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Microsatellite variation in potato landraces from the island of La Palma

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Abstract

Nineteen microsatellite markers were used to fingerprint a set of 19 potato landraces from the island of La Palma (Canary Islands). These landraces represent relicts of early introductions from South America, although most are commonly cultivated by local farmers. The SSR primers detected 62 polymorphisms, 13 of which were present in all landraces. Several accession- and group-specific markers were detected. Jaccard similarity coefficients were estimated from the molecular data and UPGMA cluster analysis was performed. Some cultivars with related common names clustered together. In some cases, e.g., for the 'Conejera' landrace, the molecular patterns were discrepant with previous species assignments, suggesting the need for a more detailed morphological and comparative study of these accessions.

Additional key words: characterization, genetic diversity, germplasm, *Solanum tuberosum,* SSR markers.

Resumen

Caracterización mediante microsatélites de variedades locales de patata de la isla de La Palma

Se han empleado 19 marcadores microsatélites para identificar un conjunto de variedades locales de patata de la isla de La Palma (Islas Canarias). Dichas variedades locales representan a las primeras introducciones originadas en América del Sur, cultivadas la mayor parte de ellas en la isla por agricultores locales. Los iniciadores SSR generaron 62 polimorfismos, 13 de los cuales estaban presentes en todos las variedades locales. Se han detectado varias entradas y grupos de alelos específicos. Se han estimado coeficientes de similaridad a partir de los datos moleculares y se ha realizado un análisis de grupos. Generalmente, los grupos de variedades locales con nombres comunes se han agrupado conjuntamente. Sin embargo, en algunos casos como en el de la variedad local 'Conejera', los patrones moleculares han mostrado discrepancias con asignaciones anteriores de especie, lo que sugiere la necesidad de un estudio morfológico y comparativo más detallado de estas entradas.

Palabras clave adicionales: caracterización, diversidad genética, germoplasma, marcadores SSR, *Solanum tuberosum.*

Introduction

During colonial times, the Canary Islands were the first point of entry into Europe of goods from South America, including novel plant species such as the potato. Modern potato cultivars are very different to this original material, but in the Canary Islands a number of Andean introductions have been maintained and cultivated since the 16th century. Local cultivars can therefore be found that are very similar to the native material brought from Peru (Zubeldia *et al*., 1955). These cultivars provide lucrative crops for local growers since, despite their low yields, they demand high prices.

The first references to the presence of potatoes in the Canary Islands date from 1567 (for Grand Canary Island) and 1574 (for Tenerife) (Hawkes and Francisco-Ortega, 1993). In 1868, Alvarez-Rixo published the first inventory of Tenerife's potatoes. Zubeldia *et al*. (1955) assigned the traditional cultivars of the Canary Islands to the following taxa: *Solanum tuberosum* subsp. *andigena, S. tuberosum* subsp. *tuberosum*, and *S. chaucha*

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(formerly *S. mamilliferum*). *Solanum chaucha* is a triploid hybrid between the tetraploid *S. tuberosum* subsp. *andigena* and the diploid *S. stenotonum* (Zubeldia, 1955). Marrero (1992) and Gil *et al.* (2000) determined the geographical distribution of the traditionally grown cultivars in the Canary Islands and evaluated their agronomic performance. Based on these studies, Rios (2002) collected accessions from Tenerife in an attempt to identify duplicates and classify the varieties. This author published a detailed morphological and ecophysiological characterization of the island's cultivars. On the island of La Palma, similar work is being carried out by the *Cabildo Insular de La Palma* (the La Palma local government). Researchers have collected 19 accessions from two places on the island for their classification. All of these accessions are thought to be morphologically different (Lorenzo R., pers. comm.), and share similar common names. On La Palma, homonyms are commonly related to the place of cultivation and the agronomic characteristics of the growing area.

