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GENETIC DIVERSITY OF THE ANDEAN TUBER CROP SPECIES *ULLUCUS TUBEROSUS* AS REVEALED BY MOLECULAR (ISSR) AND MORPHOLOGICAL MARKERS

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ABSTRACT. — Genetic diversity of ulluco (*Ullucus tuberosus*), maintained *ex situ* and originating from Peru and North Bolivia, has been analysed using morphological traits and inter-simple sequence repeat (ISSR) markers, along with passport data. With regard to morphological and ISSR variation, our study revealed a high number of morphotypes and genotypes. Morphological and molecular results were congruent and both correlated with geographical distribution. Moreover, comparison of molecular and morphological characterisation of accessions indicated the existence of intra-morphotype heterogeneity. We discuss the genetic structure of ulluco in relation to characteristics of the species and of the Andean agricultural system. Patterns of diversity probably have a geographical origin and have been modified for centuries by Andean farmers, at small and large scales. The fact that both morphological and molecular markers correlate with geographic distribution is of high interest for genetic diversity studies and for genetic resource conservation of Andean tubers and of other vegetatively propagated crop species.

KEY WORDS. — *Ullucus tuberosus*, Andean tuber, genetic diversity, ISSR, molecular markers, morphological markers.

INTRODUCTION

Scientifically neglected and vegetatively propagated crop species are cultivated all around the world in traditional subsistence farming systems, where they are of great importance for feeding millions of people in small communities. They are characterised by their ability to produce genetically identical but independent ramets, which form genets or clones (KLIMEŠ *et al.* 1997). Owing to this propagation mode, a low genetic diversity is expected. However, vegetatively propagated plants are in general not less variable than sexually reproduced ones (MC LELLAN *et al.* 1997) and, as is also observed for sexually reproduced plants, the genetic diversity found within vegetatively propagated species is often threatened with extinction because of the spread of intensive agricultural practices and changes in food habits.

The Andean tuber crop species ulluco (*Ullucus tuberosus* Caldas, Basallaceae) is of particular interest for addressing questions of conser-

vation needs and factors influencing levels of genetic diversity in the case of scientifically neglected and vegetatively propagated crops. Ancient in origin, ulluco originates from the Andean highlands. Representations of ulluco appear on ceremonial vessels of the Robles Moqo style of the Wari culture (between 400 and 700 A.D.) and on Q'ero ceremonial vessels of the post-Incan era. Its cultivation probably extended from Venezuela to northwestern Argentina and northeastern Chile in pre-Hispanic times. The region of ulluco domestication remains unknown (Hernández Bermejo & León 1992). Nowadays, ulluco is cultivated between 3 000 and 4 000 m a.s.l., within an area extending from northern Argentina to Colombia and Venezuela, between 23°S and 9°N. It is used by local people as a valuable food crop, comparable to potato. Ulluco is cultivated for its edible tubers through which it is propagated (Rousi *et al.* 1986). Regeneration from seed is rare (Lempiäinen 1989, Pietilä & Jokela 1990). According to Pietilä (1995), ulluco is an outbreeder. Varieties of ulluco are characterised by considerable morphological variation (Rousi *et al.* 1986, Rousi *et al.* 1989) and can be visually distinguished through characters of aerial parts of the plant and tubers (Arbizu 2004). In Andean farming systems, ulluco varieties are cultivated under a dynamic way of management and are frequently submitted to tuber flow in traditional rural fairs, where exchange, barter, or purchase of tubers are important sources of variation within the species (Terrazas & Valdivia 1998). The vast distribution of ulluco in the Andes and its very ancient cultivation are also reflected by the profusion of varietal names (Hernández Bermejo & León 1992). According to Tapia & Estrella (2001), ulluco is threatened by genetic erosion because of many factors such as drought, changes in food habits, intensive monocultures of introduced crop species, deforestation and human migration from rural to urban areas. In recent decades, a reduction of genetic variability of ulluco has been observed. Conservation programs have to be developed to preserve genetic resources of this valuable crop species.

