanum maglia* provided by the PFGB-INTA; Mgl-CHILE, Chilean genotype of *S. maglia*; CLM872-Ktz, *Solanum kurtzianum;* ClAlo 943-Chc, *Solanum chacoense*; TBR, *Solanum tuberosum.* The numbers above the branches are bootstrap support values.

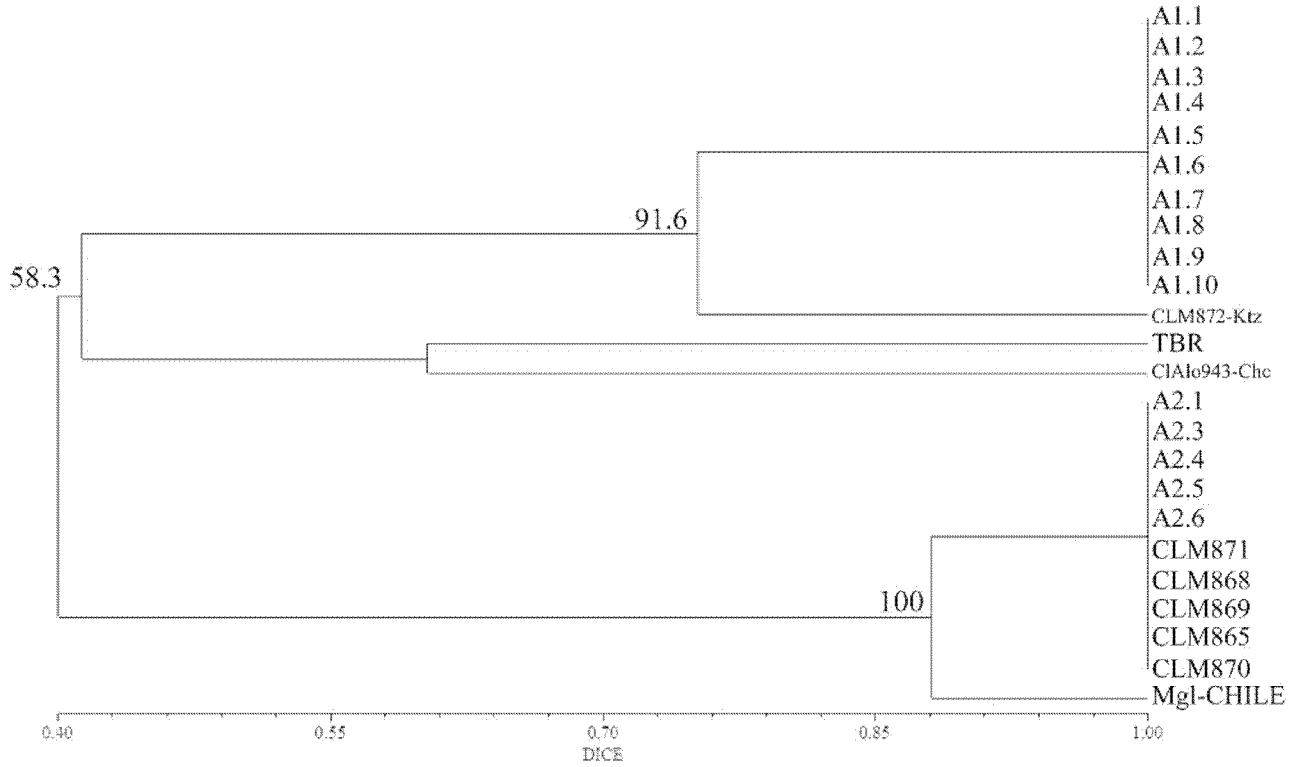
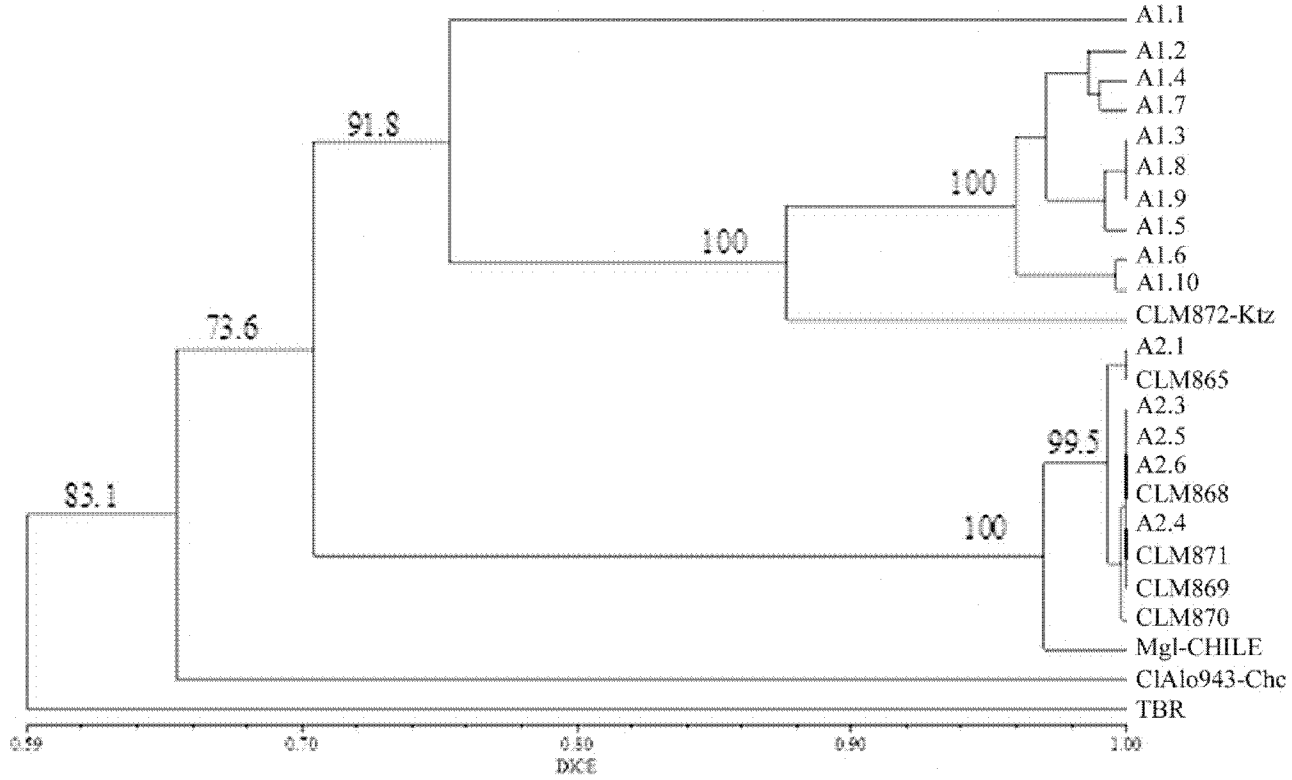


