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ORIGINAL PAPER

Pattern of genetic diversity of cultivated and non-cultivated mashua, *Tropaeolum tuberosum*, in the Cusco region of Perú

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Abstract This paper analyzes the genetic diversity of mashua cultivars grown in six communities in the Cusco region of Perú, of non-cultivated forms collected in the same region, and a sample of cultivars from the germplasm collection at the International Potato Center (CIP). From the DNA fingerprinting generated by SRAP markers, it is clear that mashua is a genetically variable crop with a range of similarity ranging from 65 to 99%. The widest range of variation was found for the most isolated community, Sayllafaya. Another important finding was that most of the non-cultivated accessions are likely feral races resulting from escape to cultivation rather than wild relatives. In general the range of variation of the cultivars from the communities and their feral relatives are not represented in the cultivars sampled of the collection maintained at the International Potato Center, Perú.

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Introduction

Mashua (*Tropaeolum tuberosum* Ruiz et Pavon spp. *tuberosum*), is a member of the angiosperm family Tropaeolaceae, the *Nasturtium* family (Ruíz and Pavón 1802; Sparre 1973; Sparre and Andersson 1991). This crop is a tetraploid ($2n = 4\times = 48$) although other numbers have been reported by several authors (Gibbs et al. 1978). It is also known as isañu (Aymara, Bolivia), cubio (Colombia), añu and ysaño, (Quechua—Perú and Bolivia) (Hernandez and León 1994; Herrera 1941), and pertains to the group of traditional, subsistence-oriented tuber crops indigenous to the Andean highlands known as ARTC—Andean Root and Tuber Crops (Arbizu and Robles 1986; Flores et al. 2003; Grau et al. 2000). The Andean Region in South America, especially Perú and Bolivia, is distinguished as one of the most important centers of crop diversity around the world, being the center of origin of at least 25 species of root and tuber crops representing a considerable range of diversity in terms of taxonomic affiliation and ecological adaptation unique in the world (Hermann and Heller 1997; National Research Council [NRC] 1989; Tapia 1993).

Because of their cold tolerance, these tubers represent one of the few cropping options at high altitudes and complement a diet based on potatoes, barley, beans, and Andean grains. The Andean tuber complex is important not only as a component in the rotational farming systems of the Andean rural communities because of their adaptation and diversification in the Andes, but also because of the pool of variation in many beneficial factors, including nutritional value and resistance to pests and diseases (NRC 1989). The highest concentration of mashua diversity occurs from central Perú to central Bolivia at altitudes between 3,500 and 3,800 m (Arbizu and Robles 1986; Hernandez and León 1994; King 1988; Rea and Morales 1980) and it has been suggested that the crop originated in the highlands of the central Andes in the vicinity of the Titicaca basin (Hodge 1946).

Sparre (1973) and Sparre and Andersson (1991) recognize two subspecies of *T. tuberosum*; the cultivated *T. tuberosum* spp. *tuberosum* and the wild *T. tuberosum* spp. *silvestre sparre*, differentiating one from the other by the smaller size in all plant parts and the lack of tubers in *T. tuberosum* spp. *silvestre*. However, this classification does not include non-cultivated tuber-forming *T. tuberosum*, such as the ''kipa isaño'' mentioned by Johns and Towers (1981) and studied by León (1964) and Ortega (2000). However, in the absence of a comprehensive study on the diversity of cultivated and non-cultivated forms of mashua, it is difficult to pinpoint the likely center of origin of the crop (Grau et al. 2000).

Mashua ranks fourth in importance in the Andean region after potato (*Solanum* spp.), oca (*Oxalis tuberosa* Molina) and ulluco (*Ullucus tuberosus* Caldas) (NRC 1989). However, the future role of mashua as a crop in the region seems to be uncertain, in spite of its nutritional properties (Hermann and Heller 1997). Apparently a range of positive nutritional attributes as well as rusticity (drought, frost and pest tolerance) and high productivity under low levels of inputs cannot compensate for its lack of attractiveness to consumers. Thus, mashua is not often seen in local markets and is known mostly to a few traditional farmers, who occasionally grow it for food or as an ornamental. The low appeal of this crop is due to its strong flavor and bitterness caused by its high content of isothiocyanates derived from glucosinolates (Drewnowski and Gomez-Carneros 2000; Grau et al. 2000; Johns and Towers 1981). On the other hand, isothiocynates have been reported to serve as cancer-protecting agents (Halkier and Du 1997; Stoner and Morse 1997; Sugie et al. 1994; Wattenberg 1987, 1992), suggesting that consumption of mashua may offer medicinal benefits as well.

The genetic variability of mashua has been described mostly in terms of morphological and agronomic traits (Arbizu et al. 1999), which has the disadvantages of being subjective and influenced by environment (Grattapaglia and Ferreira 1998; Tanksley et al. 1989). However in recent years, variation in different mashua clones has been studied at the biochemical and molecular levels using polyacrylamide gel electrophoresis (PAGE) (Shah et al. 1993), isoenzymes (Monteros 1996; Monteros et al. 1997) and RAPD markers (Ortega 2000). Mashua, similarly to other Andean crops, has suffered a progressive reduction in its genetic variability and production volume, particularly during the last decades (Grau et al. 2000; León 1964).

The objective of this paper is to determine the pattern of mashua genetic diversity in the Cusco region of Perú by sampling cultivars grown in six farming communities and feral tuber-forming accessions in neighboring areas. The Cusco region is one of the richest in genetic diversity for Andean tuber crops. A sample of cultivated mashua from other regions of Perú, maintained at the International Potato Center (CIP), was used for comparison.

Materials and methods

Plant material

Following current access procedures, including farmers communities prior consent, mashua tubers were harvested in the following communities of the Cusco region: (1) Matinga, Picol and Qqueccayoc (Zone 1 located on the District of Taray—Province of Calca) and (2) Chumpe, Poques and Sayllafaya (Zone 2 located on

the District of Lamay—Province of Calca). These accessions are maintained as a live collection at Centro Regional de Investigacion en Biodiversidad Andina, Experimental Center K'ayra of the University of Cusco, Perú (CRIBA). After visual inspection of the tubers, these were classified visually in morphotypes by one of us (CA) based on the three morphological descriptors: tuber skin color, tuber skin secondary color and tuber shape. After morphotype determination, we sampled leaves from a single accession per morphotype in October and November of 2002. A total of 92 visually distinct accessions were used for DNA isolation. Additionally, we obtained DNA from 39 tuber-bearing non-cultivated accessions of mashua collected from eight different provinces of Cusco, and also maintained as a live collection at CRIBA, (Table 1). Morphological data for the non-cultivated accessions for above three morphological traits was already available (Ortega 2000). For DNA collection from accession sampled in the communities we used a new technique, called FTA (Whatman BioScience), where leaf juice is applied to FTA filter paper cards. These extracts can be stored dry at room temperature for several months before DNA extractions. This approach was ideal for collecting DNA from remote locations, like the isolated communities in Cusco, which makes timely tissue storage and processing very difficult. From the collection held at International Potato Center (CIP), we sampled leaves from 22 cultivated mashua accessions collected from the Southern Region of Perú (departments of Cusco, Puno and Huancavelica) grown at the CIP experimental station in Huancayo. These samples were lyophilized immediately after collection. Morphological data from the Collection from CIP were not available (Table 1, Fig. 1).

