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Genetic diversity among native varieties and commercial cultivars of *Solanum tuberosum* ssp. *tuberosum* L. present in Chile

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Abstract

Background: The object of this work was to determine the genetic and allelic diversity of *Solanum* species present in Chile, assessing allelic distribution among native varieties and commercial cultivars of *Solanum tuberosum* ssp. *tuberosum* L., using microsatellite markers.
Results: A high level of allelic richness was found in the potatoes studied. The seven microsatellite markers used presented a total of 64 allelic variants among native varieties and commercial cultivars of *Solanum tuberosum* ssp*. tuberosum*. The SSR loci generated an average of 9.16 alleles/locus. The group with the highest PIC was that of native varieties collected in the Chiloe archipelago. The high PIC values found indicate that the native varieties from Chiloe have a low level of interrelation, representing wide genetic diversity.
Conclusions: The markers with the highest number of alleles in native varieties corresponded to loci STG 0016 and LEMALX. Commercial cultivars do not present the same genetic variability as native varieties, and the allelic richness of commercial cultivars is lower than that of native varieties of *S. tuberosum* ssp. *tuberosum*. Most of the native varieties were clustered in accordance with their geographical location, while commercial cultivars, were clustered in accordance with their breeding programs in Chile and Europe, with the exception of Shepody.

Keywords: dendrogram; germplasm; SSR markers.

INTRODUCTION

Chilean vascular flora is a natural resource of great scientific, economic and cultural value, mainly due to its species richness (5,105 species) and high level of endemism (45.8%) (Hoffmann et al. 2003). Chile is the origin center of *Fragaria chiloensis* (L.) Mill., *Lycopersicon chilense* Dun., *Ugni molinae* Turcz. and *Gevuina avellana* Mol., and an origin sub-center of *Solanum tuberosum* L. ssp. *tuberosum*, among other species useful to man for their edible, medicinal or ornamental properties (Spooner et al. 2005b). However, studies on genome diversity are scarce for these species, and such studies should therefore be a priority task in the near future (Jara-Seguel and Urrutia, 2012). It is broadly accepted that genetic information may be useful in the study of genetic population structure, conservation, protection, and genetic improvement, especially in those plants that are important as food resources.

The potato (*S. tuberosum*) is a member of the Solanaceae, a large plant family containing more than 3,000 species (Visser et al. 2009). It is one of the most popular food crops world-wide and has a large number of wild relatives that are extensively studied for improvement purposes. Genetic material from these relatives may assist in overcoming problems by providing resistance to abiotic factors such as

cold and salinity, and biotic factors like disease (Hijmans and Spooner, 2001; Visser et al. 2009). In Chile there are some 53,653 hectares of cultivated potato with an average yield of 312.5 qqm ha^{-1} (ODEPA, 2012). This area is mainly planted with commercial potato cultivars introduced from Europe, as well as others generated by national breeding programs. Native potato varieties are characterized by a rich diversity of tuber forms, sizes, and colours, and other phenological characteristics of interest for breeders. These native varieties have been maintained over time by small-scale farmers practicing subsistence agriculture in the Chiloe and Chonos archipelagos. Of particular interest is the Chilean endemic species *Solanum fernandezianum* Phil., Etuberosum series, which is found growing naturally on Robinson Crusoe Island in the Juan Fernández archipelago, a Chilean National Park and Biosphere Reserve (Solano et al*.* 2011). This species has been classified as endangered by Ricci (2006). The wild material of this species is highly valuable for its resistance to viruses, bacteria and frost. Hybrids generated using *S. fernandezianum* as a progenitor presented PVY hypersensitivity, PRLV tolerance, and resistance to both frost and bacterial wilt (*Ralstonia solanacearum*) (Hijmans et al*.* 2003).

Molecular markers have contributed to a greater genetic knowledge of the potato at an international level, and have been used to analyze biodiversity and for phylogenetic studies of the genus *Solanum* (Ritter et al*.* 2004; Spooner et al*.* 2005a; Solano et al*.* 2007; Spooner et al. 2007). Recently, efforts have been made to achieve a less subjective description of potato germplasms based on Random Amplified Polymorphic DNA (RAPD) (Sun et al*.* 2003; Orona-Castro et al. 2004), microsatellites (Simple Sequence Repeats - SSR) (Ashkenazi et al. 2001; Raker and Spooner, 2002; Ghislain et al. 2004; Feingold et al*.* 2005; Sukhotu et al*.* 2005; Barandalla et al*.* 2006; Ghislain et al. 2006; Ispizúa et al. 2007; Mathias et al*.* 2007; Spooner et al*.* 2007, Ghislain et al*.* 2009a; Ghislain et al. 2009b; Lung'aho et al. 2011), and Interrupted Simple Sequence Repeats (ISSR) (Milbourne et al*.* 1998; Bornet et al*.* 2002).

In countries like Chile, which have abundant genetic resources and where concern exists over their conservation and use, the long term preservation of germplasm in seed banks should be proposed to assist in the preservation of alleles, principally those considered unique. In this context, the object of this work was to determine the genetic and allelic diversity of *Solanum tuberosum* present in Chile, assessing allelic distribution among native varieties and commercial cultivars using microsatellite markers.