Defining conservation strategies requires assessment of genotypic and phenotypic diversity, as well as their geographical distribution and the factors that determine whether diversity is maintained or lost. Many efforts are made by national and international centres in Andean countries for *in situ* and *ex situ* conservation of Andean tubers resources, including ulluco (Holle 1999, Garcia & Cadima 2003). Accessions in gene banks are traditionally described using passport data and morphological characters. Lists of descriptors are available for an increasing number of plant species, like ulluco (IPGRI/CIP 2003).

The use of morphological data and other field measurements (passport and ethnobotanical data) has been of great value in crop diversity studies but presents some limitations. The most widely recognised problem is the influence of environmental conditions on morphological variation. Moreover, the level of diversity maintained in gene banks is largely expressed by the number of genotypes conserved, but diversity and heterogeneity of the material remain largely unknown. Nowadays, molecular markers can be applied to assist germplasm characterisation and to identify redundancy or intra-morphotype variation, a morphotype being defined as a set of accessions with identical morphological characters. Furthermore, molecular techniques are usually unaffected by environmental variations (Smith & Smith 1992). Among them, the inter-simple sequence repeat (ISSR) technique developed by Zietkiewicz *et al.* (1994) uses anchored arbitrary primers to amplify the intermicrosatellite region of DNA, yielding dominant markers. Compared with other PCR-based techniques, ISSR is very reproducible and generates large numbers of polymorphisms per primer (Bornet & Branchard 2001). ISSR has been successfully used to reveal polymorphism in various crops (Pradeep Reddy *et al.* 2002), including oca (*Oxalis tuberosa* Mol.), another Andean tuber crop species (Pissard *et al.* 2006, Malice *et al.* 2007).

In the present study we used ISSR in concert with morphological descriptions to screen a large number of ulluco accessions maintained in an *ex situ* collection. Considering the need to study genetic diversity of ulluco, and to define conservation strategies for this neglected crop species threatened with erosion (Tapia & Estrella

Fig. 1. Geographic distribution of 187 ulluco accessions of the ARTC collection.

2001), this work is aimed at examining the molecular and morphological diversity of ulluco, and testing the correlation between both markers and the geographical origin of accessions.

MATERIAL AND METHODS

Plant material

The samples consisted of 187 accessions of ulluco (Table 1), obtained from the Andean root and tuber crops (ARTC) collection maintained *ex situ* by the International Potato Centre (CIP, Lima, Peru). Accessions were collected in representative regions of ARTC germplasm collection and follow a longitudinal and latitudinal gradient from North Peru to North Bolivia (Table 2; Fig. 1).

Morphological characterisation

The ulluco field collection is maintained by the CIP in the rural community of La Liberdad (3 700 m a.s.l.), Junin, Peru. Morphological data were recorded from 2000 to 2005 using standard descriptor lists (IPGRI/CIP 2003). A set of 21 qualitative variables, scored as categorical characters, was used to describe the 187 accessions morphologically. The descriptors, with the number of descriptor states within parentheses, are: predominant tuber surface colour (12), secondary tuber surface colour (4), distribution of secondary

Table 1. List of the 187 ulluco accessions used for ISSR analysis, with the morphotype they belong to.