DNA extraction

CTAB Protocol: In a 1.5 ml. Eppendorf tube, approximately 0.2 g of ground lyophilized leaves was mixed with 0.7 ml of 2 × CTAB buffer and incubated at 65°C for 90 min. After incubation, 0.7 ml of chloroform was added and the tubes centrifuged at 14,000 rpm for 5 min. The supernatant was transferred into a new tube. After repeating this step once, the DNA was precipitated in 60% of the total volume of 2-propanol. The DNA was then washed with 70% ethanol, dissolved in 0.3 ml of 0.1 TE Buffer and stored at –4°C.

FTA Protocol: A 2.0 mm disc was punched from the filter paper using a Harris Micro Punch Tool (FTA Whatman Technology), washed three times with FTA Reagent using for 2 min incubations at room temperature for each wash. The disk was then washed 2 times in 10 mM TE Buffer for 2 min at room temperature. The discs were air-dried for 1 h at room temperature, and stored at –4°C.

DNA fingerprinting

We used Sequence Related Amplified Polymorphism (SRAP) molecular markers developed by Li and Quiros (2001) for DNA fingerprint of the mashua accessions. The forward primers were purified non-labeled and the reverse primers were labeled using a single infrared fluorescent dye (IRD700 or IRD800) from LI-COR. Each 10 μl PCR reaction mixture consisted of 1.6 μl of genomic DNA, 1.0 μl of 10× reaction buffer, 0.6 μl of 25 mM $MgCl_2$, 0.2 μl of 6.25 mM dNTP, 0.2 μl of *Taq*-polymerase (10 U/μl), 0.25 μl of 10 μM forward primer and 0.25 μl of 10 μM reverse primer. The first five cycles of the PCR are run at 94°C, 1 min, 35°C, 1 min, and 72°C, 1 min for denaturing, annealing and extension, respectively. The annealing temperature was then raised to 50°C for another 35 cycles. The amplicons were then separated by denaturing acrylamide gels in an automatic LI-COR IR2 4200 system and detected by fluorescence for genotyping (Li and Quiros 2001). Each sample was run twice to assure consistency and reliability of the scored bands. A size standard 50–700 bp (IRD 700 and 800) was used to estimate the size of the polymorphic bands. The bands were named by primer set used to amplify it followed by its size in bp.

Primer screening

A total of 180 different primer combinations using 30 forward and six reverse primers sequences were tested on a sample of 12

Table 1 Mashua accessions used for SRAP molecular genotyping. Name of the sample material starts with a letter and then the accessions number. Zone 1: P = Picol, M = Matinga and Q = Qqueccayoc; Zone 2: Ch = Chumpe, Pq = Poques and S = Sayllafaya; NC = Non-cultivated; and C = CIP (-x- : data unavailable)

Order	Community	Accession	Morphological traits		
			Tuber skin color	Tuber skin sec. color	Tuber shape
1	Picol	P1502	Pale yellow	Absent	Conical–long
2	Picol	P1504	Yellow	Absent	Conical
3	Picol	P1506	Orange yellowish	Absent	Long
4	Picol	P1508	Orange yellowish	Dark Purple	Conical–long
5	Picol	P1509	Orange	Red	Conical–long
6	Picol	P1511	Yellow	Pink	Conical–long
7	Picol	P1515	Red grayish	Yellow	Conical–long
8	Picol	P1517	Purple grayish	Yellow	Conical
9	Matinga	M2001	Pale yellow	Absent	Conical–long
10	Matinga	M2002	Pale yellow	Dark Purple	Conical
11	Matinga	M2004	Yellow	Dark Purple	Conical–long
12	Matinga	M2008	Orange yellowish	Dark Purple	Conical–long
13	Matinga	M2009	Orange yellowish	Absent	Conical–long
14	Matinga	M2010	Orange yellowish	Dark Purple	Conical
15	Matinga	M2013	Yellow	Red	Conical–long
16	Matinga	M2014	Pale yellow	Dark Purple	Conical–long
17	Matinga	M2015	Yellow	Dark Purple	Conical–long
18	Matinga	M2016	Pale yellow	Red grayish	Conical–long
19	Matinga	M2017	Yellow	Red	Conical–long
20	Matinga	M2020	Red grayish	Yellow	Conical–long
21	Qqueccayoc	Q2501	Pale yellow	Absent	Conical–long
22	Qqueccayoc	Q2502	Orange yellowish	Red	Conical–long
23	Chumpe	CH1001	Pale yellow	Absent	Conical–long
24	Chumpe	CH1004	Orange yellowish	Absent	Conical–long
25	Chumpe	CH1010	Orange	Absent	Conical
26	Chumpe	CH1017	Pale yellow	Dark Purple	Conical
27	Chumpe	CH1019	Orange yellowish	Dark Purple	Long
28	Chumpe	CH1020	Orange yellowish	Dark Purple	Long
29	Chumpe	CH1026	Yellow	Dark Purple	Conical–long
30	Chumpe	CH1030	Orange yellowish	Dark Purple	Long
31	Chumpe	CH1039	Yellow	Red	Conical
32	Chumpe	CH1052	Orange yellowish	Dark Purple	Conical
33	Chumpe	CH1053	Pale yellow	Dark Purple	Long
34	Chumpe	CH1054	Pale yellow	Absent	Conical–long
35	Chumpe	CH1055	Pale yellow	Dark Purple	Conical–long
36	Chumpe	CH1057	Orange yellowish	Dark Purple	Conical
37	Poques	PQ502	Pale yellow	Absent	Conical–long
38	Poques	PQ504	Yellow	Absent	Conical–long
39	Poques	PQ525	Yellow	Absent	Conical
40	Poques	PQ529	Pale yellow	Dark Purple	Conical
41	Poques	PQ532	Orange	Dark Purple	Conical–long
42	Poques	PQ534	Yellow	Absent	Conical
43	Poques	PQ547	Yellow	Dark Purple	Conical–long
44	Poques	PQ570	Orange	Red grayish	Conical–long
45	Poques	PQ573	Orange yellowish	Dark Purple	Long
46	Poques	PQ584	Yellow	Absent	Conical–long
47	Poques	PQ586	Yellow	Dark Purple	Conical–long
48	Poques	PQ588	Dark Purple	Yellow	Conical–long
49	Poques	PQ590	Yellow	Dark Purple	Conical–long
50	Sayllafaya	S1	Pale yellow	Absent	Conical–long
51	Sayllafaya	S2	Pale yellow	Absent	Conical–long
52	Sayllafaya	S4	Pale yellow	Absent	Conical
53	Sayllafaya	S7	Pale yellow	Dark Purple	Conical–long

