plant biology

Plant Biology ISSN 1435-8603

RESEARCH PAPER

Reproductive ecology and genetic variability in natural populations of the wild potato, *Solanum kurtzianum*

C. F. Marfil[1] & R. W. Masuelli[1,2]

1 Facultad de Ciencias Agrarias, Instituto de Biología Agrícola Mendoza (IBAM), Universidad Nacional de Cuyo, Mendoza, Argentina

2 Instituto Nacional de Tecnología Agropecuaria (INTA), E.E.A. La Consulta Mendoza, Argentina

Keywords

AFLP markers; genetic resources; *in situ* populations; wild crop relatives.

Correspondence

C. F. Marfil, Facultad de Ciencias Agrarias, Instituto de Biología Agrícola Mendoza (IBAM), Universidad Nacional de Cuyo, A. Brown 500 (M5528AHB) Chacras de Coria, Mendoza, Argentina.
E-mail: cmarfil@fca.uncu.edu.ar

Editor

T. Peeters

Received: 23 November 2012; Accepted: 6 June 2013

doi:10.1111/plb.12072

ABSTRACT

The cultivated potato (*Solanum tuberosum* ssp. *tuberosum*) has more than 200 related wild species distributed along the Andes, adapted to a wide range of geographical and ecological areas. Since the last century, several collection expeditions were carried out to incorporate genetic variability into the potato germplasm around the world. However, little is known about the reproductive ecology and genetic population structure of natural potato population from field studies. The aim of this work is to study, in the field, the genetic variability and reproductive strategies of populations of one of the most widely distributed potato species in Argentina, *Solanum kurtzianum*, growing in Mendoza province. AFLP markers showed that the genetic variability is mainly present among plants within populations, indicating that in the sampled populations, sexual reproduction is more relevant than clonal multiplication (by tubers). Additional evidence was obtained evaluating the genetic diversity in populations with a distribution in patches, where several genotypes were always detected. From a field study performed in the Villavicencio Natural Reserve, we found that the average number of plump seeds per fruit was 94.3, identified and calculated the foraging distance of four insect pollinators, and demonstrated the seed dispersal by storm water channels. We argue that the breeding system, the two modes of reproduction and the ecological interaction described here may have a prominent role in determining the genetic structure of *S. kurtzianum* populations, and discuss the importance of field studies on population genetics, reproductive biology and ecology to design collections and conservation strategies.

INTRODUCTION

Wild potato species 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; Santini *et al.* 2000; Davies *et al.* 2002; Oltmans & Novy 2002; Jansky & Peloquin 2005). There are many germplasm banks around the world to safeguard the potato biodiversity for present and future use. With a focus on the immediate need for conservation, efforts have initially been oriented to the acquisition of genetic resources. In many cases, this procedure resulted in collections of considerable size and unknown value. Maintaining and evaluating germplasm is expensive, and there are limits to the number of samples that can be handled effectively (Marshall & Brown 1975). Consequently, it has become increasingly important to improve the efficiency of genetic resource management.

There are many conservation challenges that benefit from the guidance and direction that genetic data can furnish. Optimised sampling strategies are needed for future collections. Knowledge on the distribution of genetic diversity in natural areas may provide relevant information to develop sampling strategies that will maximise the probability of collecting genetically distinct samples. Lack of accurate information regarding the distribution of genetic diversity among natural populations may lead to inadequate sampling during collections (IBPGR 1985).

Germplasm collections provide the raw material for programmes of plant breeding and crop improvement. However, germplasm collections are not used solely by breeders, other biologists will have different interests and different requirements. For example, the field of plant systematics has undergone a renaissance during the last 20 years and potatoes were not an exception. Great efforts have been focused on resolving the phylogenetic relationships among the more than 200 potato species described (Hawkes 1990), but these relationships are confusing, in part because of limited knowledge of the intraspecific genetic variability. Potatoes can reproduce sexually and/or asexually, and until now, the prevalent mode of reproduction in nature has not been ascertained (Camadro *et al.* 2012). Lack of information on the genetic structure of plant populations is a serious problem, since any understanding of speciation, adaptation or genetic change must take into account genetic patterns and the processes through which they are modified (Loveless & Hamrick 1984).

Solanum kurtzianum is a diploid Argentinean species with an outcrossing breeding system, which grows naturally in drier valleys and hillsides of the western and north-western Argentine Andes, in the Catamarca, La Rioja, Mendoza and San Juan provinces. It is probably the wild potato from

Argentina best adapted to dry environments, and is found in very dry sandy soils, on hillsides and sometimes in the open, but often protected by bushes, shrubs and trees (Hawkes & Hjerting 1969). In addition, this species is important for potato breeding because of its resistance to leaf-roll *Fusarium sambucinum*, nematodes, late blight, powdery mildew, *Verticillium* wilt, Colorado beetle, leaf hoppers and viruses A, F, X and Y (Hawkes & Hjerting 1969; Lynch *et al.* 2003). This species has received particular interest among naturalists and geneticists. Hijmans *et al.* (2002) processed data derived from databases of wild potato collection expeditions, and showed that out of 196 wild potato species considered, *S. kurtzianum* ranks tenth in terms of number of observations and is one of the species with major geographic distributions. Grouping analyses of SSR markers performed on *S. kurtzianum* accessions from different Argentinean provinces conserved in the Potato and Forage Germplasm Bank of the Estación Experimental Agropecuaria (EEA) Balcarce, INTA, Argentina (PFGB-INTA), showed that the accessions were not associated according to their geographic origin. For example, not all accessions from the same province grouped together, while clusters with accessions from different provinces were detected (Raimondi 2002; Raimondi *et al.* 2005; Bedogni & Camadro 2009). These studies investigated evidence of natural interspecific hybridisation between *S. kurtzianum* and other sympatric potato species from Argentina, and highlighted the importance of characterising the natural diversity of wild Argentinean potato germplasm for both fundamental and applied scientific goals. However, the authors did not discuss the utility of the genetic–geographic patterns and the genetic structure of *Solanum* populations to design conservation strategies. It has been suggested that information from genetics, ecology, population and reproductive biology must be obtained to gain a better understanding of the biodiversity of wild potato populations (Camadro *et al.* 2012). The objective of the present work is to explore several of these aspects to explain the genetic variability naturally found in *S. kurtzianum*. For this purpose, we performed a field study to evaluate the genetic variability of *in situ* populations from three regions of Mendoza, using AFLP markers, to compare the sexual *versus* asexual reproduction in populations with a distribution in patches, to characterise flower visitors, to identify climatic factors associated with seed dispersal and to compute the viability of seeds collected from 21 populations in the Villavicencio Natural Reserve.

MATERIAL AND METHODS

Sample collection

Plants of *S. kurtzianum* are distributed in discrete populations, forming patches along storm water channels, in open places or protected under bushes of *Larrea* spp., among others. We sampled individuals of *S. kurtizanum* from Mendoza (Argentina) during two collecting trips in the years 2007 and 2011. In the autumn of 2007, three regions of Mendoza were explored: Álvarez Condarco, Cerro Arco and Villavicencio Natural Reserve (Figure S1); a total of seven populations were sampled (Table 1). In each population, the total number of plants was recorded and 20% of the observed plants were sampled by collecting leaves from the apex. To avoid clonal plants originating from asexual reproduction, we sampled plants spaced about 1 m from each other in each population.