MATERIALS AND METHODS

Plant material

A collection of *S. tuberosum* consisting of 30 accessions of native varieties originating from the island of Chiloe (Table 1) and nine commercial cultivars commonly used in Chile (Table 2) was evaluated. Further, one accession of *S. fernandezianum* collected in 2002 from Plazoleta Yunque in Robinson Crusoe Island (Juan Fernandez archipelago), located at 257 m altitude (33º 39' 9.03" S, 78º 50' 45.9" W) was included.

DNA extraction

DNA was extracted from fresh young leaves. Approximately 200 mg of leaf tissue were freeze-dried and ground in liquid nitrogen with a mortar and pestle. Genomic DNA was isolated with Plant DNAzol® (Invitrogen) following the manufacturer's instructions. The pellet was recovered in 70 µl of TE buffer. The quality of the DNA was evaluated by agarose gel electrophoresis (1%), using standard DNA marker DL 15,000 bp (Biolabs). The DNA concentration was quantified using a digital Spectrophotometer (Thermo Spectronic GENESYS™ 10) based on 260 nm absorbance. DNA quality was inferred by the 260/280 nm ratio. The DNA concentration was adjusted to approximately 30 ng μL^{-1} by the addition of sterile, deionized water.

Table 1. Description of native potato collection.

Laboratory number	Accession	Name	Geographic origin
1	UCT-11Mgb	Meca gato blanca	Chiloe Island
2	UCT-14MgRe	Redonda	Chiloe Island
3	UCT-17Br	Bruja	Quinchao Island
4	UCT-6Gc	Guadacho colorado	Chonchi, Chiloe
5	UCT-24Tn	Tonta	Castro, Chiloe
6	UCT-22Cm	Clavela morada	Castro, Chiloe
7	UCT-25Gñ	Guicoña	Quellón, Chiloe
8	UCT-7Ca	Camota	Chiloe Island
9	UCT-18Mn	Michuñe negro	Chiloe Island
10	UCT-26Ach	Azul chañihue	Chiloe Island
11	UCT-27Mu	Murta	Quellón, Chiloe
12	UCT-28MiR	Michuñe rojo	Chiloe Island
13	UCT-29Mol	Molejona	Chiloe Island
14	UCT-3Cl	Clavela	Los Muermos, Chilean Mainland
15	UCT-1Ma	Michuñe azul	Chiloe Island
16	UCT-16At	Azul table	Chiloe Island
17	UCT-30Ño	Ñocha	Chiloe Island
18	UCT-19Aq	Azul de quento	Castro, Chiloe
19	UCT-2Lv	Lengua	Castro, Chiloe
20	UTC-31Ob	Ojitos blanco	Ancud, Chiloe.
21	UCT-32Ci	Cielito	Castro, Chiloe
22	UCT-20Ro	Rosada	Chiloe Island
23	UCT-33Cab	Cabrita	Chiloe Island
24	UCT-15MgRo	Meca gato rojo	Chiloe Island
25	UCT-21Ac	Azul cristalina	Chiloe Island
26	UCT-34Cor	Cordillera	Castro, Chiloe
27	UCT-35AzC	Azul caucheque	Castro, Chiloe
28	UCT-8Gb	Guadacho blanco	Ancud, Chiloe
29	UCT-9MgM	Meca gato morada	Ancud, Chiloe
30	UCT-10MgL	Meca gato morada larga	Los Muermos, Chilean Mainland
40	Sf	*S. fernandezianum*	Juan Fernandez Archipelago

Table 2. Description of commercial cultivars.

Laboratory number	Name	Geographic origin	Year of release	Parents
31	Desirée	Introduced Cultivar ZPC-Holland	1962	Urgenta x Despeche
32	Karú	Chilean Cultivar	2002	Yagana x Fanfare
33	Shepody	Introduced Cultivar Canada	1980	Bake-King x F58050
34	Baraka	Introduced Cultivar	--	SVP 50-358 x Avenir
35	Híbrida	Hybrid LT8 xTS-9	--	LT-8 x TS-9
36	Rosara	Introduced Cultivar Solana-Germany	1990	Secura x 2605 77
37	Yagana	Chilean Cultivar	1983	Hydra x 904/61
38	Pukará	Chilean Cultivar	1993	Cleopatra x Yagana
39	Rodeo	Introduced Cultivar	--	--

SSR loci and PCR amplification

A total of seven microsatellite markers were selected for their high level of polymorphism. Five of these were reported by Moisan-Thiery et al. (2005): SSR 1, STM 2005, LEMALX, STM 1097, and STM 2020, while two loci were selected from the kit proposed by Ghislain et al. (2009b): STG 0016 and STI 0030 (Table 3). All of these primers were recommended for genetic studies in potatoes due to their good stability and amplification quality, high polymorphic content and location in different chromosomes of the species (Ghislain et al. 2009b). The reaction was performed in a volume of 20 µL, with 1U of *Taq* DNA polymerase (Fermentas), 30 ng of template DNA, 2.4 µM of each primer, 0.1 µM of each dNTP, 1X of 10XPCR-buffer (20 Mm Tris, 50 mM KCl, pH 8.4), 1.2 to 2.0 mM $MgCl_2$. The mixtures were then subjected to PCR in a Tempra Thermocycler, model MG96G version 3.23, using the following program: 1 cycle of 4 min at 95ºC, followed by 30 cycles of 1 min at 95ºC, 1 min at specific annealing temperature and 2 min at 72ºC, with a final extension step of 10 min at 72ºC.