Morphotype	Accessions	Morphotype	Accessions
M4	CIP 201323, CIP 201364, CIP 201330,	M32	CIP 201012
	CIP 201319, CIP 201134, CIP 201316,	M33	CIP 201013
	CIP 201321, CIP 201352, CIP 201360,	M34	CIP 201014, CIP 201024
	CIP 201322, CIP 201328, CIP 201353,	M36	CIP 201022
	CIP 201317, CIP 201001, CIP 201315,	M37	CIP 201007
	CIP 201361, CIP 201324, CIP 201318	M38	CIP 201009
M5	CIP 201002	M39	CIP 201010
M6	CIP 201003	M40	CIP 201062
M7	CIP 201005	M41	CIP 201063
M8	CIP 201443	M42	CIP 201064
M9	CIP 201033, CIP 201049, CIP 201373,	M44	CIP 201294
	CIP 201035, CIP 201444	M45	CIP 201112, CIP 201066, CIP 201111
M11	CIP 201008, CIP 201441, CIP 201446	M46	CIP 201067
M12	CIP 201025	M47	CIP 201072
M13	CIP 201026	M48	CIP 201074, CIP 201073
M14	CIP 201387, CIP 201054, CIP 201029	M49	CIP 201432, CIP 201461, CIP 201472
M16	CIP 201021, CIP 201019, CIP 201015,	M50	CIP 201433
	CIP 201383, CIP 201011, CIP 201436	M51	CIP 201434
M17	CIP 201057, CIP 201386, CIP 201017	M53	CIP 201310
M18	CIP 201051	M54	CIP 201311
M19	CIP 201052	M55	CIP 201312
M20	CIP 201381, CIP 201053	M56	CIP 201329
M21	CIP 201058	M57	CIP 201331
M22	CIP 201430, CIP 201428, CIP 201429,	M58	CIP 201121, CIP 201332, CIP 201123
	CIP 201459, CIP 201467, CIP 201468	M64	CIP 201420, CIP 201379, CIP 201388
M23	CIP 201427	M65	CIP 201365
M24	CIP 201334, CIP 201431, CIP 201439,	M67	CIP 201466, CIP 201451, CIP 201460
	CIP 201437	M68	CIP 201452
M25	CIP 201023, CIP 201034	M69	CIP 201279, CIP 201453, CIP 201473,
M26	CIP 201016, CIP 201377, CIP 201394,		CIP 201478, CIP 201521
	CIP 201400, CIP 201036	M70	CIP 201454
M27	CIP 201359, CIP 201038, CIP 201362,	M71	CIP 201455
	CIP 201355	M72	CIP 201391
M28	CIP 201041	M73	CIP 201458
M29	CIP 201449, CIP 201438	M74	CIP 201475
M30	CIP 201440	M75	CIP 201462, CIP 201476
M31	CIP 201384, CIP 201407, CIP 201442	M76	CIP 201463

Morphotype	Accessions	Morphotype	Accessions
M77	CIP 201464	M130	CIP 201389
M78	CIP 201465	M131	CIP 201396
M79	CIP 201403, CIP 201280, CIP 201390,	M134	CIP 201397
	CIP 201469, CIP 201471	M135	CIP 201398
M80	CIP 201470	M137	CIP 201283
M82	CIP 201477	M138	CIP 201401
M87	CIP 201411	M139	CIP 201402
M99	CIP 201115	M140	CIP 201404
M100	CIP 201116	M141	CIP 201405
M101	CIP 201120	M144	CIP 201406
M102	CIP 201122	M145	CIP 201408
M109	CIP 201147	M146	CIP 201409
M110	CIP 201148	M147	CIP 201410
M111	CIP 201149	M148	CIP 201419, CIP 201415, CIP 201412
M112	CIP 201150	M149	CIP 201414, CIP 201413
M113	CIP 201418, CIP 201153	M150	CIP 201417, CIP 201422
M120	CIP 201448	M152	CIP 201421
M122	CIP 201075	M154	CIP 201285
M124	CIP 201277	M155	CIP 201293
M125	CIP 201375	M156	CIP 201289
M126	CIP 201376	M157	CIP 201292
M127	CIP 201380	M159	CIP 201416
M128	CIP 201393, CIP 201382	M162	CIP 201378
M129	CIP 201385		

tuber surface colour (4), tendency to show chimeras (2), tuber shape (4), cortex colour (8), central cylinder colour (5), plant habit (2), stem elongation (4), stem colour (4), stem wing pigmentation (2), leaf shape (4), foliage colour (3), abaxial leaf colour (3), petiole colour (4), flowering habit (4), shape of the inflorescence axis (2), colour of the inflorescence axis (3), sepals colour (3), tepals colour (4) and flower tendency to show more than five petals (2). Characterisation allowed the definition of morphotypes, which are defined as groups of accessions originally collected from different geographical areas, sharing identical morphological characters but not necessarily the same genetic structure.