The second collecting trip took place in the autumn of 2011, and we sampled in Villavicencio Natural Reserve (Mendoza, Argentina). In contrast to the sampling performed in 2007, we designed a strategy to distinguish whether plant patches originated from: (i) genetically identical tubers clonally propagated, or (ii) genetically different botanical seeds maintained by tubers over time. We sampled three populations from three different 30 × 30 cm plots (Figure S2). All plants of *S. kurtzianum* growing in these squares were removed from the soil. We observed that each stem grew from an individual tuber. From these three populations, a total of 14 plants (Table 1) were sampled by collecting leaves from the apex, and were compared genetically. In addition, we collected fruits from 21 different populations of *S. kurtzianum* to evaluate seed production and viability (Table 1). The number of plump seeds per fruit was recorded and the percentage germination was established. To break dormancy, the seeds were pre-soaking in 1500 ppm gibberellic acid for 24 h. Germination was achieved by placing 40 seeds obtained from different fruits of each population on moistened filter paper in Petri dishes, in a growth chamber (16 h photoperiod and 25 °C). After 4 weeks, the number of germinated seeds was counted.

AFLP analysis

The AFLP reactions were performed twice for each sample, and only stable and repeatable patterns were used for analysis. Total DNA was extracted from leaves according to Dellaporta *et al.* (1983). After spectrophotometric measurement of DNA concentration (GeneQuant RNA/DNA Calculator; Pharmacia Biotech, Uppsala, Sweden), the DNA was diluted in 1× TE buffer to 100 $ng \cdot \mu l^{-1}$. For each sample, 500 ng of DNA were digested overnight at 37 °C with 2 U *Eco*RI and 1 U *Mse*I (New England Biolabs, Beverly, MA, USA). Two pairs of oligonucleotides were annealed at 65 °C for 10 min, 37 °C for 10 min and 25 °C for 10 min to generate an *Eco*RI-specific adaptor (5′-CTC GTA GA C TGC GTA CC-3′ and 5′-AAT TGG TAC GCA GTC TAC-3′) and an *Mse*I-specific adaptor (5′-GAC GAT GAG TCC TGA G-3′ and 5′-TAC TCA GGA CTC AT-3′). These adaptors and the digested DNA were ligated at 22 °C for 3 h with 1 U T4 DNA ligase (Promega, Madison, WI, USA). The adaptor-ligated template DNA was amplified using PCR with a set of pre-selective primers with one selective nucleotide (*Eco*RI + 1, 5′-AGA CTG CGT ACC AAT TC A-3′ and *Mse*I+1, 5′-GAC GAT GAG TCC TGA GTA AC-3′). The PCR programme consisted in 19 cycles at 94 °C for 30 s, 56 °C for 30 s and 72 °C for 2 min. The pre-amplification mixture was diluted threefold with sterile distilled water, and 2 μl were used for selective amplification. Two forms of selective amplifications were performed for samples obtained in each collecting trip (Table 1).

We employed fluorescent AFLP analysis for those samples collected in 2007. Four primer combinations with different specific 3 bp overhangs were used to amplify AFLP fragments. The primer combinations were E-AGC/M-CTA, E-AGC/M-CTT, E-AAG/M-CAC and E-AAG/M-CTG, using the same core primers as in the pre-amplification, adding two selective nucleotides at the 3′-end. The PCR amplification temperature profile was performed with an initial touch down protocol: 94 °C for 30 s, 65 °C for 30 s and 72 °C for 1 min for 12 cycles;

Table 1. *Solanum kurtzianum* populations under study.

region	population name	year expedition	location	altitude (m)	no. sampled plants (n)	AFLP analysis[a]	fruit collections (fruit number)	fruit dispersion study	pollinator observations
Álvarez Condarco	AC	2007	S33°02′36″ W69°02′57″	1126	5	fAFLP	No	No	No
Cerro Arco	CA1	2007	S32°51′44″ W68°56′03″	1099	5	fAFLP	No	No	No
	CA2	2007	S32°51′39″ W68°56′24″	1103	2	fAFLP	No	No	No
	CA3	2007	S32°51′44″ W68°56′06″	1112	4	fAFLP	No	No	No
Villavicencio Natural Reserve	V2	2007	S32°34′48″ W68°55′59″	1096	4	fAFLP	No	No	No
	V3	2007	S32°32′05″ W68°56′39″	1249	7	fAFLP	No	No	No
	V4	2007	S32°31′50″ W68°56′56″	1235	4	fAFLP	No	No	No
	A	2011	S32°34′42″ W68°56′10″	1078	6	ssAFLP	No	No	No
	B	2011	S32°32′05″ W68°56′59″	1261	5	ssAFLP	No	No	Yes
	C	2011	S32°28′59″ W68°55′46″	1309	4	ssAFLP	No	No	No
	P1	2011	S32°32′04″ W68°57′00″	1255	–	–	Yes (7)	No	Yes
	P2A	2011	S32°32′05″ W68°57′01″	1256	–	–	Yes (10)	No	Yes
	P2B	2011	S32°32′05″ W68°57′02″	1256	–	–	Yes (9)	No	Yes
	P3	2011	S32°32′04″ W68°57′02″	1259	–	–	Yes (13)	No	Yes
	P4	2011	S32°32′05″ W68°57′02″	1259	–	–	Yes (11)	No	Yes
	P5A	2011	S32°32′05″ W68°57′02″	1259	–	–	Yes (15)	No	Yes
	P5B	2011	S32°32′05″ W68°57′02″	1259	–	–	Yes (14)	Yes	Yes
	P6	2011	S32°31′48″ W68°56′51″	1236	–	–	Yes (10)	No	No
	P7	2011	S32°31′48″ W68°56′51″	1235	–	–	Yes (19)	Yes	No
	P8	2011	S32°31′47″ W68°56′51″	1238	–	–	Yes (18)	Yes	No
	D	2011	S32°32′03″ W69°01′19″	2166	–	–	Yes (14)	No	No
	EA	2011	S32°32′02″ W69°01′19″	2180	–	–	Yes (10)	No	No
	EB	2011	S32°32′02″ W69°01′19″	2180	–	–	Yes (9)	No	No
	2166M	2011	S32°32′05″ W69°01′32″	2166	–	–	Yes (7)	No	No
	QH1	2011	S32°30′51″ W69°00′34″	1667	–	–	Yes (3)	No	No
	QH2	2011	S32°30′52″ W69°00′33″	1713	–	–	Yes (9)	No	No
	QH3	2011	S32°30′06″ W69°01′10″	1910	–	–	Yes (9)	No	No
	QH4	2011	S32°30′06″ W69°01′10″	1910	–	–	Yes (4)	No	No

Table 1. Continued

region	population name	year expedition	location	altitude (m)	no. sampled plants (n)	AFLP analysis[a]	fruit collections (fruit number)	fruit dispersion study	pollinator observations
	QH5	2011	S32°30′05″ W69°01′19″	1935	–	–	Yes (13)	No	No
	QH6	2011	S32°30′05″ W69°01′22″	2010	–	–	Yes (10)	No	No
	1228M	2011	S32°31′47″ W68°56′56″	1228	–	–	Yes (20)	No	No
	TRQ1	2011	S32°31′48″ W68°56′54″	1234	–	–	No	Yes	Yes
	T3Q2	2011	S32°31′47″ W68°56′56″	1225	–	–	No	Yes	Yes
total	33	–	–	–	fAFLP: 31 plants from 7 populations ssAFLP:15 plants from 3 populations		234 fruits from 21 populations	5	10

[a]Two AFLP analyses were performed: fluorescent AFLP (fAFLP), and silver stained AFLP (ssAFLP).