Molecular markers are useful in cultivar identification, the analysis of biodiversity (Ritter *et al*., 2005), and for examining the phylogenetic relationships related to autoploidy and amphidiploidy in potato (Martinez-Zapater and Oliver, 1984). Microsatellite markers (SSR) are particularly helpful since they are highly polymorphic, represent co-dominant markers, and are generally well-conserved across related species. Moreover they are simple to use in PCR (Powell *et al*., 1996). The first SSR study of potato was based on DNA sequences from public databases (Veilleux *et al*., 1995). SSR markers have subsequently been used to study genetic relationships among *S. tuberosum* cultivars (Provan *et al.*, 1996; Schneider and Douches, 1997). Milbourne *et al*. (1998) published a set of 112 potato SSRs located on all 12 chromosomes of the genome. Ashkenazi *et al.* (2001) used SSRs to study phylogenetic distances among wild and cultivated potato species. Raker and Spooner (2002) were able to distinguish *S. tuberosum* subsp. *tuberosum* from *S. tuberosum* subsp. *andigena* using SSR markers. Ghislain *et al*. (2004) used 22 SSR markers to analyse over 900 native potato accessions from the Andes belonging to eight different *Solanum* species, and finally Barandalla *et al*. (2006) used them to classify 41 local potato cultivars from 10 locations on Tenerife.

The aim of the present study was to use SSR markers to analyse and compare the molecular relationships among potato landraces from the island of La Palma.

Material and Methods

Molecular analyses were performed on 19 potato landraces collected on the island of La Palma (28º40'N/ 17º37'W; 2426 m maximum altitude) by the *Cabildo Insular de La Palma*, which have yet to be fully morphologically characterised. The commercial variety 'Kennebec' (*S. tuberosum*) and the Tenerifian accessions 'Peluca Blanca' (*S. tuberosum* subsp. *tuberosum*), 'Bonita Colorada' (*S. tuberosum* subsp. *andigena*) and 'Yema de Huevo' (*S. chaucha*) were used as references.

Table 1 shows the accession codes, common names, places of origin and the current taxonomic assignment of each entry. Based on their common names, groups of local varieties such as 'Marcialas' and 'Corraleras' were distinguished. Other landraces such as 'Moruna', 'Malgara', 'Morada', 'Colorada' and 'De año' represented individual entries.

DNA extraction was undertaken using the DNAeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). Concentrations were determined by electrophoresis in 0.7% agarose gels and comparison to a 1kb DNA ladder standard (Invitrogen, Carlsbad, CA, USA). Nineteen of the 22 SSR primers used by Ghislain *et al*. (2004) were employed in the present work (Table 2). One of the primers of each SSR was modified with a 5'-end M13 extension (Steffens *et al.,* 1993). A complementary M13 primer labelled with the fluorescent infrared dye IRD800 (LI-COR, Lincoln, Nebraska, USA) was added to each PCR reaction. This helped reduced the number of labelled primers. PCR reactions were performed in a 10 µl volume containing 1 µl of 10 × PCR buffer, 200 µM of dNTPs, 2.5 mM of $MgCl_2$, 0.2 µM of each primer (forward and reverse), 0.18 µM of $M13^{IRD800}$ primer (MWG, Genotec, Spain), 0.5 units of Taq polymerase (LINUS, Teknovas, Spain), and 30 ng of genomic DNA. PCR was carried out in a Robocycler Gradient 96 thermocycler (Stratagene, La Jolla, CA, USA) using the following cycling profile: 2 cycles of 1 min at 94ºC, 2 min at 63ºC and 35 s at 70ºC, 18 touchdown cycles of 45 s at 94ºC, 45 s at 62ºC (–1ºC each 2 cycles), and 45 s at 70ºC, 20 cycles of 30 s at 92ºC, 30 s at 53ºC, and 1 min at 70ºC, plus a final elongation step of 5 min at 72ºC. Amplification products were separated on 6% denaturing polyacrylamide gels. SSR fragments were detected using a LI-COR 4200-S1 DNA Sequencer (LI-COR Biosciences, Eversberg, Germany), and fragment analysis performed following the manufacturer's instructions (LI-COR, 1997).