Multiple correspondence analysis (MCA) and calculation of Gower's distance between accessions were performed using SAS 9.1 (SAS Institute Inc.). The correlation between morphological and geographical Euclidean distance matrices was assessed with the Mantel test using PASSAGE ver. 1.1 (ROSENBERG 2001).

MOLECULAR CHARACTERISATION

Genomic DNA was isolated (DELLAPORTA *et al.* 1983) from fresh leaf material collected in 2003. Sixteen primers, taken from the literature (PREVOST & WILKINSON 1999, JOSHI *et al.* 2000, MCGREGOR *et al.* 2000), were tested for PCR amplification. Annealing temperature was optimised for each primer using the gradient temperature option of the thermal cycler. Ten of the 16 tested primers, which gave clear polymorphisms and reproducible banding patterns, were selected to assess genetic variability of the accessions (Table 3). ISSR amplifications were performed in 25 µL containing 5 ng DNA, 1× buffer (New England

Table 2. Number of accessions according to country and department where they were collected.

Country	Department	Number of accessions
Peru	Amazonas	4
Peru	Cajamarca	36
Peru	Piura	14
Peru	Ancash	26
Peru	La Libertad	12
Peru	Junín	10
Peru	Lima	5
Peru	Pasco	29
Peru	Apurimac	1
Peru	Ayacucho	2
Peru	Cusco	29
Peru	Puno	11
Bolivia	La Paz	5
Bolivia	Oruro	2
Bolivia	Potosi	1
Total		187

BioLabs), 2.5 mM $MgCl_2$, 400 µM dNTPs, 0.25 µM primer, 0.2 µg μL^{-1} BSA, and 1.4 U *Taq* polymerase. The thermal cycler (PTC-200, MJ Research Inc.) was programmed for an initial denaturation step of 1 min at 94°C followed by 35 cycles of 1 min at 94°C, 1 min at optimised annealing temperature (Table 3) and 4 min at 72°C, and a final extension step of 7 min at 72°C. Amplification products were separated on 1.8% TAE agarose gels, stained with ethidium bromide and visualised under UV light. Sixty accessions (30% of all) were reamplified with each of the selected primers to test reproducibility of the entire banding pattern. Clear, unambiguous and reproducible ISSR markers were scored (1 for presence, 0 for absence). Principal component analysis (PCA) was performed using SAS ver. 9.1 (SAS Institute Inc.). Jaccard's distances between accessions were calculated using TREECON (VAN DE PEER & DE WACHTER 1994). Spatial genetic structure was analysed with autocorrelation analysis using SPAGeDI ver. 1.2 (HARDY & VEKEMANS 2002). To calculate kinship coefficients between accessions, inbreeding was estimated using HICKORY ver. 1.0 (HOLSINGER & LEWIS 2003). The correlation between molecular and geographical Euclidean distance matrices was assessed with the Mantel test using PASSAGE ver 1.1 (ROSENBERG 2001).

CONGRUENCE BETWEEN MORPHOLOGICAL AND ISSR MARKERS

A Mantel test was performed to analyse the correlation between molecular and morphological distance matrices, calculated from Euclidean distance between the 187 accessions, using PASSAGE ver. 1.1 (ROSENBERG 2001).

The majority of morphotypes are represented in ARTC collection by one to three accessions (Table 1), which complicates the analysis of congruence between morphological and molecular data and the identification of intra-morphotype variability. In order to infer robust relationships between morphotypes and genotypes in ulluco, a cluster analysis was performed on morphotypes represented by four or more accessions. Cluster analysis was performed using the UPGMA algorithm (unweighted pair-group method) and Jaccard's distance using TREECON (VAN DE PEER & DE WACHTER 1994).

RESULTS

MORPHOLOGICAL AND MOLECULAR DIVERSITY

Analysis of morphological data divided the 187 accessions into 108 morphotypes (42.2%

Table 3. Selected ISSR primers and number of amplified fragments obtained from DNA amplification of ulluco.