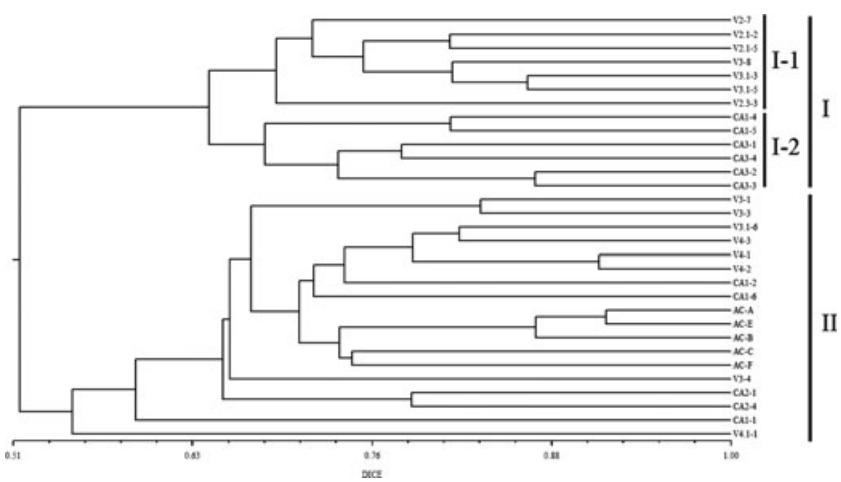

Fig. 1. UPGMA phenogram analysis of *Solanum kurtzianum* based on AFLP markers. Labels AC, CA and V indicate plants sampled in Álvarez Condarco, Cerro Arco and Villavicencio Natural Reserve, respectively.

in each cycle the annealing temperature was decreased by 0.7 °C, followed by 23 cycles: 94 °C 30 s, 56 °C 30 s and 72 °C 1 min. Then, 1.0 µl of the amplified products were mixed with 8.5 µl of Hi-Di™ Formamide and 0.5 µl DNA size standards (Genescan-500 ROX; Applied Biosystems, Foster City, CA, USA). Following a 2-min denaturing step at 90 °C, the reactions were loaded, and analysed on an automatic DNA sequencer (ABI PRISM 3130; Applied Biosystems). To minimise gel electrophoresis artefacts, each labelling reaction was run in duplicate. GenMapper software (Applied Biosystems) was used to determine the length of the sample fragments by comparison to the DNA fragment length size standards included with each sample. In the analysis, we used those fragments between 100 and 600 bp in length and with a fluorescence value above 50 arbitrary units on the ABI sequencer. Only peaks found on both duplicates were used in the subsequent analysis.

For those samples collected in 2011, a conventional AFLP protocol was performed as previously described by Marfil *et al.* (2011), in which the amplified fragments were separated by denaturing polyacrylamide gel electrophoresis, and silver stained. The following three primer combinations were used: E-ACG/M-CAA, E-ACA/M-CAT and E-AAG/M-CTG.

Phenetic analyses

Cluster analyses were performed with NTSYSpc software version 2.10t (Rohlf 1992). Pair-wise comparisons were used to generate a similarity matrix based on the Dice coefficient (Sneath & Sokal 1973). Clusters were generated from the matrices using the unweighted pair group method arithmetric mean (UPGMA) method of the SAHN module. A principal coordinates analysis (PCA) was performed for a data set of samples collected in 2007: a minimum spanning tree (MST) was calculated and projected over the principal coordinates.

Analysis of molecular variance

An analysis of molecular variance (AMOVA) was used to partition the observed variation in three components: within populations, among populations, and among geographic regions (Excoffier *et al.* 1992). Variance components were tested for significance with a non-parametric resampling approach using 1023 permuted data sets. AMOVA analyses were performed using the Arlequin software version 3.1 (Excoffier *et al.* 2005).

Characterisation of flower visitors

For 3 days in February 2011 in the Villavicencio Natural Reserve, we monitored 10 populations of *S. kurtzianum* during the morning to learn about pollinating agents (Table 1). Insects visiting flowers of *S. kurtzianum* were caught, pinned and identified to genus level with the assistance of researchers from the Ecological Interactions Laboratory, Argentine Institute of Dryland Research, CONICET CCT-Mendoza, Argentine. In addition, we had access to an entomological collection of the Ecological Interactions Laboratory, which contained several specimens for each genus of the insect pollinators we collected.

To infer the foraging distance of pollinators, we measured the distance between the wing bases. For each insect taxon, at least five individuals were measured using a stereoscopic microscope (Nikon SMZ645, Melville, New York, USA). Three different estimates of foraging distance of pollinators were calculated according to Greenleaf *et al.* (2007): typical homing distance, maximum homing distance and maximum feeder training distance.

Characterisation of seed dispersal

We employed an empirical method to detect if storm water channels participate in the process of seed dispersal. Mature fruits from five different populations of *S. kurtzianum* growing in Villavicencio Natural Reserve (Table 1) were painted with red nail polish on two different occasions: 13 and 25 February 2011. Painted fruits were checked weekly for presence or absence. Daily precipitation values were provided by the National Meteorological Service (Meteorological Information Center) from the nearest meteorological station 'El Plumerillo', located approximately 40 km from this reserve.

RESULTS

The AFLP analysis was performed to explore the genetic diversity of 31 plants of *S. kurtzianum* collected in three geographic regions from the province of Mendoza (Argentina) in the autumn of 2007. The four primer combinations produced a total of 300 AFLP fragments, 8 (2.6%) of which were not repeatable between replicates and were discarded from the analysis. Of the 292 analysed fragments, 13 (4.5%) were monomorphic. The AFLP analysis indicated that all sampled plants were unique genotypes, *i.e.,* no two plants had 100% similarity. The most similar plants were AC-A and AC-E (Dice coefficient = 0.92) from Álvarez Condarco and V4-1 and V4-2 (Dice coefficient = 0.91) from Villavicencio Natural Reserve (Fig. 1).