Table 1. Number of SSR polymorphisms in 19 accessions of potato landraces from La Palma, three cultivars from Tenerife, and a commercial cultivar of *S. tuberosum* (cv. Kennebec)

Accession code	Common name	Place of origin	Previous species assignment[1]	Detected SSR polymorphisms
PS-LP1	Jaragana	La Piedra, La Palma	—	33
PS-LP2	Marciala blanca	La Piedra, La Palma	*S. tuberosum* subsp. *tuberosum*	34
PS-LP3	Corralera colorada	Buenavista, La Palma	*S. tuberosum* subsp. *andigena*	24
PS-LP4	Moruna	La Piedra, La Palma	*S. tuberosum* subsp. *tuberosum*	37
PS-LP5	Marciala rayada	La Piedra, La Palma	*S. tuberosum* subsp. *tuberosum*	32
PS-LP6	Corralera tijarafera	Buenavista, La Palma	*S. tuberosum* subsp. *andigena*	31
PS-LP7	Cecilia	La Piedra, La Palma	*S. tuberosum* subsp. *tuberosum*	31
PS-LP8	Siciliana	La Piedra, La Palma	*S. tuberosum* subsp. *tuberosum*	35
PS-LP9	Conejera blanca	Buenavista, La Palma	—	23
PS-LP10	Marciala colorada	Buenavista, La Palma	*S. tuberosum* subsp. *tuberosum*	36
PS-LP11	Malgara	La Piedra, La Palma	*S. tuberosum* subsp. *tuberosum*	34
PS-LP12	Conejera	Buenavista, La Palma	—	35
PS-LP13	Corralera legítima	Buenavista, La Palma	*S. tuberosum* subsp. *andigena*	26
PS-LP14	Morada	La Piedra, La Palma	*S. tuberosum* subsp. *tuberosum*	34
PS-LP15	Corralera blanca	La Piedra, La Palma	*S. tuberosum* subsp. *andigena*	27
PS-LP16	Negra	La Piedra, La Palma	*S.tuberosum* subsp. *tuberosum*	34
PS-LP17	Marciala negra	La Piedra, La Palma	*S. tuberosum* subsp. *tuberosum*	35
PS-LP18	Colorada	Buenavista, La Palma	—	32
PS-LP19	De año	Buenavista, La Palma	—	28
		Cultivars		
Knn	Kennebec	NEIKER, Vitoria	*S. tuberosum* subsp. *tuberosum*	31
BcO	Bonita colorada	La Orotava, Tenerife	*S. tuberosum* subsp. *andigena*	26
LbO	Peluca blanca	La Orotava, Tenerife	*S. tuberosum* subsp. *tuberosum*	32
N-T	Yema de huevo	Tacoronte, Tenerife	*S. chaucha*	33

[1] Zubeldia (1955).

Each band or fragment on the gels was scored for presence or absence. These data were used to calculate similarity coefficients between the 19 accessions. A cophenetic matrix was produced from the tree matrix and compared to the distance matrix generated by the Jaccard coefficients (Jaccard, 1901). Cluster analyses were performed using the UPGMA method (Rohlf, 2001) and employing NTSYS-PC software (Rohlf, 2001; supplied by Exeter Software, Setauket, NY, USA). The polymorphism index content (PIC), a measure of allelic diversity, was calculated according to Nei's coefficient (Nei, 1973), $PIC = 1-\Sigma(p_i^2)$, where p_i is the frequency of the i^{th} polymorphism detected in the germplasm. The polymorphism frequency distribution was analysed using the PROC UNIVARIATE procedure of the SAS program (SAS, 2000).

Results

Table 2 shows the polymorphisms detected by each SSR. The landraces showed a total of 62 SSR fragments, ranging from one (monomorphic) to six (polymorphic) per SSR. Only 13 of them were present in all materials; the other 49 showed a varying degree of polymorphism among accessions. PIC values ranged from 0 to 0.78; STM0030 had a high PIC value and a maximum of five markers.

Several accessions showed specific bands (Table 3). 'Corralera tijarafera' (PS-LP6), 'Moruna' (PS-LP4), 'Cecilia' (PS-LP7) and 'Marciala colorada' (PS-LP10), showed a specific polymorphism. Other specific polymorphisms included STM2030_207, which was present only in PS-LP1 and PS-LP18, and STM0037_84, which was present only in 'Marcialas' and accession PS-LP14. Polymorphisms STM0019_205 and STM2013_142 were present in all accessions except in the 'Corraleras' (PS-LP3, PS-LP6 and PS-LP15). Polymorphism STM1052_213 was present in all accessions except for 'Cecilia' (PS-LP7), and STM0037_75 was present in all accessions except for 'Cecilia' (PS-LP7) and 'Morada' (PS-LP14). Finally, STM1104_164 was present in all accessions except for PS-LP1 and PS-LP18 (see Table 3 for all).