Table 3. General description of SSRs used in genetic characterization of the potato collection.

Locus	Repeat motif	Approximate fragment size (bp)	N° of alleles	Polymorphism Information contend	Power discrimination	Primer sequences forward (F) and reverse (R.)	Source
SSR1	$(TCAC)_n$	230-194	11	--	0.91	F.5'GATGAGATGAGATATGAAACAACG3' R.5'GCAATTTCTCTTGACACGTGTCACTGAAAC3'	Moisan-Thiery et al. (2005)
STM2005	(CTGTTG)	193-160	5	--	0.79	F.5'TTTAAGTTCTCAGTTCTGCAGGG3' R.5'GTCATAAACCTTTACCATTGCTGGG3'	Moisan-Thiery et al. (2005)
LEMALX	$^n(ATT)_n$	140-120	4	--	0.84	F.5'CTCACCCACAAAGAAAATTC3' R.5'CTAACAAACATTGTACAACAATAATC3'	Moisan-Thiery et al. (2005)
STM1097	$(CGTTT)_n$	281-234	6	--	0.82	F.5'GTTCACAGCCTTCGTGAACG3' R.5'ATTCAAACTCAGCCAGCAGC3'	Moisan-Thiery et al. (2005)
STM2020	$(TAA)_n$	193-160	10	--	--	F.5'CCTTCCCCTTAAATACAATAACCC3' R.5'CATGGAGAAGTGAAAACGTCTG3'	Moisan-Thiery et al. (2005)
STG0016	$(AGA)_n$	137-174	14	0.773	--	F.5'AGCTGCTCAGCATCAAGAGA3' R.5'ACCACCTCAGGCACTTCATC3'	Ghislain et al. (2009b)
STI0030	$(ATT)_n$	94-137	17	0.811	--	F.5'TTGACCCTCCAACTATAGATTCTTC3' R.5'TGACAACTTTAAAGCATATGTCAGC3'	Ghislain et al. (2009b)

Electrophoresis of PCR products

After thermal cycling, an aliquot of 7 µL loading dye was added to 14 µL of reaction product. The products were then denatured for 5 min at 95ºC and loaded in a 6% denaturing polyacrylamide gel (19:1 acrylamide: bisacrylamide, Tris pH 8.0, 8 M Urea) buffered with 1X TBE using 60 W constant power for 3 hrs, depending of the PCR size product. Finally, gels were silver stained using the method described by Bassam et al. (1991).

Data analysis

SSR marker alleles were scored for presence (1) or absence (0) of the band for each accession. The approximate allele was determined by comparison of its mobility across the gel with the molecular size marker GeneRuler^{TM} 50 bp DNA Ladder (Biolabs). The total number of alleles and the average number of alleles per locus between pairs of genotypes were then calculated. The total number of alleles was calculated as the sum of all alleles detected for all loci. The average number of alleles per locus was estimated by the total number of alleles divided by the total number of loci. (Equation 1), where K = number of loci and n_i = number of alleles detected per locus.

$$n = (1/K) \sum_{i=1}^{k} ni$$

[Equation 1]

Polymorphic information content (PIC)

For the measurement of each marker locus polymorphism, the polymorphic information content (PIC) was calculated according to Equation 2, where p_i, is the frequency of the i^{th} allele detected over all accessions and *N* represents the total number of alleles observed at the locus (Nei, 1973). The polymorphic information content (PIC) parameter is frequently used to measure the discriminatory power of loci, with values ranging between 0 and 1.

$$PIC = 1 - \sum_{i=1}^{N} (p_i)^2$$

[Equation 2]

Genetic similarity and cluster analysis

Genetic distances between individuals were calculated using the similarity coefficient based on presence or absence of alleles. Genetic analysis was performed using the DARwin 5.0 program (Pierrer and Jacquemoud-Collet, 2006). A dissimilarity matrix was calculated using Sokal and Sneath 2 coefficient (Equation 3).

$$Dij = \frac{2(b + c)}{a + 2(b + c)}$$

[Equation 3]

Where “i” and “j” are the indices of the two genotypes compared, “a” is the number of cases over all loci where an allele is present in both genotypes, and “b” and “c” are the number of cases where an allele is present in one genotype and absent in the other.

A bootstrap analysis was conducted using 1000 replicates with DARwin software, with 60% minimal proportion of valid data required for each unit pair. The dendrogram was constructed using the Neighbour Joining method (Saitou and Nei, 1987). This method estimates phylogenetic trees, attempting to define a tree that is usually close to the true phylogenetic tree. In the present work the tree was rooted using *S. fernandezianum* as an out group. This method is proposed for reconstructing phylogenetic trees from evolutionary distance data. The principle of this method is to find pairs of operational taxonomic units (OTU) that minimize the total branch length at each stage of OTU clustering, starting with a starlike tree. The branch lengths as well as the topology of a parsimonious tree can quickly be obtained using this method.