The phenetic analyses indicated that plants of *S. kurtzianum* did not group according to their geographic distribution, except for plants collected in Álvarez Condarco. In the UPGMA analysis, plants were arranged into two main groups with a genetic similarity coefficient of 0.51 (Fig. 1, Groups I and II). Group I contained two sub-groups: Group I-1 with seven plants from Villavicencio Natural Reserve and Group I-2 with six plants from Cerro Arco (Fig. 1). Group II included plants from Álvarez Condarco, Cerro Arco and Villavicencio Natural Reserve, and only the plants collected in Álvarez Condarco formed a separate sub-group. The spatial ordination of genotypes in the PCA was consistent with the results of the UPGMA analysis (Fig. 2). Groups I-1 and I-2 were also recovered by the PCA analysis. In contrast, plants from Group II, showed a more dispersed distribution in the PCA analysis. Most plants from Group II (14 plants) clustered together while four plants (V3-1, V3-4, CA1-1 and CA2-4) were isolated (Fig. 2). The three-first coordinates explained 39% of the total observed variation.

The AMOVA showed that the differences in molecular variance among regions were not significant (Fig. 3A). From the total molecular variance, the largest percentage (73.89%) was due to variation within populations, statistically significant at the 0.001% level. A smaller percentage (21.11%) corresponded to variation among populations, which was statistically significant at the 0.001% level.

To gather more evidence about the reproductive biology of *S. kurtzianum*, we performed a second collecting trip and sampled plants within a 30 × 30 cm square from three additional populations (Fig. 4 and Figure S2). The three primer combinations produced a total of 176 AFLP fragments, 77 (44%) of which were monomorphic. In the replicates of each sample, identical patterns were observed. We found two or more different genotypes of *S. kurtzianum* growing in each population. In population A, four genotypes and two duplicates (100% similarity) were detected out of six sampled plants. Population B included five plants, of which three genotypes and two duplicates were observed. We did not observe duplicates in population C (Fig. 4). The AMOVA performed on the plants collected in

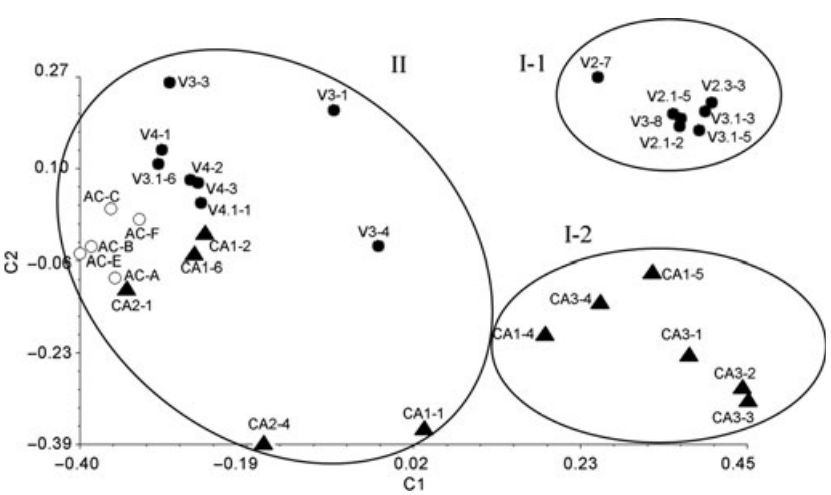

Fig. 2. Principal coordinates analyses of *Solanum kurtzianum* based on nuclear AFLP markers. Labels AC (white circles), CA (triangles) and V (black circles) indicate plants sampled in Álvarez Condarco, Cerro Arco and Villavicencio Natural Reserve, respectively. Groups I-1, I-2 and II correspond to the cluster labels in Fig. 1.

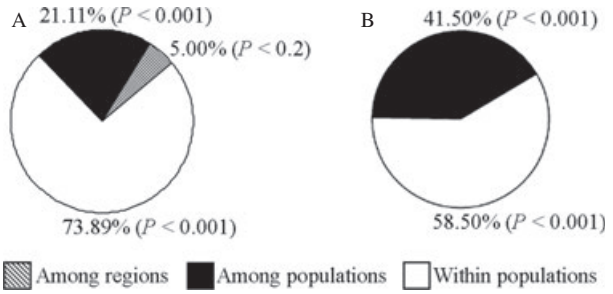

Fig. 3. Results of analysis of molecular variance (AMOVA) inferred from AFLP markers in *Solanum kurtzianum*. (A) Proportions of total variance from seven populations sampled in three different regions (Álvarez Condarco, Cerro Arco and Villavicencio Natural Reserve). (B) Proportions of total variance from three populations sampled in Villavicencio Natural Reserve. The *P*-value is the probability of obtaining an equal or more extreme value by chance alone, estimated from 1023 permutations.

these three populations indicated that the largest percentage of the molecular variance (58.5%) was due to variation within populations, statistically significant at the 0.001% level. The variation detected among populations was 41.5%, statistically significant at the 0.001% level (Fig. 3B).

During three trips to the Villavicencio Natural Reserve, we observed four different insects feeding on flowers of *S. kurtzianum*. Three bees (*Augochloropsis* sp., *Bombus* sp. and *Lonchopria* sp.) and one hoverfly (*Copestylum* sp.) species were identified (Table 2). All three bee species were observed emitting buzzes of about 1 s each on *S. kurtzianum* flowers. Individuals of *Bombus* sp. and *Lonchopria* sp. performed one buzzing per visited flower, while individuals of *Augochloropsis* sp. emitted from one to four buzzes per flower. The range of calculated flight distance varied from 0.1 to 0.6 km, 1.8–3.9 km, 0.4–1.2 km and 0.3–1.0 km for *Augochloropsis* sp., *Bombus* sp., *Lonchopria* sp. and *Copestylum* sp., respectively (Table 2).

We also studied the production and viability of seeds of *S. kurtzianum*, by collecting three to 20 fruits from 21 population distributed at different locations within the Villavicencio Natural Reserve (Table 1). The number of seeds per fruit varied between 39 and 197, with an average of 144. The percentage of plump seeds per fruit varied between 77.2% and 99.1%, with an average of 94.3%. The percentage germination varied between 8% and 68.4%, with an average of 47.5% (Fig. 5).

To characterise seed dispersal in *S. kurtzianum*, we painted fruits on two occasions for five populations from Villavicencio Natural Reserve (see Material and Methods; Fig. 6). All 75 fruits painted on 13 February 2011 disappeared after rainfall of 36 mm on 23 February 2011 (Fig. 6). On the other hand, many of the 78 fruits from the same five populations painted on 15 February 2011 remained for a longer period in their original sites. We observed a reduction of 28% of painted fruits after rainfall of 17 mm on 15 March 2011. However, after 70 days (5 May 2011), 68% of the fruits were found in their original site, under plants where the fruits were painted (Fig. 6).

In addition, we checked for biotic agents that may have a role in seed dispersal. Leafcutter ants were observed carrying fruit pieces containing seeds at two different locations (Figure S3). Leafcutter ant individuals were collected from each site and were identified as *Acromyrmex lobicornis* by a specialist of the Ecology of Desert Communities Research Group, Argentine Institute of Dryland Research, CONICET-CCT Mendoza, Argentina. We then searched for seeds from *S. kurtzianum* by sampling ant refuse from the external dumps. A total of 1000 cc of ant refuse was washed with tap water using a sieve with mesh size of 0.27 mm. All seeds were germinated on moistened filter paper in Petri dishes for subsequent identification of plantlets. No plants were identified as *S. kurtzianum*.