Table 2. Polymorphisms detected by SSR markers in 19 potato landraces from La Palma

SSR marker	No. of SSR fragments	No. of monomorphic SSR fragments	Detected SSR markers (in bp) and their absolute frequencies[1] *(in brackets)*	PIC[2]
STM1058	1	1	114 *(19)*	0.00
STM1049	2	1	179 *(2)* 188 *(14)*	0.22
STM3023	2	1	172 *(18)* 192 *(16)*	0.50
STPoAc58	2	0	227 *(16)* 243 *(2)*	0.20
STM0019	4	0	180 *(1)* 188 *(6)* 205 *(17)* 232 *(3)*	0.54
STM1017	2	1	128 *(19)* 137 *(4)*	0.29
STM1031	3	2	256 *(19)* 260 *(19)* 278 *(4)*	0.58
STM1052	3	0	205 *(9)* 213 *(18)* 223 *(15)*	0.64
STM1053	2	1	167 *(11)* 171 *(19)*	0.46
STM1106	3	0	153 *(9)* 156 *(1)* 189 *(8)*	0.55
STM2022	4	1	176 *(12)* 185 *(1)* 188 *(17)* 233 *(4)*	0.61
STM2030	3	2	177 *(19)* 179 *(19)* 207 *(2)*	0.55
STGBSS	3	1	127 *(9)* 130 *(18)* 133 *(8)*	0.62
STM0030	5	0	141 *(9)* 143 *(4)* 149 *(6)* 157 *(4)* 165 *(7)*	0.78
STM1064	4	1	183 *(13)* 188 *(19)* 191 *(8)* 193 *(4)*	0.68
STM1104	3	0	161 *(3)* 164 *(17)* 173 *(8)*	0.54
STM2013	5	0	142 *(16)* 146 *(11)* 154 *(8)* 158 *(6)* 166 *(11)*	0.77
STM3012	5	1	162 *(18)* 192 *(4)* 194 *(8)* 198 *(4)* 204 *(5)*	0.71
STM0037	6	0	72 *(2)* 75 *(17)* 77 *(8)* 82 *(4)* 84 *(6)* 91 *(1)*	0.72
Total	62	13		

[1] Missing data means some totals may not reach 19. [2] PIC: polymorphism information contents.

Table 1 shows the total number of SSR polymorphisms detected in each genotype. That part of the *S. tuberosum* subsp. *andigena* germplasm represented by 'Corraleras' apparently has fewer polymorphisms than *S. tuberosum* subsp. *tuberosum* germplasm (represented by the 'Marcialas').

Figure 1 shows the dendrogram obtained by UPGMA clustering using the amplification results for the 19 SSRs. The cophenetic matrix derived from the cluster analyses was in good agreement with the original similarity matrix ($r^2 = 0.956$). Setting the cut-off point for the average similarity coefficient at 0.78 distinguished seven groups. The first was formed by 'Moruna', 'Jaragana', 'Colorada' and the Tenerifian cultivar of *S. tuberosum* subsp. *tuberosum* 'Peluca Blanca'. All the accessions of this group belong to *S. tuberosum* subsp. *tuberosum.* The second group included four accessions from La Palma ('De año', 'Corralera Blanca', 'Corralera

Table 3. Accession- and group-specific SSR polymorphisms detected in potato landraces from La Palma

SSR	Specific polymorphism (bp)	Accession of group for which the SSR marker is specific
STM2030	207	Sixth group (PS-LP1 and PS-LP18)
STM2013	142	All accessions except 'Corraleras' (PS-LP3, PS-LP6 and PS-LP15)
STM1052	213	All accessions except 'Cecilia' (PS-LP7)
STM2022	185	'Corralera tijarafera' (PS-LP6)
STM0037	75	All accessions except PS-LP7 and PS-LP14
STM0037	84	'Marcialas' and PS-LP14
STM0037	91	'Moruna'
STM1104	164	All accessions except sixth group (PS-LP1 and PS-LP18)
STM1106	156	'Cecilia' (PS-LP7)
STM0019	180	'Marciala' PS-LP10
STM0019	205	All accessions except 'Corraleras' (PS-LP3 and PS-LP6)