RESULTS

Allelic frequency distributions

A total of 64 allelic variants were observed in the potato species evaluated, using seven SSR markers. For the STM 2005 marker, the fragments identified as alleles -7, -8, -10 and -12 were present in respectively 93%, 68%, 55% and 82% of *S. tuberosum* native accessions. Alleles -8 and -10 were present in 100% and 90% of *S. tuberosum* commercial cultivars. For this marker, allele -5 (approximately 150 bp) was unique to the diploid species *S. fernandezianum*. The SSR1 marker

revealed the presence of eight alleles, many of which were quite common. The STM 1097 marker was represented by five alleles, of which allele -1 (approximately 224 bp) was found exclusively in two native accessions of *S. tuberosum*. The LEMALX marker showed a total of 11 alleles between 120 and 140 bp for *S. tuberosum* material, of which allele -1 (around 118 bp) was unique to the diploid species *S. fernandezianum.* The STI 0030 marker was very polymorphic, with a total of nine alleles. Allele -5 (approximately 98 bp) was unique to *S. fernandezianum*. For the STG 0016 marker, alleles -2, -3, -6, -7 and -11 were unique to native potato materials. For the STM 2020 marker, allele -6 (approximately 156 bp) was unique to native potato accessions, being present in 33% of these. Another fairly common allele, although not exclusive to the native material, was allele -4. Alleles -3, -4 and -5 were present in 56%, 33% and 56% of commercial cultivars included in this study, while allele -1 (approximately 140 bp) was present only in *S. fernandezianum*.

The group exhibiting the highest number of alleles was the native varieties of *S. tuberosum,* with 57 alleles, corresponding to 89% of all the alleles detected. The group of commercial cultivars generated a total of 39 alleles, corresponding to 60.9% of the total found. A total of 21 alleles were found to be unique to the group of native potatoes, corresponding to 33% of the total number of alleles, while 34 (53% of the total) were shared with commercial cultivars included in this study. Only five alleles (8% of the total) were specific to the group of commercial varieties, and absent from the native potatoes. This indicates that the native material may constitute a rich source of alleles for *S. tuberosum.* The species *S. fernandezianum* generated a total of eight alleles, corresponding to 12.5% of all the alleles found. Of these, two alleles were shared with native potatoes from Chiloe Island, while two different alleles were shared with commercial cultivars and not with the native potatoes. The other four alleles were unique and exclusive alleles of this diploid species, endemic to Juan Fernandez Island (Table 4).

Table 4. Total and average number of alleles, percentage of total number and exclusive alleles.

Species	Total allele number	Average allele number	Percentage of total	Exclusive alleles	Percentage of exclusive alleles
S. tuberosum ssp. *tuberosum*	--	--	--	--	--
Native varieties	57	8.14	89.0	21.0	33.0
Commercial cultivars	39	5.57	60.9	5.0	8.0

The SSR loci generated an average of 9.16 alleles/locus. The number of alleles obtained per marker ranged between 5 (STM 1097) and 12 (LEMALX). The native accessions of *S. tuberosum* averaged 8.14 alleles per locus, commercial cultivars of *S. tuberosum* 5.57 alleles per locus, and the diploid species *S. fernandezianum* averaged 1.6 alleles per locus.

The markers with the highest number of alleles among the native potato accessions corresponded to loci STG 0016 and LEMALX, with 12 and 10 alleles respectively (Table 5). Among the commercial cultivars, the markers STG 0016, LEMALX and STM 2005 generated the greatest number of alleles, numbering seven alleles each. Allelic richness observed at the different loci was correlated between the two tetraploid origins ($r = 0.91$, $Pvalue_{5\ dof} = 0.0024$), which is consistent with the known result that allelic diversity is highly dependent on the SSR marker loci and the repetitive motif. *S. fernandezianum* was found to be heterozygous at three loci (STM 2005, LEMALX and STI 0030), homozygous at two loci (STM 2020 and STG 0016), while no band was amplified at the two remaining loci (Figure 1).

Table 5. Number of alleles per locus.

SSR locus	Total alleles	*S. tuberosum* spp. *tuberosum*		*S. fernandezianum*	Approximate fragment size (bp)
		Native varieties	Commercial cultivars		
STM 2020	6	5	4	1	160-140
SSR 1	8	8	5	--	220-172
STM 2005	12	9	7	2	193-110
LEMALX	12	10	7	2	140-118
STM 1097	5	5	3	--	280-224
STG 0016	12	12	7	1	166-125
STI 0030	8	8	6	2	112-85

Polymorphic information content (PIC)

Table 6 shows the polymorphic information content (PIC) for data obtained with the selected seven SSR markers. PIC values ranged from 0.63 to 0.89. The marker STM 1097 presented the lowest diversity for all groups, with a PIC value of 0.63 in the native varieties and 0.44 in the commercial cultivars. Conversely, the most diverse was the STG0016 marker with a PIC of 0.87 and 0.80 in the native varieties and commercial cultivars respectively (Table 7). The group with the highest PIC was the native varieties collected in the Chiloe archipelago, followed by those belonging to commercial cultivars currently in cultivation on the mainland in Southern Chile. The high PIC values found indicate that the native material from Chiloe has a low level of interrelation, representing wide genetic diversity. On the other hand, the sole and exclusive alleles found for the species *S. fernandezianum* show the original genotype status of this species from the Juan Fernandez archipelago.