DISCUSSION

Reproductive ecology of *S. kurtzianum* populations from the Natural Reserve Villavicencio

Lack of information about reproductive biology, the role of pollinators and seed set of wild potatoes is apparent (Andersson & de Vicente 2010; Camadro 2012; Camadro *et al.* 2012). Our results contribute to understanding of the reproductive ecology of a wild potato species, *S. kurtzianum*. If gene flow between populations over a certain distance was sufficiently restricted, subpopulations should be distinguished. This study indicates that gene flow was not restricted between populations of *S. kurtzianum* within each location (Cerro Arco and Villavicencio Natural Reserve) because the genotypes did not group according their collection site. Pollinator flight behaviour and seed dispersal could enable gene flow and participate in establishment of the genetic structure observed in these

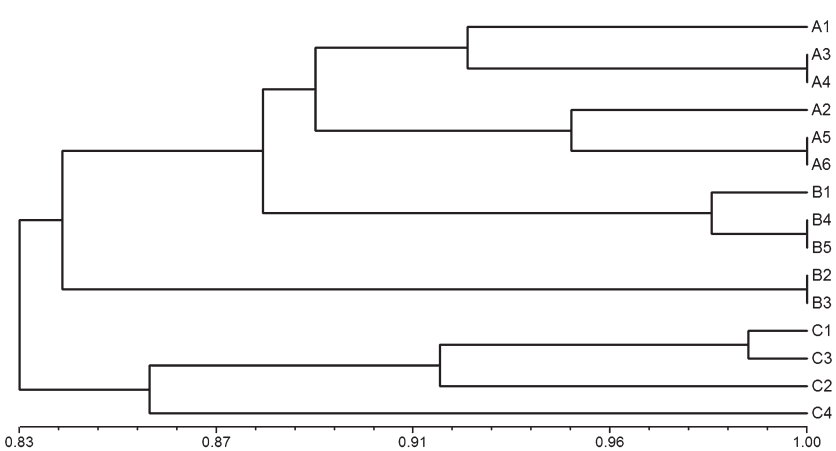

Fig. 4. Sexual *versus* asexual reproduction in patch populations of *Solanum kurtzianum*. Cluster analysis of three populations of *S. kurtzianum* from Villavicencio Natural Reserve based on AFLP markers. Labels A, B and C correspond to sampled populations.

Table 2. Distance between the wing bases and estimated foraging distances for pollinators of *Solanum kurtzianum*.

species	no. collected and identified individuals[a]	no. measured individuals[b]	mean distance between the wing bases (mm)	predicted typical homing distance (km)[c]	predicted maximum homing distance (km)[c]	predicted maximum feeder distance (km)[c]
Augochloropsis sp.	5	42	1.664	0.1	0.2	0.6
Bombus sp.	2	5	3.820	1.8	3.9	3.9
Lonchopria sp.	1	7	2.343	0.4	0.8	1.2
Copestylum sp.	4	24	2.171	0.3	0.6	1.0

[a]Insects collected by the authors on flowers of *Solanum kurtzianum* from Villavicencio Natural Reserve.
[b]Additional individuals of the same species presented at the entomological collection of the Ecological Interactions Laboratory were used to characterise the distance between the wing bases.
[c]Distances calculated according to equations presented in Greenleaf *et al.* (2007).

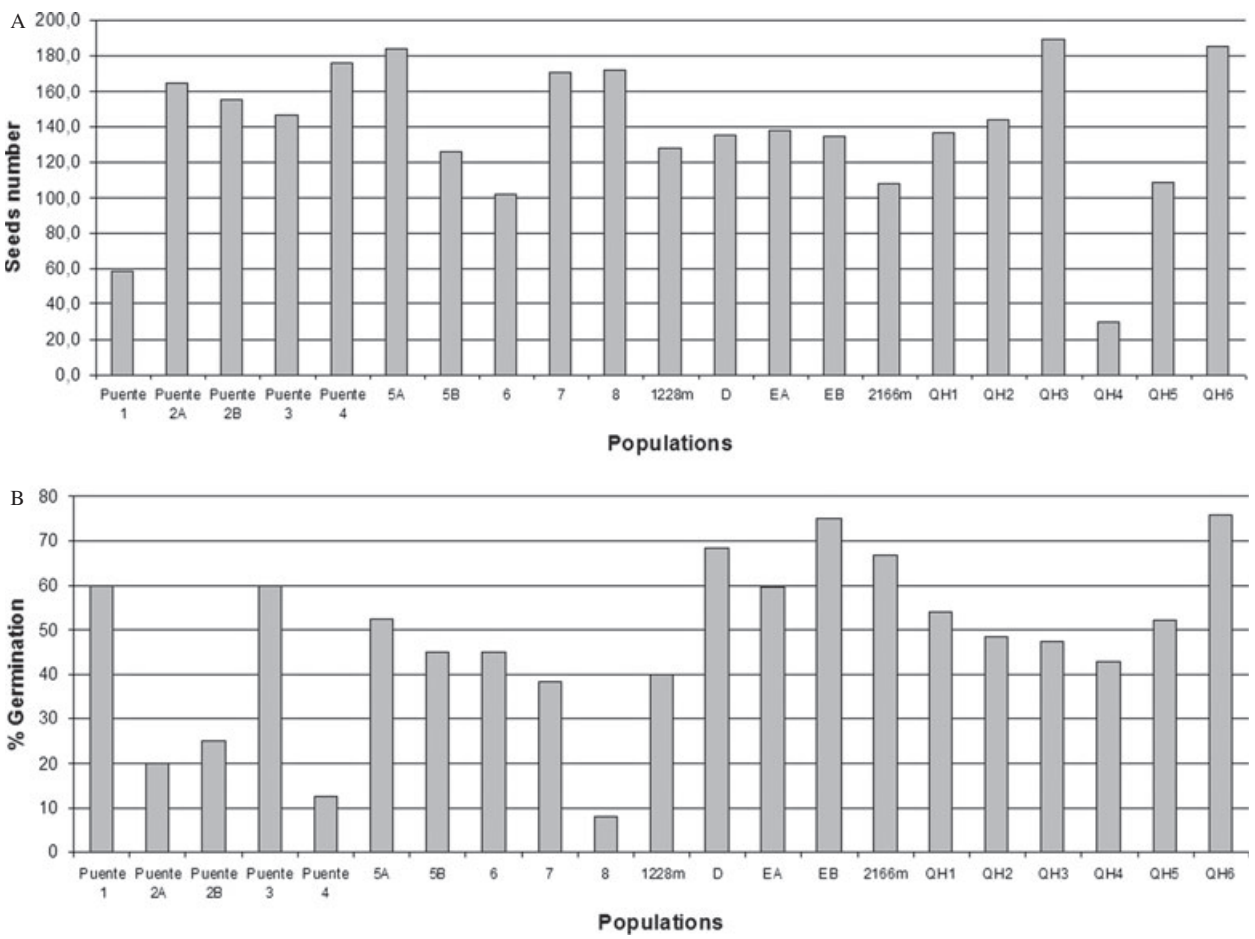

Fig. 5. Seed viability in *Solanum kurtzianum* populations from Villavicencio Natural Reserve. (A) Number of plump seeds per fruit. (B) Germination percentage per population. The *x*-axis labels show names of the sampled populations.

populations. We observed four insect species that visit flowers of *S. kurtzianum* and performed a study on the seed set and distribution in nature.