Table 1 continued

Order	Community	Accession	Morphological traits		
			Tuber skin color	Tuber skin sec. color	Tuber shape
54	Sayllafaya	S10	Pale yellow	Dark Purple	Conical–long
55	Sayllafaya	S11	Yellow	Dark Purple	Conical–long
56	Sayllafaya	S13	Pale yellow	Dark Purple	Conical–long
57	Sayllafaya	S19	Orange yellowish	Dark Purple	Long
58	Sayllafaya	S22	Yellow	Absent	Conical–long
59	Sayllafaya	S23	Orange	Absent	Conical
60	Sayllafaya	S34	Orange	Absent	Conical–long
61	Sayllafaya	S47	Orange	Absent	Conical
62	Sayllafaya	S54	Orange	Absent	Conical–long
63	Sayllafaya	S62	-x-	-x-	-x-
64	Sayllafaya	S64	Yellow	Absent	Conical–long
65	Sayllafaya	S65	-x-	-x-	-x-
66	Sayllafaya	S66	Orange yellowish	Red	Conical–long
67	Sayllafaya	S67	-x-	-x-	-x-
68	Sayllafaya	S68	Pale yellow	Dark Purple	Conical–long
69	Sayllafaya	S69	Orange yellowish	Red	Conical–long
70	Sayllafaya	S77	Orange yellowish	Dark Purple	Conical–long
71	Sayllafaya	S79	-x-	-x-	-x-
72	Sayllafaya	S80	Orange	Red	Conical–long
73	Sayllafaya	S81	-x-	-x-	-x-
74	Sayllafaya	S82	Pale yellow	Red	Conical
75	Sayllafaya	S83	Orange	Absent	Conical
76	Sayllafaya	S85	-x-	-x-	-x-
77	Sayllafaya	S89	Orange	Absent	Conical
78	Sayllafaya	S90	-x-	-x-	-x-
79	Sayllafaya	S92	-x-	-x-	-x-
80	Sayllafaya	S94	Orange yellowish	Dark Purple	Conical
81	Sayllafaya	S96	Orange yellowish	Dark Purple	Long
82	Sayllafaya	S106	Orange yellowish	Dark Purple	Conical–long
83	Sayllafaya	S121	-x-	-x-	-x-
84	Sayllafaya	S130	-x-	-x-	-x-
85	Sayllafaya	S135	Orange yellowish	Dark Purple	Conical–long
86	Sayllafaya	S138	-x-	-x-	-x-
87	Sayllafaya	S144	Orange	Dark Purple	Conical–long
88	Sayllafaya	S145	-x-	-x-	-x-
89	Sayllafaya	S148	Red grayish	Yellow	Conical–long
90	Sayllafaya	S149	-x-	-x-	-x-
91	Sayllafaya	S150	Red grayish	Yellow	Conical–long
92	Sayllafaya	S153	Dark Purple	Absent	Conical–long
93	Taray	NC1	Yellow	Dark Purple	Conical–long
94	Taray	NC2	Yellow	Absent	Conical–long
95	Cusco	NC5	Yellow	Dark Purple	Long
96	Cusco	NC7	Pale yellow	Absent	Conical–long
97	Sicuani	NC8	Pale yellow	Dark Purple	Long
98	Sicuani	NC9	Pale yellow	Dark Purple	Long
99	Sicuani	NC10	Yellow	Dark Purple	Long
100	Sicuani	NC11	Yellow	Absent	Long
101	Marangani	NC18	Pale yellow	Dark Purple	Long
102	Marangani	NC19	Pale yellow	Absent	Long
103	Marangani	NC20	Yellow	Absent	Ovoid
104	Marangani	NC21	Yellow	Absent	Long
105	Marangani	NC22	Yellow	Absent	Long
106	Marangani	NC23	Yellow	Absent	Conical–long
107	Marangani	NC24	Pale yellow	Dark Purple	Conical–long
108	Pisac	NC26	Pale yellow	Absent	Conical–long