Table 6. Polymorphic information content.

Locus	Repeat motif	Polymorphic information content
STM2020	(TAA)	0.76
SSR1	(TCAC)	0.77
STM2005	(CTGTTG)	0.78
LEMALX	(ATT)	0.87
STM1097	(CGTTT)	0.63
STG0016	(AGA)	0.89
STG0030	(ATT)	0.83

Table 7. Polymorphic information content at seven microsatellites markers for native varieties and commercial cultivars.

Material groups	STM 2020	SSR1	STM 2005	LEMALX	STM 1097	STG 0016	STI 0030	Average
Native varieties	0.70	0.79	0.77	0.86	0.66	0.87	0.81	0.78
Commercial cultivars	0.68	0.68	0.68	0.79	0.44	0.80	0.77	0.69

Genetic distance

The longest distance was between the native varieties and commercial cultivars, and *S. fernandezianum*. The average genetic dissimilarity between *S. fernandezianum* and the accessions of *S. tuberosum* was 0.96, ranging from 0.90 to 1.00. Accessions UCT-34Cor and UCT-26Ach diverged markedly from the rest of the native varieties and commercial material included in this study. The average genetic dissimilarity among the accessions of native varieties evaluated was 0.78, with the dissimilarity values ranging from 0.12 between UCT-10MgL and UCT-1Ma, to 0.96 between UCT-34Cor and UCT30Ño (Figure 2). The average genetic dissimilarity among commercial cultivars was estimated a little lower, at 0.69 but this was still remained quite high, evidence of a broad genetic base of the commercial cultivars used in the south of Chile. The minimum and maximum dissimilarities among commercial cultivars were evaluated at 0.35 and 0.84 respectively. The average dissimilarity between the native varieties and commercial cultivars evaluated was 0.84, with a minimum of 0.55 and a maximum of 0.96.

Cluster analysis

Based on the dissimilarity matrix, cluster analysis by Neighbour Joining, using the seven polymorphic loci, resulted in the dendrogram shown in Figure 3. The Neighbour Joining clustering analyses separated the native varieties from the commercial cultivars and from *S. fernandezianum* almost

completely. Most of the native varieties were clustered in accordance with their geographical location, and thus might have a background of genetic similarity. Commercial cultivars were clustered in accordance with their breeding programs in Chile and Europe, with the exception of the cultivar Shepody (33) which is of North American origin (Canada). Furthermore, the high polymorphic information content (PIC) values found within groups indicated that the native varieties of Chiloe as well as the commercial cultivars represented a wide genetic diversity. Few genotypes within each group were related. Based on the dissimilarity matrix, the genotypes UCT-1Ma (15) and UCT-10MgL (30) were the closest (dissimilarity coefficient of 0.12). They grouped with two other close varieties UCT-310b (20) and UCT-9MgM (29). Interestingly, these four varieties were also grouped together based on morphological distances (data not shown). Among the commercial varieties, Karú (32) and Pukará (38) on the one hand, and Baraka (34) and Yagana (37) on the other, formed two different groups of close varieties. Other varieties appeared quite distant from one another.