Pollen is the only food reward offered by *Solanum* spp., since their flowers offer no nectar (Buchmann & Cane 1989). In addition, the anthers of these plants require sonication by insects to release pollen, a specific plant–insect mechanism called 'buzz pollination' (Buchmann & Hurley 1978). Bumblebees and their relatives are considered the main pollinators of potato (Batra 1993), although Schittenhelm & Hoekstra (1995) found that hoverflies were the predominant flower visitors in multiplication fields of diploid tuber-bearing *Solanum* species. On ten monitored populations of *S. kurtzianum*, the more common visitors were *Augochloropsis* sp. and *Copestylum* sp. The latter hoverfly species was not observed performing buzz pollination, and therefore may play only a minor role in the pollination of *S. kurtzianum*. Bamberg *et al.* (2009) have discussed the role of pollinators in establishing the differences in RAPD polymorphism between tuber collection and seed collection for two wild potato species. In *S. kurtzianum*, the average number of plump seeds produced per fruit in natural populations from Villavicencio Natural Reserve is about twice that

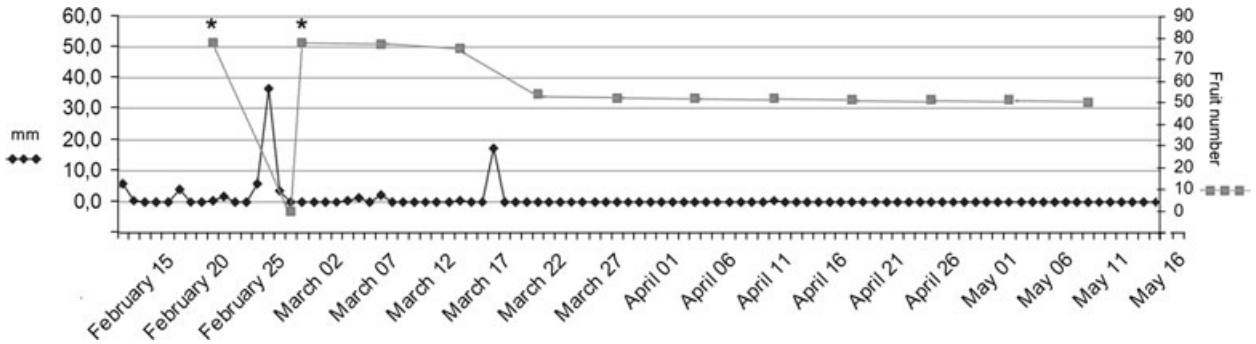

Fig. 6. Seed dispersal through storm water channels in Villavicencio Natural Reserve. Weekly monitoring of painted fruits and daily rainfall values. Asterisks indicate days when fruits were painted.

obtained from controlled hand-pollinated intraspecific crosses (94.3 *versus* 48.8, respectively; Raimondi 2002), revealing the importance and efficacy of the pollinators described here in the generation of new genetic combinations in natural habitats. Based on the most conservative estimates of pollinator flight capacity, plants (genotypes) of *S. kurtzianum* located 100 m apart could be cross-pollinated by *Augochloropsis* sp. On the other hand, the interaction between *S. kurtzianum–Bombus* sp. would allow cross-pollination between individuals 1800 m apart. As far as we know, the extent of pollen flow in wild potato populations has not been studied. The inferred distances reported in this work are in accordance with studies performed in field trials, which showed that pollen flow ranged from 20 m to more than 1000 m (van Soest 1983; McPartlan & Dale 1994; Skogsmyr 1994).

Fruiting pedicels of *S. kurtzianum* are articulated (like all members of section *Petota*), and easily dislodged by mechanical means such as birds, wind or rain. According to our results, precipitation of 17 and 36 mm are able to remove, respectively, 32% and 100% of the mature fruits from their original site. This mode of seed dispersal would have two implications: (i) fruits washed into streams allow a long dispersal distance for seeds, and (ii) the seeds are dispersed in groups and can germinate together when a suitable place is reached. Not only the fruits, but also tubers can be dispersed by storm water channels and establish a new populations. However, we did not find evidence of this in the present study. We argue that tuber dispersal would be secondary compared to the thousands of seeds (fruit) dispersed in watercourses. In either case (seeds or tubers), dispersal associated with precipitation can explain the distribution of *S. kurtzinaum* populations along watercourses.

Populations of *S. kurtzianum* are distributed both within dry watercourses and away from them. We propose two dispersal modes for those plants found far from watercourses. The first is related to biotic agents. Two colonies of leafcutters ants (*A. lobicornis*) were identified foraging on two populations of *S. kurtzianum*. According to our results, leafcutters ants may carry pieces of fruits up to 13 m to their nests (data no shown), but we did not find seeds in the refuse of the ant external dump. Studies focused on evaluation of dropping rates of fruit pieces during removal and transport could clarify the participation of this ant in the dispersion of *S. kurtzianum* seeds. The second dispersal mode could be fruits or seeds carried by wind. Dry berries of *S. kurtzianum* collected on 5 May 2011 weighed 0.17–0.48 g (data not shown) and could be sufficiently light to be dispersed by the zonda. The zonda is a warm and extremely dry wind that occurs east of the Andes Cordillera and can reach speeds of 54–72 $km \cdot h^{-1}$ at ground level (Seluchi *et al.* 2003). If this phenomenon were operating, it would have the same implication as the fruit dispersal through watercourses: the distribution of seeds in groups, and then the possibility that several individuals become established in an appropriate place.

The distribution of genetic diversity, both within and among natural populations, results from the joint action of mutation, migration, selection and drift, four evolutionary processes that operate within the historical and biological context of each plant species (Loveless & Hamrick 1984). In potato, to explain the lack of association between genetic and geographic distance, several authors have suggested that the small size of potato populations may make stochastic events more important in the partitioning of genetic diversity (del Rio *et al.* 2001; Ghislain *et al.* 2006; Spooner *et al.* 2009). These events include environmental changes, demographic factors and genetic drift. However, ecological factors affecting reproduction and dispersal are likely to be particularly important in determining genetic structure (Loveless & Hamrick 1984). In *S. kurtzianum*, the breeding system, the two modes of reproduction and the ecological interaction described here may have a prominent role in the establishment and maintenance of high diversity within populations and low differentiation among populations. Plant breeding systems have been identified as a major factor influencing genetic structure (Loveless & Hamrick 1984). *S. kurtzianum* (like most wild potato species) is allogamous, a breeding system that promotes pollen movement between individuals and permits alleles to be shared widely among populations, thus reducing the differentiation among them. Seed dispersal through storm water channels may decrease genetic structure by providing genetically diverse founding populations. In addition, the potatoes presented seed dormancy (Simmonds 1964), a process that increases the effective population size, reduces the decay of genetic variance and retards local subdivision (Loveless & Hamrick 1984). Clonally reproducing populations may increase differentiation and subdivide populations into clonal patches. However, we did not find purely clonal populations with a distribution in patches. We show that the populations maintain a seed bank in the soil, allowing the establishment of new genotypes over time. These genotypes, which are sexually established, are retained clonally through tubers, even under very unfavourable conditions, such as dry seasons or defoliation by diseases and pests.