Table 1 continued

Order	Community	Accession	Morphological traits		
			Tuber skin color	Tuber skin sec. color	Tuber shape
109	Pisac	NC29	Pale yellow	Dark Purple	Long
110	Pisae	NC30	Pale yellow	Absent	Slim-twisted
111	Pisac	NC32	Pale yellow	Absent	Conical–long
112	San Sebastián	NC36	Dark Purple	Dark Purple	Slim-twisted
113	Pisac	NC37	Dark Purple	Dark Purple	Slim-twisted
114	Pisac	NC38	Yellow	Dark Purple	Slim-twisted
115	Pisac	NC39	Yellow	Dark Purple	Slim-twisted
116	Pisac	NC40	Yellow	Dark Purple	Slim-twisted
117	Pisac	NC41	Yellow	Dark Purple	Slim-twisted
118	Pisac	NC42	Yellow	Dark Purple	Slim-twisted
119	Pisac	NC43	Yellow	Dark Purple	Long
120	Paucartambo	NC45	Pale yellow	Dark Purple	Slim-twisted
121	Acos	NC49	Yellow	Absent	Conical–short
122	Acos	NC50	Yellow	Absent	Conical–short
123	Colquepata	NC53	Pale yellow	Absent	Slim-twisted
124	Colquepata	NC54	Pale yellow	Dark Purple	Slim-twisted
125	Taray	NC57	Pale yellow	Absent	Long
126	Taray	NC58	Yellow	Absent	Conical–long
127	Taray	NC59	Pale yellow	Pink	Long
128	Taray	NC60	Pale yellow	Dark Purple	Ovoid
129	Paruro	NC61	Yellow	Absent	Conical–long
130	Carhuayo	NC63	Dark Purple	Absent	Slim-twisted
131	Sicuani	NC64	Pale yellow	Dark Purple	Long
132	Cusco	C3018	-x-	-x-	-x-
133	Cusco	C3020	-x-	-x-	-x-
134	Cusco	C3021	-x-	-x-	-x-
135	Cusco	C3022	-x-	-x-	-x-
136	Puno	C3038	-x-	-x-	-x-
137	Puna	C3039	-x-	-x-	-x-
138	Puno	C3040	-x-	-x-	-x-
139	Puno	C3042	-x-	-x-	-x-
140	Puno	C3043	-x-	-x-	-x-
141	Puno	C3044	-x-	-x-	-x-
142	Puno	C3045	-x-	-x-	-x-
143	Cusco	C3050	-x-	-x-	-x-
144	Huancavelica	C3065	-x-	-x-	-x-
145	Huancavelica	C3074	-x-	-x-	x-
146	Huancavelica	C3095	-x-	-x-	-x-
147	Huancavelica	C3096	-x-	-x-	-x-
148	Huancavelica	C3097	-x-	-x-	-x-
149	Huancavelica	C3098	-x-	-x-	-x-
150	Huancavelica	C3099	-x-	-x-	-x-
151	Huancavelica	C3100	-x-	-x-	-x-
152	Huancavelica	C3101	-x-	-x-	-x-
153	Cusco	C3126	-x-	-x-	-x-

accessions from different origin in order to select the most polymorphic.

Data scoring and interpretation

We scored the markers obtained as presence (1) or absence (0) of a polymorphic band to construct similarity indexes. For that purpose we used the Sequential agglomerative hierarchal nested cluster analysis—SAHN (Seneath and Sokal 1973) in the NTSYS-pc package version 2.1 (Rohlf 2000) to construct an Unweighted pair-group method with arithmetic mean (UPGMA) tree matrix of nested clusters based on similarity calculated applying

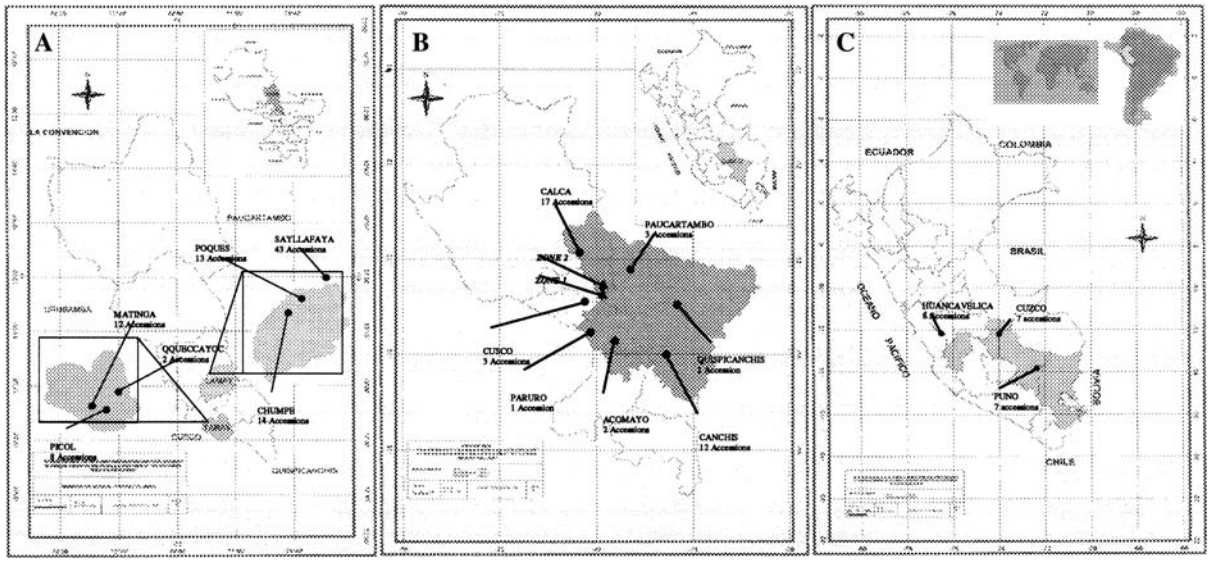

Fig. 1 (**A**) Map of Calca, Cusco, Perú showing the geographical distribution of the mashua cultivated accessions from the villages in Zone 1 and Zone 2. (**B**) Map of Cusco showing the geographical distribution of non-cultivated mashua accessions, and (**C**) map of Perú showing the geographical distribution of mashua accessions from the CIP Collection

the Simple matching (SM) as similarity coefficient of resemblance. We also used for comparison the Jaccard coefficient due to the mixture of dominant and co-dominant markers produced by SRAP (Li and Quiros 2001). The goodness-of-fit of the tree to the data was tested by cophenetic correlation in order to transform the UPGMA matrix into a matrix of ultrametric distances using the COPH module in the NTSYS-pc package. This test is commonly used as an alternative to the bootstrap analysis when NTSYS package is the software selected for the clustering assessment. A Common Principal Component Analysis (CPCA) using the 153 × 153 Similarity Matrix (NTSYS-pc 2.10) was carried on to plot the accessions into a two-dimension coordinate graph according to the eigenanalysis of the covariance matrix. The Arlequin Ver. 2.000 Program (Schneider et al. 2000), an Analysis of Molecular Variance (AMOVA) procedure was applied to estimate the variance components for SRAP phenotypes. Variance was partitioned among individuals (within population), among populations within groups, and among groups. These variance components were tested using 1,000 permutations. For this purpose a Population Structure was placed where communities Picol, Matinga and Qqueccayoc were grouped under the group named as Zone 1, Chumpe, Poques and Sayllafaya in the group named Zone 2, non-cultivated relatives were named as the Non-Cultivated Relatives Group; and the cultivated samples from CIP in the group CIP Collection Group. Finally, a UPGMA dendogram based on 16 morphological traits generated by Duran (2005) was used for a congruency analysis with the molecular dendrogram. The coefficient of similarity used for the morphological dendrogram was the Simple Matching Coefficient (SM) included in the NTSYS-pc package version 2.1 (Rohlf 2000; Duran 2005).