Primer	Sequence 5' → 3'	Reference	Annealing temperature	Amplified fragments	
				Reproducible	Polymorphic
1	BDB-$(ACA)_5$	Mc Gregor *et al.* 2000	46.0°C	12	5
2	DD-$(CCA)_5$	Mc Gregor *et al.* 2000	54.3°C	12	4
3	VHV-$(GT)_7$	Mc Gregor *et al.* 2000	53.5°C	8	4
4	DBD-$(AC)_7$	Mc Gregor *et al.* 2000	47.4°C	14	5
5	BDB-$(CAC)_5$	Mc Gregor *et al.* 2000	55.4°C	5	1
6	$(AG)_8$-YT	Prevost & Wilkinson 1999	47.4°C	6	5
7	$(AC)_8$–G	Prevost & Wilkinson 1999	43.9°C	7	2
8	$(AG)_8$-C	Joshi *et al.* 2000	47.9°C	13	8
9	$(GA)_8$-C	Joshi *et al.* 2000	49.4°C	9	6
10	$(GA)_8$-T	Joshi *et al.* 2000	47.4°C	8	4
Total				94	44
Mean polymorphism					46.8%

Note. B = G, T or C; D = G, A or T; H = A, T or C; Y = C or T.

redundancy; Table 1). As described in the literature (Rousi *et al.* 1986, Rousi *et al.* 1989), our results confirmed the large spectrum of tuber colours and shades found in *Ullucus tuberosus* and the high degree of phenotypic diversity (Table 4). With the exception of plant habit and abaxial leaf colour, morphological characters showed variation between morphotypes. Morphological distance within morphotype was evidently 0, as all accessions belonging to a morphotype showed identical morphological characters. The average morphological distance between morphotypes was 0.294 ± 0.102, with distance ranging from 0.004 to 0.724.

ISSR analysis performed with 10 primers revealed 94 reproducible fragments (Table 3). From these, 44 were polymorphic, which corresponds to a mean polymorphism of 46.8%. Molecular markers detected 184 genotypes out of 187 accessions (1.6% redundancy). The average genetic distance between accessions was 0.192 ± 0.048, with a range from 0 to 0.333.

Geographical distribution of diversity

Accessions are projected in an MCA plot (Fig. 2), with PC1 and PC2 accounting for 10.15% and 7.99% of total variance, respectively. This analysis failed to identify clear groups and underlined the continuous distribution of morphological variability. The Mantel test indicated a low but significant correlation between morphological and geographic data ($r = 0.153$, $P < 0.001$).

Principal component analysis (PCA) of ISSR data (Fig. 3) supported the morphological analysis. The position of accessions was defined by the first two principal coordinates PC1 and PC2, which explained 13.44% and 7.96% of the total variation, respectively. From the PCA analysis, neither discontinuity nor clear clustering appeared between locations of collections. A low but significant correlation was observed between genetic and geographic data using the Mantel test ($r = 0.192$, $P < 0.001$). Spatial autocorrelation statistics showed a strong decline in Moran's *I* values, with positive values from distance classes 1 to 4 and negative values above distance class 4 (Fig. 4). Positive values found at short distances mean that neighbouring accessions have a higher genetic relatedness than random pairs of accessions, whereas negative values occurring at larger distances indicate an "isolation-by-distance" phenomenon within the studied collection. The

Table 4. Proportions (%) of accessions falling into the different categories of morphological characters (IPGRI/CIP 2003).