Sampling strategies for *ex situ* conservation

Sampling strategies for natural populations are of fundamental importance for *ex situ* conservation of the natural genetic diversity. A criterion usually adopted by collectors is that the genetic similarity between populations decreases with geographic distance. However, this trend is not apparent for wild potato populations. Weak associations of genetic variability to geographic distance have been shown for the wild potatoes *Solanum jamesii* and *Solanum fendleri* (del Rio *et al.* 2001), *Solanum sucrens*e (del Rio & Bamberg 2002), *Solanum acaule* and *Solanum albicans* (McGregor *et al.* 2002) and *Solanum tuberosum* L. Phureja Group (Ghislain *et al.* 2006). Thus, geographic origin would not be a useful parameter in gauging inter-population genetic diversity for germplam banks (del Rio & Bamberg 2002). In potato, several studies have found significant variation among accessions within species and among plants within accessions for economically important traits, and have demonstrated that neither taxonomic relationships nor biogeographic factors consistently predict resistance to diseases and pests (Jansky *et al.* 2006, 2008; Spooner *et al.* 2009).

Results obtained with microsatellite markers showed that accessions of wild potato species *S. kurtzianum* and *Solanum spegazzinii* (Bedogni & Camadro 2009) and of *S. kurtzianum* and *Solanum chacoense* (Raimondi 2002) maintained by PFGB-INTA, did not group according to their geographic origin. A similar study performed with AFLP markers on natural populations of *Solanum gourlayi* and *Solanum spegazzinii* showed that higher percentages of the molecular variation were found within accession (Erazzú *et al.* 2009), in agreement with Bedogni & Camadro (2009).

Here, we found that the major part of the genetic variation of *S. kurztianum* is found within populations: 73.89% when three regions and seven populations were considered, and 58.5% when three populations from Villavicencio Natural Reserve were evaluated (Fig. 3A and B, respectively). The cluster analyses did not group genotypes according to their geographic origin, except for samples from Álvarez Condarco, which could be due to the small sample size. These results are relevant for the development of sampling strategies of wild potatoes for *ex situ* conservation in germplasm banks. *S. kurtzianum*, as in many of the wild tuber-bearing *Solanum* species, is found in the Andes, where populations separated by short distances are likely to experience very different climates and, consequently, different selection pressures. This species grows in four Argentinean provinces, along a transect that is approximately 900-km long (Hijmans *et al.* 2002). We argue that it would be more advisable to focus efforts on sampling many populations from a few locations, rather than the more time-consuming strategy of collecting from broad geographic regions. Regions with contrasting micro-environments should be selected (*e.g.,* the Villavicencio Natural Reserve with an area of 72,000 ha and and an altitude range from 700 to 3000 m a.s.l. is of great interest for potato collectors).

In situ conservation

In potato, del Rio *et al.* (1997a) showed that standard germplasm bank techniques are sufficiently thorough and that there has been minimal loss or change of genetic diversity in *ex situ* germplasm. However, germplasm banks fail to preserve the dynamic variability found in natural populations. Results obtained using RAPD markers in the wild potato species *S. jamesii* and *S. fendleri* showed that populations re-collected in different years at the same site and maintained in germplasm banks were significantly different for both species (del Rio *et al.* 1997b). This finding indicates that genetic variability preserved at the germplasm bank does not necessarily represent that of the natural population. Thus, *in situ* conservation is highly valuable to preserve the continuously changing genetic variability of wild potatoes. Given that the highest genetic variability was found within wild potato populations (Bedogni & Camadro 2009; Erazzú *et al.* 2009; and this study), *in situ* conservation of potatoes from a single area would already preserve a large portion of the total variability.

ACKNOWLEDGEMENTS

This work was supported by grants from the Secretaría de Ciencia, Técnica y Posgrado, Universidad de Cuyo, Argentina. We are grateful to Diego Vázquez, Natalia Chajof and Rodrigo Pol (Instituto Argentino de Investigaciones de las Zonas Aridas, CONICET-CCT-Mendoza, Argentina) for insect identification, and M. Virginia Sánchez-Puerta (Instituto de Biología Agrícola de Mendoza, CONICET-CCT Mendoza, Argentina) for criti'cal reading of the manuscript.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Map location of the three collecting sites in the province of Mendoza (Argentina).

Figure S2. Representative photos showing the sampling strategy of a population by collecting all plants within a 30 × 30 cm plot (left) and observing that each stem originated from a separate tuber (right, white arrows point to the original tuber). B1 to B5 indicate the five sampled plants.

Figure S3. Individuals of the cutter ant *Acromyrmex lobicornis* carrying fruits of *Solanum kurtzianum*.

REFERENCES

Andersson M.S., de Vicente M.C. (2010) *Gene flow between crops and their wild relatives*. The John Hopkins University Press, Baltimore, MD, USA.

Bamberg J., del Rio A., Moreyra R. (2009) Genetic consequences of clonal versus seed sampling in model populations of two wild potato species indigenous to the USA. *American Journal of Potato Research*, **86**, 367–372.

Batra S.W.T. (1993) Male-fertile potato flowers are selectively buzz-pollinated only by *Bombus terricola* in upstate New York. *Journal of the Kansas Entomological Society*, **66**, 252–254.

Bedogni M.C., Camadro E.L. (2009) Morphological and molecular evidence of natural interspecific hybridization in the diploid potato *Solanum kurtzianum* from Argentina. *Botany*, **87**, 78–87.

Buchmann S.L., Cane J.H. (1989) Bees assess pollen returns while sonicating *Solanum* flowers. *Oecologia*, **81**, 289–294.

Buchmann S.L., Hurley J.P. (1978) A biophysical model for buzz pollination in angiosperms. *Journal of Theoretical Biology*, **72**, 639–657.

Camadro E.L. (2012) Relevance of the genetic structure of natural populations, and sampling and classification approaches for conservation and use of wild crop relatives: potato as an example. *Botany*, **90**, 1065–1072.

Camadro E.L., Erazzú L.E., Maune J.F., Bedogni M.C. (2012) A genetic approach to the species problem in wild potato. *Plant Biology*, **14**, 543–554.

Davies C.S., Ottman M.J., Peloquin S.J. (2002) Can germplasm resources be used to increase the ascorbic acid content of stored potatoes? *American Journal of Potato Research*, **79**, 295–299.

Dellaporta S.L., Wood J., Hicks J.B. (1983) A plant DNA minipreparation: version II. *Plant Molecular Biology*, **1**, 19–21.