Results and discussion

Primer screening

Eight primer combinations out of the 180 tried were selected on the basis of the number of polymorphisms detected in the samples. These selected primers yielded 120 polymorphic markers, which were easily resolved and reproducible. The number of polymorphic products per primer

combination ranged from 6 (DC1-ODD22) to 26 (DC1-ODD24) with an average of 15 polymorphic fragments per primer (Table 2). Each of these primer combinations was able to generate consistent fingerprint profiles across the eight populations (Picol, Matinga, Qqueccayoc, Chumpe, Poques, Sayllafaya, non-cultivated accessions and CIP Collection). The size distribution of the polymorphic SRAP markers ranged from 61 (primer combination DC1-ODD15) to 556 bp (primer combination SA12-ODD9) with an average of 247 bp.

Data scoring, fragments frequencies and clustering interpretation

The clustering analysis was based on the 120 polymorphic markers (Fig. 2) varying in frequency among populations. The dendrogram obtained from the Simple-matching similarity matrix by the UPGMA analysis grouped the 153 accessions into two major clusters. Cluster 1 included most of the accessions from CIP and cluster 2, which was further divided into 11 sub-clusters, included primarily the cultivated accessions from the six communities in Cusco plus the rest of the accessions from CIP and the non-cultivated accessions (Fig. 3). We did not detect any duplicates in the collections (accessions sharing the same genotype [SRAP profile] but morphotyped as different probably because of the limitations of the visual classification). The similarity among accessions ranged from 62 to 99% (Fig. 3).

Cluster 1: Seventeen accessions from the CIP collection were grouped in this cluster (markers such as E1-OD26,174 D1-OD24,218 and D1-OD24,2O7 were more frequent among the CIP collection; similarly, markers D1-OD24,483; S12-OD8,416; D1-OD22,172; D1-OD22,204; E2-OD17,348; S12-OD8,313; E2-OD17,465; D1-OD24,483 and S12-OD8,202 were absent or at a low frequency, and were also informative to separate CIP accessions from the rest of the mashua samples used in this study). In addition, a clear geographic separation was shown among accessions from Huancavelica which were separated from the ones from Cusco and Puno by the frequency of markers such as: S12-OD5,98; D1-OD24,147; S12-OD9,458; D1-OD15,448 and D1-OD22,258 (more frequent in Huancavelica); and S12-OD5,278; D1-OD24,91; S12-OD5,74; D1-OD15,508; S12-OD9,166; S12-OD9,197; E2-OD17,112; E2-OD17,476 and S12-OD5,250 (more infrequent in Huancavelica). Other markers, for example: S12-OD5,76 (more frequent in Puno); S12-OD5,343 (less frequent in Puno); S12-OD9,288 (absent in Puno and Huancavelica) separated accessions of Puno from the rest of the CIP accessions. In general the range of variation of the cultivars from the communities and the non-cultivated accessions in the Cusco region are not represented in the cultivars of the CIP germplasm collection.

Cluster 2 was the larger cluster and included cultivated accessions from the six communities in Cusco plus the rest of the accessions from CIP

Table 2 List of primer combinations selected for SRAP fingerprinting analysis

Primer combination			Number of polymorphic markers
Forward	Reverse	Sequence	
SA12		5′-TGA GGT GTA AAG AAG GCA TCC-3′	
	ODD5	5′-TCT CGT TGA TAA GGT ACA CA-3′	16
	ODD8	5′-CAC AAG TCG CTG AGA AGG-3′	12
	ODD9	5′-AGT TCC TCA GAC GCT ACC-3′	22
DC1		5′-TAA ACA ATG GCT ACT CAA G-3′	
	ODD15	5′-GCG AGG ATG CTA CTG GTT-3′	7
	ODD22	5′-TAC ACC AGC CAA GGA TGC-3′	6
	ODD24	5′-GAT GCT TCT CGT CCA CAA-3′	26
EM2		5′-GAC TGC GTA CGA ATT CTG C-3′	
	ODD17	5′-GTT AGT ATC AAG GTT AGA GTT-3′	20
EM1		5′-GAC TGC GTA CGA ATT CAA T-3′	
	ODD26	5′-CTA TCT CTC GGG ACC AAA C-3′	11

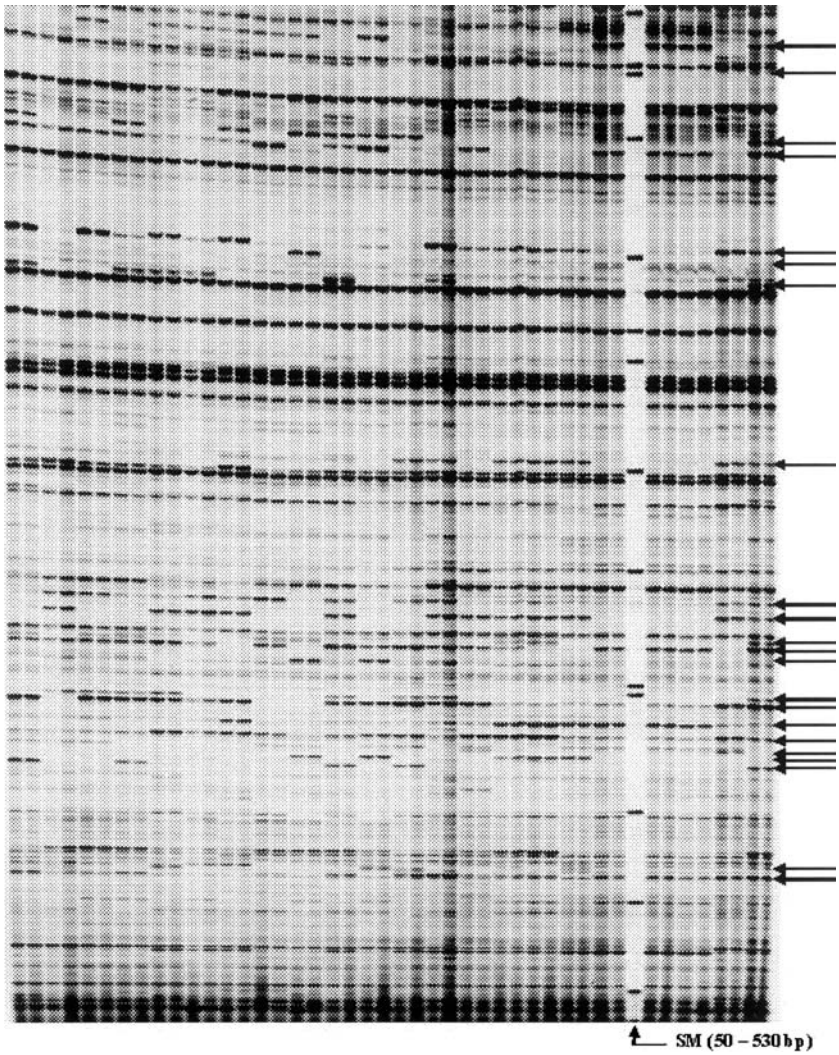

Fig. 2 SRAP amplicons produced by ODD9-SA12 (700) primer combination in 22 mashua accessions (each accession with its repetition to verify the level of reproducibility achieved). Slight differences in the loading of the lanes caused different densities of bands. Bands were therefore scored simply as present or absent. Arrows show 22 polymorphic markers. SM = Size marker