DISCUSSION

Chile is considered to be an origin sub-center of the cultivated potato, with native and endemic species distributed among the mainland and islands (Spooner et al. 2005b). Two species found are *S. fernandezianum*, endemic to the Juan Fernandez archipelago, and over a hundred native varieties of *S. tuberosum* from the Chiloe archipelago. In this study, 64 allelic variants were identified by their approximate sizes, ranging between 110 and 289 bp. The sizes of the alleles found in this study for *S. tuberosum* were within the range reported by other authors (Moisan-Thiery et al*.* 2005; Mathias et al*.* 2007; Ghislain et al. 2009a; Ghislain et al*.* 2009b). A total of 21 alleles were found specific to native varieties, while only five were specific to the commercial cultivars. In this study, *S. fernandezianum* presented a lower number of alleles per locus compared with *S. tuberosum*, due to the diploid status of the species. This lack of amplification may indicate divergences between *S. fernandezianum* and *S. tuberosum*. *S. tuberosum*, leading to modification of the flanking sequences themselves and/or insertions of sufficiently long DNA fragments to prevent any PCR amplification. Nevertheless, 50% of the alleles (4) found in this species were exclusive to *S. fernandezianum*. Data obtained in this species showed that the number of alleles per locus ranged from 5 to 12. McGregor et al. (2000), using five microsatellite loci, detected 39 alleles in 39 genotypes. Raker and Spooner (2002), report a total of 208 alleles from 18 microsatellite loci spread throughout all 12 chromosomes of the potato. In their study, microsatellite loci separated subsp. *tuberosum* from subsp. *andigenum,* and cultivated from wild species. They found that allelic distributions varied by ploidy, taxonomy and taxonomic distance, and that most of the Chilean populations of subsp. *tuberosum* are distinct from subsp. *andigena* and other wild and cultivated diploid and tetraploid populations. Similar results were obtained by Barandalla et al*.* (2006), evaluating 41 local cultivars in Tenerife Island, to compare cultivars of *S. tuberosum* ssp. *tuberosum,* with cultivars of *S. tuberosum* subsp. *andigena* and *S. chaucha*. A total of 67 alleles were observed, 12 of them present in all cultivars. Several accession- and group-specific alleles were detected. On the other hand, Feingold et al*.* (2005) obtained a range from 1 to 16 alleles using 61 microsatellites in 30 genotypes. Moisan-Thiery et al*.* (2005) found an average of 6.6 alleles per locus for 286 potato cultivars in France with five SSR loci, allowing the creation of a database comprising the allele composition of every cultivar. Mathias et al. (2007) found that 21 SSR markers showed up scorable products, with allele numbers ranging between 2 and 17. Ghislain et al*.* (2009b) reported a range from 2 to 21 for 742 potato landraces with 51 SSR markers. Moreover, Marchezi et al*.* (2010) detected that the number of alleles per locus varied from 1 to 27, with a total of 127 alleles, for 14 potato cultivars in Brazil, while Martinez et al*.* (2010) found a total of 90 alleles with 16 markers, with markers ranging between three and nine alleles, among 16 native potatoes from the district of Anco, Ayacucho, in Peru, using microsatellite markers. The total number of alleles found, the number of alleles per locus and the unique restricted alleles show the remarkable value of native varieties. Furthermore, the sole and exclusive alleles found for the species *S. fernandezianum* are evidence that this original genotype is endemic to Juan Fernandez Island. Commercial cultivars do not have the same genetic variability as native varieties. The allelic richness of commercial cultivars is lower than that of native varieties of *S. tuberosum*, with commercial cultivars presenting fewer alleles than native varieties on average. Alleles which are common to both types have different allelic frequencies. In addition, commercial cultivars have some alleles that are absent in native varieties. For example, for the STG 0016 marker, alleles -2, -3, -6, -7 and -11 were unique to native varieties. Allele -2 was unique to accessions UCT-11Mgb, UCT-14MgRe, UCT-7Ca, UCT-18Mn, UCT-26Ach; allele -6 was unique to accessions UCT-11Mgb, UCT- 6Gc, UCT-24Tn, UCT-22Cm, UCT-28MiR, UCT-3Cl, UCT-19Aq, UCT-33Cab, and UCT-15MgRo; and allele -11 was unique to accession UCT-28MiR. For the STM 2020

marker, allele -6 (approximately 156 bp) was unique to native varieties, being present in 33% of these. This can be explained by selection by farmers and by the maintenance of varieties based on factors such as yield, disease resistance, longevity in storage, and taste. As a consequence, a great diversity has been developed of cultivated varieties that are ecologically versatile (Raker and Spooner, 2002).

The polymorphic information content (PIC) confirms that SSR markers are highly informative. These values are consistent with the reports of Ispizúa et al. (2007) and Martinez et al. (2010) and higher than those published by Atencio et al. (2009) and Ghislain et al. (2009b). Martinez et al. (2010) studied the genetic diversity of potato landraces of north-western Argentina with four SSRs, finding PIC values which ranged from 0.80 to 0.92. Ispizúa et al. (2007), with four SSRs found PIC values ranging from 0.80 to 0.92 for potato landraces of north-western Argentina. When the PIC values were analyzed for each SSR and for each local variety, some markers were more informative than others. Atencio et al. (2009) evaluated the polymorphism of a local variety "Collajera" (*S. tuberosum* ssp. *andigena*) at the provincial level (Jujuy) versus Andean farmer level. They obtained PIC values from 0.31 to 0.72. On the other hand, Ghislain et al. (2009b), reported PIC values of 0.25 to 0.88 in 742 potato landraces evaluated with 51 SSR markers. This is consistent with previous results of Marchezi et al. (2010), who observed that a set of only two microsatellites was able to identify and differentiate 14 potato cultivars in Brazil. Reid and Kerr (2007) used six microsatellites to identify approximately 400 genotypes of potato. Our results also coincide with those reported by Moisan-Thiery et al. (2005), who report that sequential amplifications with SSR1, STM2005, LEMALX, STM1097 and STM2020, all markers used in the present study, are able to completely differentiate all the commercial cultivars (286) in France. Molecular markers based on Simple Sequence Repeats (SSR) are a very efficient tool for potato identification and can be very useful for germplasm conservation and management. The high allelic richness observed in the potato accessions shows the value of native varieties in comparison with commercial cultivars, suggesting that may constitute an interesting source of alleles for potato breeding programs.