Fig. 4. Cluster analysis based on AFLP markers. Labels A1 and A2, plants collected in areas 1 and 2, respectively; CLM865 to CLM871, plants of *Solanum maglia* provided by the PFGB-INTA; Mgl-CHILE, Chilean genotype of *S. maglia*; CLM872-Ktz, *Solanum kurtzianum;* ClAlo 943-Chc, *Solanum chacoense*; TBR, *Solanum tuberosum.* The numbers above the branches are bootstrap support values.



rately, only four fragments were polymorphic in plants from area 2 and plants of *S. maglia* from PFGB-INTA. On the other hand, 112 fragments were polymorphic in plants from area 1. Seventy-eight of these polymorphic fragments were present in plant 1.1. Eighteen fragments observed in plant 1.1 and absent in the other nine plants collected in area 1 were specific of *S. maglia*.

Seeds were obtained in controlled reciprocal crosses between plants of areas 1 and 2 (Table 3). Additionally, pollen–pistil compatibility was studied by fluorescence microscopy. In the A2.3 × A1.1 cross, pollen tubes grew normally down to the base of the style.[3] These results indicate that reproductive isolation among plants from areas 1 and 2 is incomplete, and that gene exchange between them is possible.

Table 3. Genotypic combinations and the number of fruit and seeds in inter-area controlled crosses.

Genotypes			
♀	♂	No. of crosses	No. of fruits (seeds/fruit)
A1.3	A2.3	2	2 (99)
A1.3	A2.5	2	0
A1.4	A2.5	4	0
A1.4	A2.6	2	0
A2.1	A1.1	1	0
A2.3	A1.1	1	1 (25)
A2.3	A1.3	2	1 (92)
A2.3	A1.4	1	1 (126)
A2.5	A1.4	3	1 (84)

Discussion

The only record of *S. maglia* in Argentina is for Quebrada La Cumbre, Mendoza (Spooner and Clausen 1993), and the accessions from that gorge are apparently one clone (Ispizúa et al. 1999). Considering that the species could be endangered in Argentina, we decided to explore the gorge in search of genetic variability and found two populations separated by 500 m. To identify the plants, we used morphological and molecular (AFLP and SSR) markers and compared them with *S. kurtzianum*, collected near the entrance of the gorge (see Fig. 1), and to a Chilean plant of *S. maglia*.

The morphological and molecular data indicated that genetic differences were present among the plants analyzed from the two sampling areas.These differences were consistent for grouping plants from area 1 with the genotype of *S. kurtzianum* and plants from area 2 with accessions of *S. maglia* from PFGB-INTA, supporting the identity of plants from area 1 as *S. kurtzianum* and plants from area 2 as *S. maglia*, except for plant 1.1 as discussed below.