and the non-cultivated accessions. This cluster branched in 11 sub-clusters. Sub-cluster C consisted mostly of accessions from Zone 2 along a few CIP accessions from Cusco. The non-cultivated accession from this cluster originated in Pisac, which is not very far from Zone 2. Around 60% of the accessions present in this cluster are from Sayllafaya, showing a clear differentiation between this community and those at Chumpe and Poques, all three in Zone 2. Until last year, Sayllafaya was fairly isolated from the other two communities since it could be reached only by foot (7 h walk). Between cluster 1 and sub-cluster C fell one cultivated accession (C3018) that did not cluster with any others, and two sub-clusters A and B, each of which includes two non-cultivated accessions that as such are morphologically different from the cultivated samples (Ortega 2000) but not remarkably different from the other non-cultivated accessions. Sub-cluster D included mostly non-cultivated accessions and a few cultivated samples from the three communities in Zone 1. Most of the non-cultivated accessions in this cluster are from Taray—Cusco, not very far Zone 1. The rest of the non-cultivated accessions are mainly from the provinces of Acomayo, Quispicanchis, Marangani and Paucartambo (Cusco). Sub-cluster G included mostly the non-cultivated accessions from Sicuani together with four cultivated accessions from both Zone 1 and

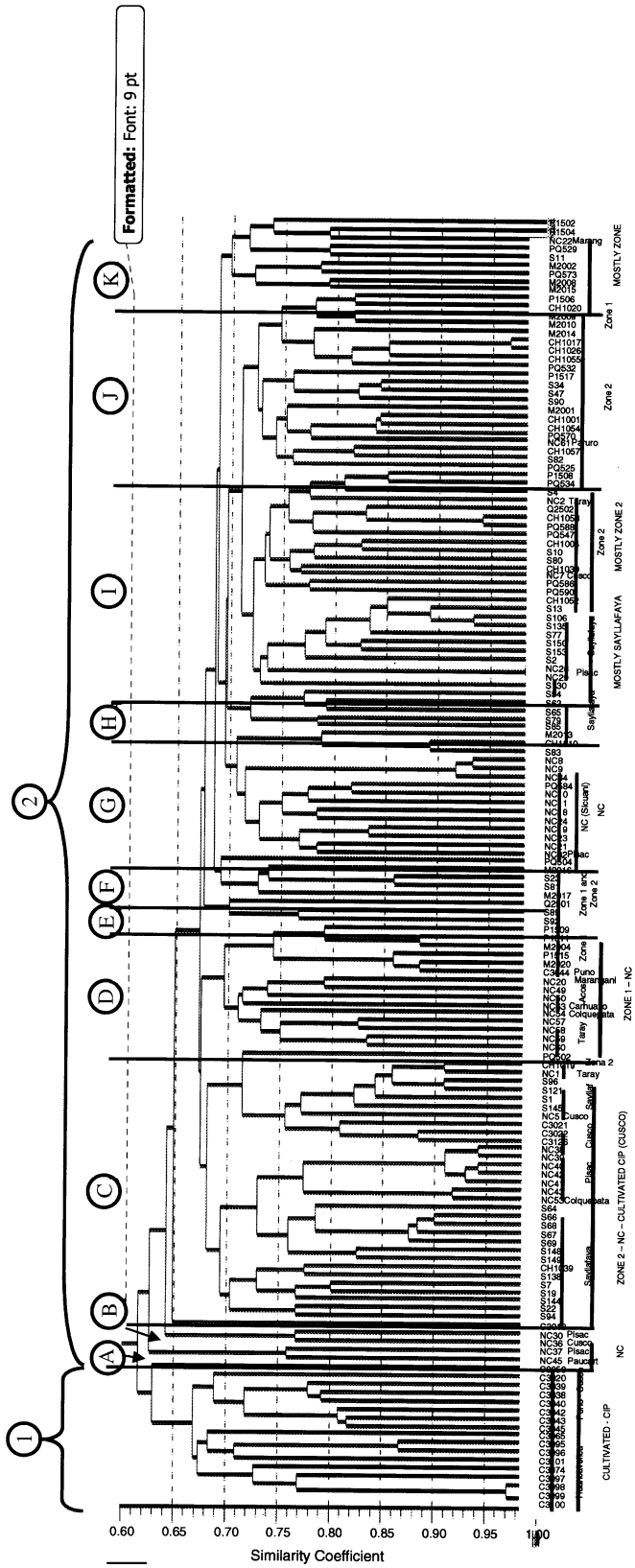

Fig. 3 Dendrogram showing clustering among mashua accessions based on SRAP polymorphic markers. Clusters enumerated from 1 to 7. Name of the sampled material starts with a letter according to its origin followed by accessions number (Table 1). P = Picol, M = Matinga, Q = Qqueccayoc, Ch = Chumpe, Pq = Poques, S = Sayllafaya, NC = Non-cultivated and C = CIP. Scale is similarity based on simple matching coefficient of resemblance