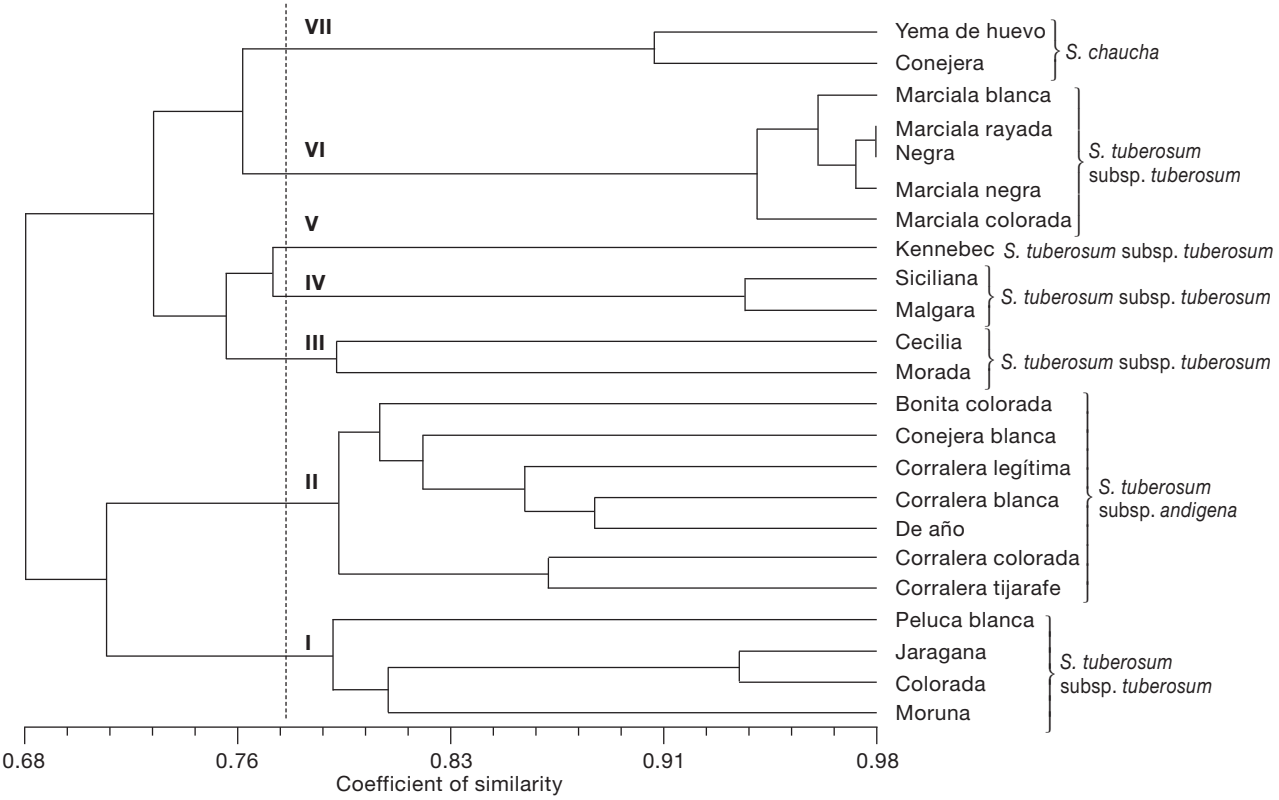

Figure 1. Potato landraces from La Palma: dendrogram of the cluster analysis based on the Jaccard similarity coefficient, using the UPGMA clustering method.

Legítima' and 'Conejera Blanca'), 'Bonita Colorada' (*S. tuberosum* subsp. *andigena*) from Tenerife, and two 'Corraleras' (Colorada and Tijarafera) from La Palma. All the group 2 accessions belong to *S. tuberosum* subsp. *andigena*. The accessions 'Morada' (PS-LP14) and 'Cecilia' (PS-LP7) formed the third group, belonging to *S. tuberosum* subsp. *tuberosum*. The fourth group comprised two entries, 'Siciliana' and 'Malgara', which are thought to belong to *S. tuberosum* subsp. *tuberosum*. The fifth group was formed by the commercial cultivar of *S. tuberosum,* 'Kennebec', and the sixth cluster contained the 'Marcialas', which belong to *S. tuberosum*. subsp. *tuberosum*. The seventh and last group was formed by the accession 'Conejera' from La Palma and the cultivar 'Yema de Huevo' from Tenerife, both of which belong to *S. chaucha*.