The SSR data clearly separated the populations from areas 1 and 2, but in contrast with AFLP markers, the analysis with SSR markers did not detect intrapopulation genetic variability. Our results indicate that there are limits in using SSR markers developed in the cultivated potato *S. tuberosum* by Ashkenazi et al. (2001) and Feingold et al. (2005) in studies of genetic variability in wild species. Similarly, the use of SSRs developed for tetraploid *S. tuberosum* in wild diploid potato species was apparently inadequate to find differences among species and accessions (Bedogni and Camadro 2009). The analysis of microsatellite structure in related species of plants and mammals has shown that highly polymorphic microsatellites in one species generally have shorter repeats in the other species, therefore exhibiting less variability (Ellegren et al. 1995; van Treuren et al. 1997).

Plants from area 2 were morphologically and genetically more homogeneous than plants from area 1. In the AFLP analysis, plants from area 2 clustered with plants of *S. maglia* of PFGB-INTA and the genetic similarity within the group ranged from 0.96 to 1.00. These results indicate that plants collected in area 2 have genotypes closely related to those of the accessions of the PFGB-INTA and some of them are clones.

The genetic variability detected in plants from area 1 was higher than in plants from area 2, as revealed by morphological and molecular (AFLP) analyses. The new accession from area 1 assigned to *S. kurtzianum* as a result of the present study is the first of this species ever described growing into the gorge. In addition to the morphological and molecular data, plants were collected on dry soil, in an open field without protection of bushes, shrubs, or trees. This is consistent with the description of *S. kurtzianum* elsewhere in Argentina, growing principally in very dry sandy soils, on hillsides, and sometimes in the open (Hawkes and Hjerting 1969).

As a result of the expeditions carried out in 1991, 2006, and 2007, we observed (*i*) a reduction in the number of plant of *S. maglia* growing at the time of the first expedition in comparison with the last two, since five populations could be sampled in 1991 but only one in the latter; and (*ii*) drastic changes in the habitat of the gorge caused by a fire in 2005 that affected the north slope and by intense cattle grazing pressure. Therefore, it is possible that in a few years, the sampled population could be either extinct or in danger of extinction in Argentina. Another possibility could be the substitution of *S. maglia* by *S. kurtzianum*, the species that would be best adapted to the novel scenario described in Quebrada La Cumbre.

Plant 1.1 was distinctive from other plants collected in area 1; in fact, it was grouped apart from the other plants of that area in the morphological analysis and this grouping was confirmed by the AFLP markers analysis. The AFLP analysis indicates introgression of *S. maglia* into the genome of plant 1.1; 18 fragments (7.5%) present in plant 1.1 and absent in the others nine plants from the same area were specific of *S. maglia*. In addition, plant 1.1 presented unique fragments not observed in the other plants evaluated. This variability for AFLP markers could be explained by assuming a hybrid origin of plant 1.1. The appearance of novel AFLP fragments, absent in both parental genotypes, has been reported in interspecific synthetic potato hybrids (Ercolano et al. 2004; Marfil et al. 2006). Also, unique RFLP fragments, absent in the parental species *S. kurtzianum* and *Solanum microdontum* were observed in the natural diploid hybrid *Solanum* × *rechei* (Clausen and Spooner 1998). Interspecific hybridization is a source of adaptive variation

[3] See supplementary Fig. 1 at http://botany.nrc.ca.

and functional novelties, and homoploid hybridization has been proposed as the main mechanism involved in the origin and evolution of the diploid potato species (Masuelli et al. 2009). To obtain additional evidence, the sexual compatibility among plants of areas 1 and 2 was established. In the interspecific, reciprocal crosses, seeds were formed in both directions in approximately 50% of the combinations. This is an indication that reproductive isolation between the two species is incomplete, and therefore, gene exchange between them is possible, as was observed by Ispizúa et al. (1999) in controlled crosses.

Pollen flow between tuber-bearing *Solanum* species in nature is dependent on bumblebee pollination; these insects can typically forage over 100–1750 m (Walther-Hellwig and Frankl 2000), thus it is highly likely that they could cover the distance of 500 m that separates area 1 from area 2 in Quebrada La Cumbre. Based on our results, we speculate that the dominant reproduction system in the gorge is asexual, by tubers, but occasionally pollen flow occurs among plants of both species resulting in few hybrids plants. The fittest hybrids are maintained by asexual reproduction but can also reproduce sexually, interbreeding among themselves and with plants of the parental population. In this way, hybridization and introgression provide the means by which the genetic variability of the populations in the gorge can be increased. However, in the material evaluated only one hybrid was described and future collection expeditions are necessary to obtain more evidence about the evolutionary implications of hybridization events between *S. maglia* and *S. kurtzianum* in this gorge.

These results have implications for the conservation of wild potatoes. *Solanum maglia* is apparently at risk of extinction in Quebrada La Cumbre; however, part of this genetic erosion could be ameliorated by the introgression of *S. maglia* genes into *S. kurtzianum*, a species that is probably better adapted to dry environments than any other Argentinian wild potato (Hawkes and Hjerting 1969).

The morphological and genetic analyses of contemporary in situ populations of wild potato species in this gorge permit the establishment of baseline data for future monitoring, with the aim of devising sampling and conservation strategies of these genetic resources for studying the continuously evolving genetic diversity of wild potato populations.

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