2. This geographical area appears to hold a distinct diversity pool from the rest of non-cultivated accessions and from cultivated accessions grown in Zone 1 and Zone 2. Between sub-clusters D and G fell sub-clusters E and F, including accessions from Zone 1 to 2 without any morphological or geographical separation. Sub-cluster H included five accessions from Sayllafaya; accessions from this community also appeared in sub-clusters C and I. Thus, the widest range of cultivar variability seem to be present in Sayllafaya, which is the most isolated from the rest of communities and from major city markets (91.50 km from Cusco and inadequate roads). Similar to sub-cluster C, the accessions from Sayllafaya in sub-cluster I tended to group together along with some other accessions from Zone 2. The accessions from Poques and Chumpe clustered together. This might be due the trade of some, if not all, accessions cultivated in these two neighboring communities (marker E1-OD26,107 was more frequent in these two communities than the rest of the mashua accessions including their neighbor community Sayllafaya). Thus, this indicates extensive germplasm exchange by farmers of these three communities. This clustering was associated with a high frequency of markers: D1-OD24,288; D1-OD24,207; S12-OD8,357 and D1-OD24,160 in Sayllafaya compared with Chumpe and Poques. These markers are also almost absent in the accessions from Zone 1 which is useful in separating these two geographical regions. Sub-cluster J included mostly a mix of cultivars from all the communities in both zones, which could be explained by seed trading and exchange taking place at the Cusco and Calca fairs and markets. However, the direct exchange between these two zones is quite limited, which can be appreciated by the relative small number of accessions per community in this cluster. Finally, sub-cluster K included accessions from Zone 1 that appear to be the most differentiated from collection held at CIP (lowest similarity = 62%). It shows a similar grouping pattern than sub-cluster D, which includes also accessions from Picol and Matinga, except for the absence of non-cultivated accessions. Additionally, Picol seems to be more differentiated from the rest of the mashua accessions based on the high frequency in Picol of markers D1-OD24,328; D1-OD24,323; D1-OD24,321 and D1-OD24,240; which are almost absent in its neighbors Matinga and Qqueccayoc. As on the previous clusters, there was no common morphological traits relationship shared by the accessions grouped in the same cluster.

From this analysis it is clear that the cultivated and the non-cultivated accessions collected in Taray and Pisac, not far from the cultivated fields of Zone 1 and Zone 2, respectively, have similar genetic backgrounds. These accessions are commonly considered ''wild'' relatives (Sparre and Andersson 1991), in spite of the fact that their ability to tuberize puts them well within the range of genetic variation of the cultivated ones (León 1964). Therefore, in spite their rustic ecological and isolated geographical conditions (Ortega 2000); they are most likely escapes from cultivation which is common for this crop according to León (1964), and hereafter referred to as ''feral''. However, markers E2-OD17,358; D1-OD24,265; E2-OD17,374; D1-OD24,483 and E2-OD17,182; are present almost exclusively in non-cultivated accessions suggesting that they might be related to adaptive traits such as morphological changes in response to the environment or deleterious traits in an agronomic setting, i.e. spindly slim-twisted tubers. The cophenetic correlation between the UPGMA-Tree matrix and the Simple-Matching Similarity Matrix was $r = 0.67244$, indicating that the fit of the data to the resulting tree was low enough to warrant caution on the interpretation. Other coefficients gave similar values: Jaccard's ($r = 0.70386$) and Dice ($r = 0.62598$). Nonetheless, the interpretation described above is supported by the following morphological clustering, principal components and variance analysis.

Clustering based on morphological traits

In the morphological dendrogram (Duran 2005), the most divergent clusters were joined at a similarity value of 38%, whereas in the molecular analysis that value was 62%, indicating that the population appears to be more diverse based on visible traits than genetic ones. All, except for two of the non-cultivated accessions were separated from the cultivated mashuas in the first cluster,

which is not surprising considering that this visual evaluation was based on traits describing these accessions as ''wild'', such as different tuber shape and skin color resulting perhaps from adaptability to adverse conditions. The second cluster is conformed by the cultivated population from the six villages and two non-cultivated accessions which means that the degree of morphological differentiation is not significantly noticeable in these individuals of different populations (data not shown). Also 26 accessions could be considered morphologically as the same genotype, however based on the molecular markers these were similar but not identical.

Principal components analysis

This analysis shows how the CIP collection accessions are separated from the rest of the samples from both the communities and non-cultivated accessions (Fig. 4). Interestingly, the first three principal components explain only the 17% of the accumulated variation. However, using only the two principal components for a two-dimensional plotting of the accessions, there is enough information about the population that allows differentiating the accessions held at CIP, non-cultivated relatives and samples from Sayllafaya, which confirms the clustering analysis by UPGMA. On the other hand, the information is insufficient in order to cluster some non-cultivated accessions with their cultivated relatives, possibly due to an association of these two principal components with morphological traits.

AMOVA analysis

The largest source of diversity comes from within population variation, which accounts for the significant 87.71% of the total variance (P-value < 0.05) (Table 3). This large within-community variation comes mostly from the wide