Predominant tuber surface colour											
1	2	3	4	5	6	7	8	9	10	11	12
5.3	17.1	7.0	5.3	0.5	29.4	0.0	15.0	4.8	5.3	0.5	9.6
Secondary tuber surface colour											
1	2	3	4								
63.6	1.6	9.6	25.1								
Distribution of secondary tuber surface colour											
1	2	3	4								
63.6	17.6	0.5	18.2								
Tendency to show chimeras											
1	2										
69.0	31.0										
Tuber shape											
1	2	3	4								
55.6	18.2	23.5	2.7								
Cortex colour											
1	2	3	4	5	6	7	8				
5.3	17.6	51.9	7.0	3.7	4.8	0.5	9.1				
Central cylinder colour											
1	2	3	4	5							
9.1	43.3	3.7	43.9	0.0							
Plant habit											
1	2										
100.0	0.0										
Stem elongation											
1	2	3	4								
79.1	19.3	1.6	0.0								
Stem colour											
1	2	3	4								
94.7	0.9	2.3	2.1								
Stem wing pigmentation											
1	2										
46.5	53.5										

Leaf shape			
1	2	3	4
0.5	96.8	1.6	1.1
Foliage colour			
1	2	3	
22.5	74.3	3.2	
Abaxial leaf colour			
1	2	3	
100.0	0.0	0.0	
Petiole colour			
1	2	3	4
57.2	41.7	1.1	0.0
Flowering habit			
1	2	3	4
0.0	13.4	73.3	13.4
Shape of the inflorescence axis			
1	2		
20.9	79.1		
Colour of the inflorescence axis			
1	2	3	
84.5	15.5	0.0	
Sepals colour			
1	2	3	
34.8	32.6	32.6	
Tepals colour			
1	2	3	4
61.0	34.2	4.8	0.0
Flower tendency to show more than five petals			
1	2		
96.3	3.7		

inbreeding coefficient was estimated as 0.10. The estimation of kinship coefficients, however, is robust to errors made on the assumed inbreeding level (HARDY 2003), and additional analyses with variable levels of assumed inbreeding produced almost identical autocorrelograms (data not shown).

CONGRUENCE BETWEEN MORPHOLOGICAL AND MOLECULAR MARKERS, AND INTRA-MORPHOTYPE VARIABILITY

The congruence between morphological and molecular descriptions of accessions was analysed using the Mantel test. We found a significant

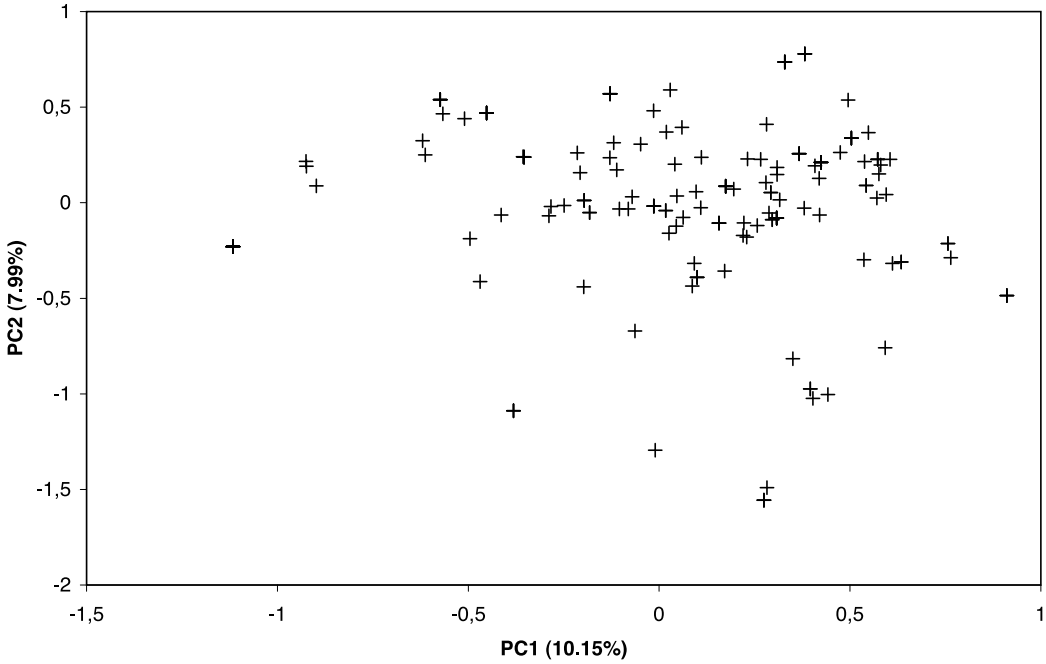

Fig. 2. MCA plot based on morphological characterisation.