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Figures

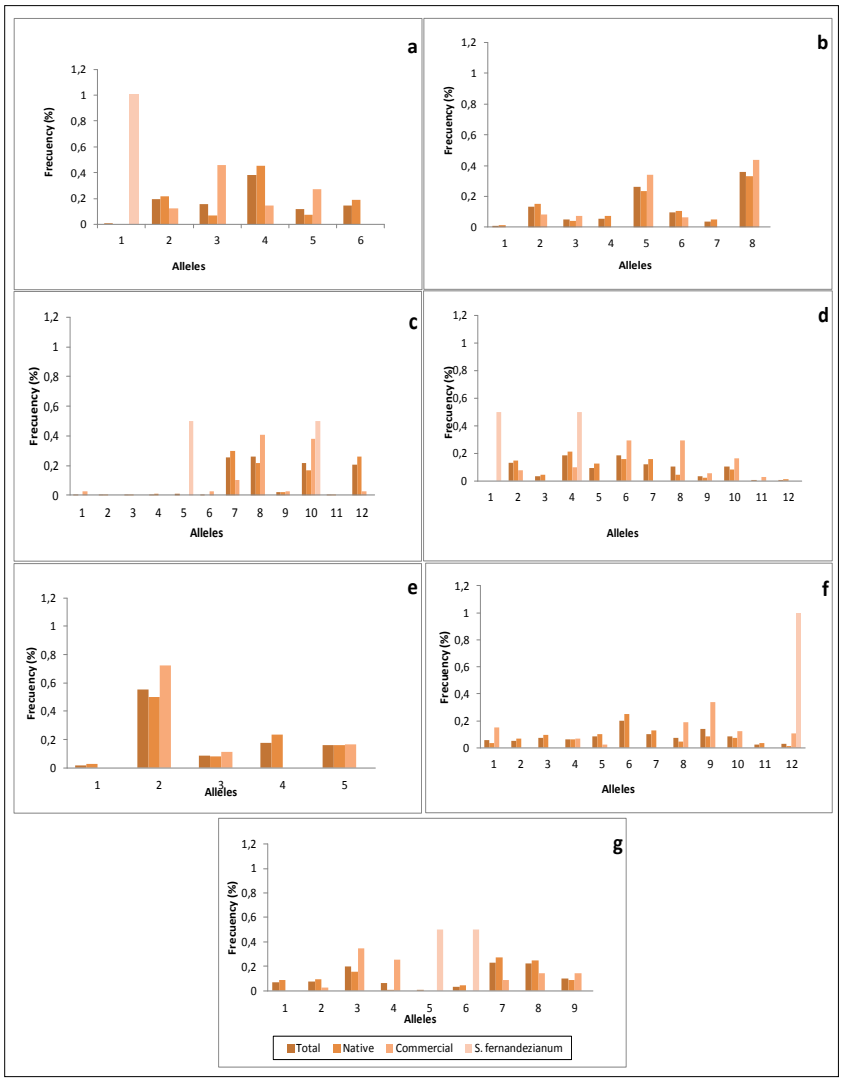

Fig. 1 Allelic frequency for *S. tuberosum* ssp. *tuberosum* and *S. fernandezianum* for loci (a) STM 2020, (b) SS R1, (c) STM 2005, (d) LEMALX, (e) STM 1097, (f) STG 0016 and (g) STI 0030.

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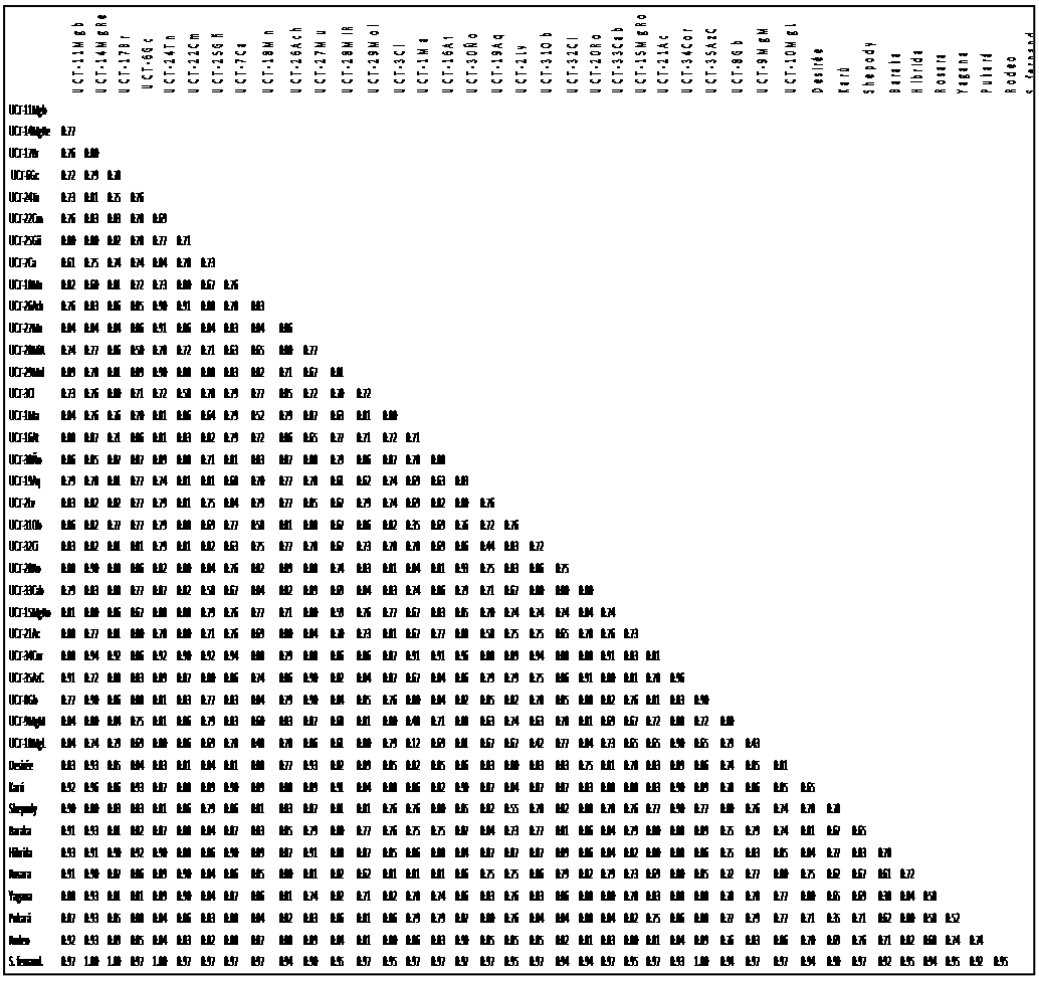