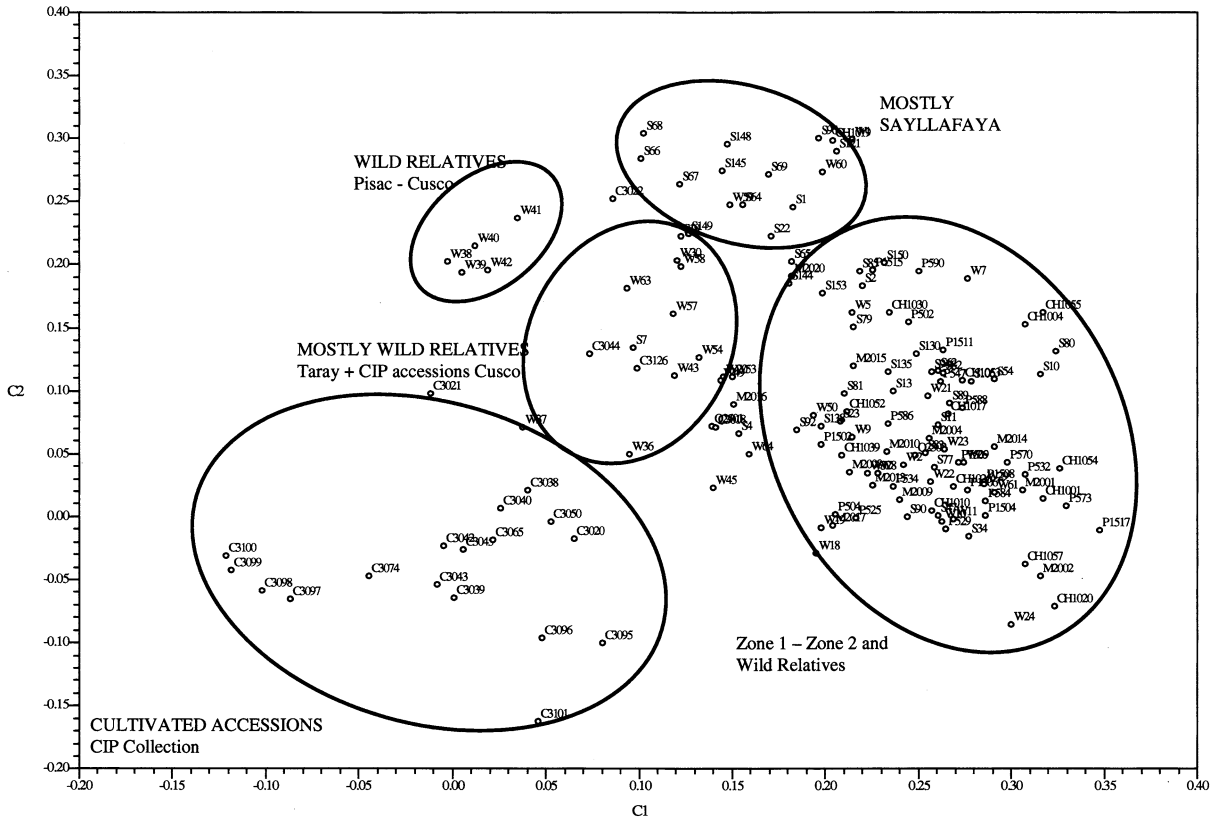

Fig. 4 Two-dimensional clustering of accessions based on the two Principal Components that accounted for just the 17% of the accumulated variation

Table 3 Analysis of molecular variance (AMOVA) for the extraction of components of SRAP variation within population structure. Fixation indices: FST: 0.12288 < 0.1500 moderate divergences in population

Source of variation	df	Sum of squares	Variance components	Percentage of variation
Among groups	3	272.024	1.10763	5.43 NS
Among population within groups	4	142.396	1.39710	6.85**
Within population	145	2592.378	17.87847	87.71**
Total	152	3006.797	20.38320	

Significance level: *$P < 0.05$, **$P < 0.01$, NS = not significant

variation between individual cultivars grown in the same community, especially Sayllafaya which contributed 43 different morphotypes (Table 1). Additionally, the genetic divergence value (FST statistics) = 0.12288 shows a moderated differentiation among all the populations. However, from the FST statistics among populations (Table 4) and from the *P*-values significance analysis (Table 5), it is apparent that there is a considerable and significant divergence (FST > 0.15) between non-cultivated accessions and their cultivated relatives. This is supported by the ''among populations within groups'' variability which, even though it is not particularly high (6.85%) is significant at a 0.05 level of significance (*P*-value < 0.0001).

In general, it seems that all the populations have a moderate divergence between each other. In Zone 1, Qqueccayoc has a moderate to great divergence (FST > 0.05) from the rest of the populations. Moreover, these data might be not reliable because of the reduced number of accessions sampled from this population, but it could also respond to the reduced diversity maintained in that community. In both Picol and Matinga, FST values show a moderate differentiation from the other populations but seem genetically more related to Chumpe and Poques in Zone 2, even though the differentiation is significant. In Zone 2, Chumpe and Poques have a negative non significant (*P*-value > 0.05) FST value = 0.01112 (negative), which shows a great

Table 4 Pairwise differences between populations according to individual FST values (1,023 permutations)

	Picol	Matinga	Qqueccayoc	Chumpe	Poques	Sayllafaya	Feral	CIP
Picol	0.000							
Matinga	0.108**	0.000						
Qqueccayoc	0.171**	0.139**	0.000					
Chumpe	0.079**	0.078**	0.113*	0.000				
Poques	0.036*	0.070**	0.143*	–0.011 NS	0.000			
Sayllafaya	0.110**	0.108**	0.106*	0.079**	0.071**	0.000		
Wild	0.213**	0.190**	0.161*	0.204**	0.179**	0.190**	0.000	
CIP	0.071**	0.106**	0.115*	0.083**	0.049**	0.074**	0.164**	0.000

Significance level: *$P < 0.05$, **$P < 0.01$, NS = not significant

Table 5 Matrix of FST *P*-values (Significance level 0.05 after 1,023 permutations)

	Picol	Matinga	Qqueccayoc	Chumpe	Poques	Sayllafaya	Wild	CIP
Picol								
Matinga	< 0.00001							
Qqueccayoc	< 0.00001	< 0.00001						
Chumpe	0.00098	< 0.00001	0.03809					
Poques	0.02246	< 0.00001	0.01592	0.72461				
Sayllafaya	< 0.00001	< 0.00001	0.02832	< 0.00001	< 0.00001			
Wild	< 0.00001	< 0.00001	0.02832	< 0.00001	< 0.00001	< 0.00001		
CIP	0.00293	< 0.00001	0.02441	< 0.00001	0.00195	< 0.00001	< 0.00001	

genetic similarity between these communities that is correlated to their geographical location. Both are neighbor communities, therefore the results indicate that they share a very similar genetic background. Finally, the collection maintained at CIP is moderately divergent from the other populations, greatly divergent from the non-cultivated population, but more similar to the Chumpe population.

Conclusions

From the molecular marker fingerprinting study based on eight SRAP primer combinations analyzed by clustering, morphological analysis, principal component analysis, and AMOVA, we conclude that the mashua population studied is genetically variable. The population sampled for this study ranged in similarity from 65 to 99%, and a moderate to highly divergent FST value of 0.12288.

The highest level of variation was found in the most isolated community, Sayllafaya, which indicates that the growers there value and maintain the genetic variability of this crop in an effective way. It is in this community that there is the largest number of cultivars that contribute to the highest percent of the total molecular variance. This community could serve as a reservoir of genetic variation by supplying other communities with tuber seed. Efforts for in situ conservation could be easily channeled there, where there is already a culture for maintaining genetic variation of tuber crops as part of their legacy from their antecessors.

Tuber-bearing non-cultivated accessions have been commonly considered as ''wild'' originating from escapes to cultivation (León 1964) and their intermingling with the cultivated accessions in most of the clusters generated in our study supports that hypothesis. However, we cannot exclude the possibility that some of the cultivars originate by selection from these accessions through independent domestications. Genetic diversity might have been increased on the farmers' fields through sexual reproduction and somatic mutations in tuber-seeds (color and shape) generating a rich morphological diversity maintained mostly during generations due asexual multiplication. The different morphological traits in the non-cultivated accessions such as spindly slim-twisted tubers might be the result of their adaptive response to harsh environments.

In general, the range of variation of the cultivars from the communities and their feral relatives are not represented in the cultivars of germplasm collection maintained at CIP. However, there are some cultivated accessions from Cusco in the collection held at CIP that are clustered together with the communities and feral material. This indicates possible gene flow between these accessions, considering that they are quite different from those collected from other regions, such as Huancavelica and Puno.

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