Discussion

The dendrogram of the cluster analysis shows that the landraces from La Palma are all different. In some cases they have similar names – but this is because they share the place or method of cultivation. For example, the group of 'Corraleras' is formed by four landraces (PS-LP3, PS-LP6, PS-LP13 and PS-LP15), all of them different, yet they share a similar name because they are commonly cultivated in vineyards. The accessions from La Palma were recently collected and morphological studies on them are as yet incomplete. Accessions PS-LP7, PS-LP9 and PS-LP12 were introduced to the island in the 20th century and are not traditionally cultivated (Lorenzo R., pers. comm.). The accessions PS-LP9 and PS-LP12 are both 'Conejeras' but are different molecularly and morphologically. Traditional names often give ethnobotanical and ethnoagricultural information, which is useful in conserving genetic plant resources, but this work helps to clarify the relationship and origin of some of the accessions from La Palma, and suggests they should be renamed.

In the dendrogram (Fig. 1), group VI (the 'Marcialas') represents *S. tuberosum* subsp. *tuberosum* germplasm. The members of this group are morphologically very different from those of other groups, with more stems, few flowers and berries, and bigger leaves (Rios, 2002). This group includes five different landraces: PS-LP2,

PS-LP5, PS-LP10, PS-LP16 and PS-LP17. The *S. tuberosum* subsp. *andigena* accessions clustered in the second group. These have asymmetric flower morphology and their tubers are orange or purple (Huaman and Spooner, 2002). The 'Conejera' accession (PS-LP12) is triploid and belongs to *S. chaucha* (Chico, 1986), along with 'Yema de Huevo' from Tenerife (Barandalla *et al.,* 2006).

A variety of molecular markers have been used to examine potato genetic diversity, as aids in taxonomic studies, or simply to search for effective fingerprinting tools. Ghislain *et al.* (1999) analysed the intraspecific variation of *S. phureja* using random amplified polymorphic DNA (RAPD) markers. Spooner *et al.* (2005a) used AFLP markers in a phylogenetic analysis of 365 potato accessions, and Bornet *et al.* (2002) compared cultivars from Europe and Argentina using inter-simple sequence repeat (ISSR) markers. SSR markers are highly polymorphic and show good reproducibility. Milbourne *et al.* (1997) compared three types of PCR-derived markers to estimate variability among 16 potato cultivars and concluded that microsatellites offer an effective means of analysing genetic distances between potato varieties. Spooner *et al.* (2005b) used microsatellites to test two competing hypotheses regarding the origin of the modern cultivated potato, and detected clusters of Indian, Andean and Chilean landraces. These authors used 13 different SSRs, all of which except one were employed in the present study. According to Ghislain *et al.* (2004), each system has its advantages and disadvantages, depending on the genetic distances of the populations in question and the nature of the problem addressed.

In the present study, nearly all the Ghislain *et al*. (2004) SSR markers were also used. When comparing the different SSR studies of potato, it is clear that various sets of SSR markers have been used; it would be a good idea to agree on a fixed set of primer combinations. In this way all accessions could be labelled with an SSR «barcode» and identification across databases would be easy. Differences in mobility could be adjusted for by including appropriate reference materials in analyses. The present cluster analysis is based on the Jaccard similarity coefficient, since the goodness of fit was slightly better than that offered by the Dice coefficient. Bornet *et al.* (2002) observed identical agglomerations with both coefficients when analysing cauliflower lines with ISSR markers.

More detailed studies are needed to shed light on the origin of these landraces. The tracking of the detected specific fragments in larger potato germplasm collections would be very interesting. Collections of related Andean accessions are available at the CIP (Lima, Peru). A comparison of SSR patterns might help in identifying the origins of the local landraces of the Canary Islands and help analyse their adaptive evolution in different habitats.

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