Fig. 2 Dissimilarity matrix using Sokal and Sneath 2 coefficient calculated with DARwin 5.0 program.

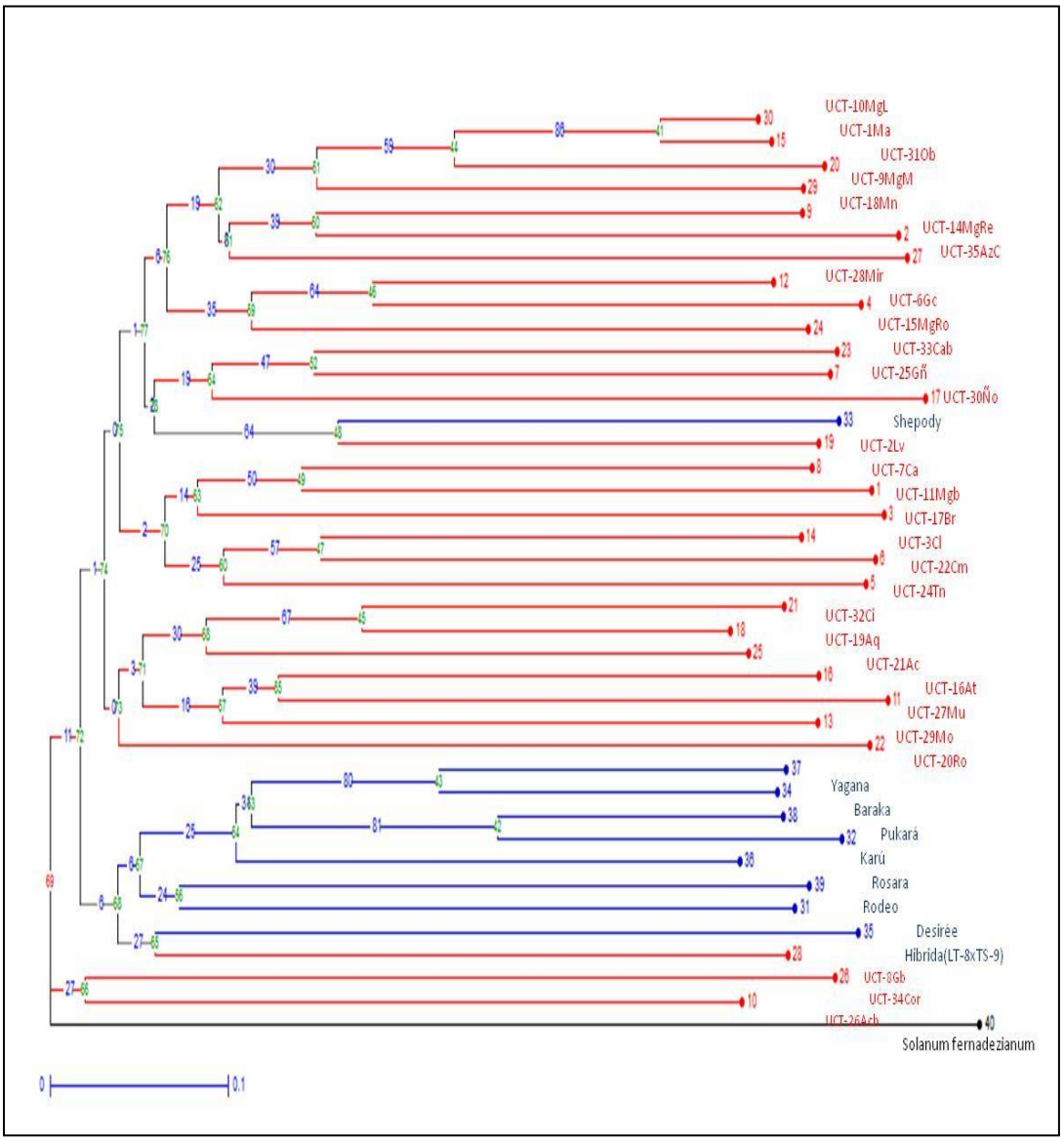

Fig. 3 Rooted dendrogram of native varieties, commercial cultivars and *S. fernandezianum* product of the allelic variation of seven microsatellite markers.