Erazzú L.E., Camadro E.L., Clausen A.M. (2009) Persistence over time, overlapping distribution and molecular indications of interspecific hybridization in wild potato populations of Northwest Argentina. *Euphytica*, **168**, 249–262.

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

Excoffier L., Smouse P., Quattro J. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Ghislain M., Andrade D., Rodriguez F., HIjmans R.J., Spooner D.M. (2006) Genetic analysis of cultivated potato *Solanum tuberosum* L. Phureja Group using RAPDs and nuclear SSRs. *Theoretical and Applied Genetics*, **113**, 1515–1527.

Greenleaf S.S., Williams N.M., Winfree R., Kremen C. (2007) Bee foraging ranges and their relationship to body size. *Oecologia*, **153**, 589–596.

Hawkes J.G. (1990) *The potato: evolution, biodiversity and genetic resource*. Smithsonian Institution Press, Washington, DC, USA.

Hawkes J.G., Hjerting J.P. (1969) *The potatoes of Argentina, Brazil, Paraguay and Uruguay. A biosystematic study*. Oxford University Press, London, UK.

Hijmans R.J., Spooner D.M., Salas A.R., Guarino L., de la Cruz J. (2002) *Atlas of wild potatoes*. International Plant Genetic Resources Institute, Rome, Italy.

International Board of Plant Genetic Resources (IBPGR) (1985) Ecogeographical surveying and *in situ* conservation of crop relatives: report of an IBPGR Task Force, Washington, DC. 30 July–1 August 1984. IBPGR, Rome, Italy.

Jansky S.H., Peloquin S.J. (2005) Advantages of wild diploid Solanum species over cultivated diploid relatives in potato breeding programs. *Genetic Resources and Crop Evolution*, **56**, 669–674.

Jansky S.H., Simon R., Spooner D.M. (2006) A test of taxonomic predictivity: resistance to white mold in wild relatives of cultivated potato. *Crop Science*, **46**, 2561–2570.

Jansky S.H., Simon R., Spooner D.M. (2008) A test of taxonomic predictivity: resistance to early blight in wild relatives of cultivated potato. *Phytopathology*, **98**, 680–687.

Loveless M.D., Hamrick J.L. (1984) Ecological determinants of genetic structure of plant populations. *Annual Review of Ecology and Systematics*, **15**, 65–95.

Lynch D.R., Kawchuk L.M., Chen Q., Kokko M. (2003) Resistance to *Fusarium sambucinum* in wild and cultivated *Solanum* species. *American Journal of Potato Research*, **80**, 353–358.

Marfil C.F., Pigni N.B., Camadro E.L., Masuelli R.W. (2011) Evidence of gene flow between natural sympatric populations of the wild diploid potatoes *Solanum maglia* and *Solanum kurtzianum* from Argentina. *Botany*, **89**, 55–64.

Marshall D.R., Brown A.H. (1975) Optimum sampling strategies in genetic conservation. In: Frankel O.H., Hawkes J.G. (Eds), *Crop genetic resources for today and tomorrow*. Cambridge University Press, Cambridge, UK, pp 53–80.

McGregor C.E., van Treuren R., Hoekstra R., van Hintum T.H.J.L. (2002) Analysis of the wild potato germplasm of the series Acaulia with AFLPs: implications for *ex situ* conservation. *Theoretical and Applied Genetics*, **104**, 146–156.

McPartlan H.C., Dale P.J. (1994) An assessment of gene transfer by pollen from field-grown transgenic potatoes to non-transgenic potatoes and related species. *Transgenic Research*, **3**, 216–225.

Oltmans S.M., Novy R.G. (2002) Identification of potato (*Solanum tuberosum* L.) haploid × wild species hybrids with the capacity to cold-chip. *American Journal of Potato Research*, **79**, 263–268.

Raimondi J.P. (2002) Caracterización de las especies silvestres diploides Solanum kurtzianum y Solanum ruiz-lealii para su utilización en el mejoramiento genético de la papa. Ph.D. thesis, Universidad Nacional de Mar del Plata, Argentina, pp 32–58.

Raimondi J.P., Peralta I.E., Masuelli R.W., Feingold S., Camadro E.L. (2005) Examination of the hybrid origin of the wild potato *Solanum ruiz-lealii* Brücher. *Plant Systematics and Evolution*, **253**, 33–51.

del Rio A.H., Bamberg J.B. (2002) Lack of association between genetic and geographic origin characteristics for the wild potato *Solanum sucrense* Hawkes. *American Journal of Potato Research*, **79**, 335–338.

del Rio A.H., Bamberg J.B., Huaman Z. (1997a) Assessing changes in the genetic diversity of potato gene banks. 1. Effects of seed increase. *Theoretical and Applied Genetics*, **95**, 191–198.

del Rio A.H., Bamberg J.B., Huaman Z., Salas A., Vega S.E. (1997b) Assessing changes in the genetic diversity of potato genebanks. 2. *In situ* vs *ex situ*. *Theoretical and Applied Genetics*, **95**, 199–204.

del Rio A.H., Bamberg J.B., Huamán Z., Salas A., Vega S.E. (2001) Association of ecogeographical variables and RAPD marker variation in wild potato populations of the USA. *Crop Science*, **41**, 870–878.

Rohlf F.J. (1992) *NTSYS-pc numerical taxanomy and multivariate system*. Exeter Software, Setauket, NY, USA.

Ross H. (1986) *Potato breeding – problems and perspectives*. In: Journal of Plant Breeding, Supplement 13. Paul Parey, Berlin, Germany, 132 pp.

Santini M., Camadro E.L., Marcellán O.N., Erazzú L.E. (2000) Agronomic characterization of diploid hybrid families derived from crosses between haploids of the common potato and three wild Argentinean tuber-bearing species. *American Journal of Potato Research*, **77**, 211–218.

Schittenhelm S., Hoekstra R. (1995) Recommended isolation distances for the field multiplication of diploid tuber-bearing *Solanum* species. *Plant Breeding*, **114**, 369–371.

Seluchi M.E., Norte F.A., Satyamurty P., Chou S.C. (2003) Analysis of three situations of the Foehn effect over the Andes (zonda wind) using the Eta–CPTEC regional model. *Weather and Forecasting*, **18**, 481–501.

Simmonds N.W. (1964) The genetics of seed and tuber dormancy in the cultivated potatoes. *Heredity*, **19**, 489–504.

Skogsmyr L. (1994) Gene dispersal from transgenic potatoes to conspecifics: a field trial. *Theoretical and Applied Genetics*, **88**, 770–774.

Sneath P.H.A., Sokal R.R. (1973) *The principles and practice of numerical classification*. WH Freeman, San Francisco, CA, USA.

van Soest L.J.M. (1983) An easy and reliable method for the rejuvenation of self-incompatible tuber-bearing *Solanum* species. *Euphytica*, **32**, 601–605.

Spooner D.M., Jansky S.H., Simon R. (2009) Tests of taxonomic and biogeographic predictivity: resistance to disease and insect pests in wild relatives of cultivated potato. *Crop Science*, **49**, 1367–1376.