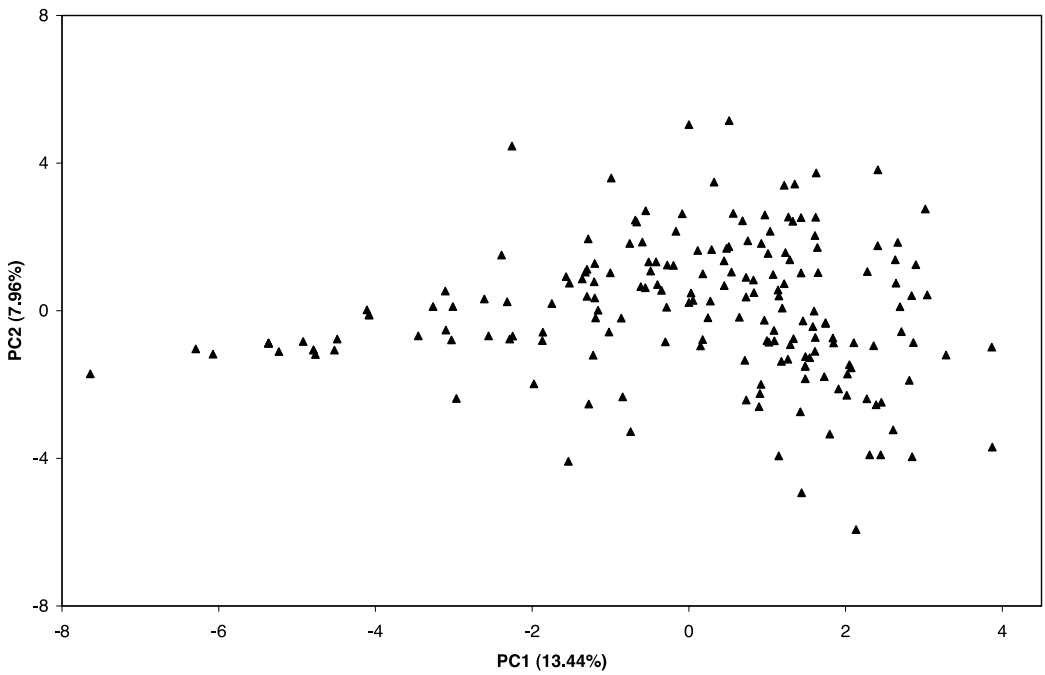

Fig. 3. PCA plot based on molecular (ISSR) characterisation.

positive relationship between morphological and genetic distance matrices ($r = 0.293$, $P < 0.001$).

The UPGMA cluster analysis using Jaccard's genetic distance (Fig. 5) took into account 57 accessions (nine morphotypes, each represented by four or more accessions). Accessions are identified by their initial number (CIP201xxx), country of origin (PER), department where they were collected, and morphotype (Mxx).

ISSR markers indicated intra-morphotype variability. Accessions belonging to the same morphotype showed higher similarity and usually clustered close to each other, compared to accessions belonging to different morphotypes. All morphotypes but two (M27 and M79) showed one or two accessions that clustered independently from the others. The mean genetic distance between accessions was 0.171 ± 0.063, with a range from 0 to 0.289. The average morphological distance between the nine morphotypes was 0.373 ± 0.097 with distance ranging from 0.167 to 0.523, while the average genetic distance within morphotypes was 0.115 ± 0.026, with distance ranging from 0.061 (morphotype M4) to 0.150 (morphotype M79).

DISCUSSION

Ulluco diversity has been studied before, using morphological data, but never extensively for large germplasm collection fingerprinting. Studies using molecular techniques are relatively scarce. In this work we aimed at performing a genetic diversity analysis of 187 accessions maintained *ex situ* in the ARTC collection, using ISSR molecular markers in concert with morphological and passport data.

Mean distances, taken as indicators of diversity, appear quite low (0.294 and 0.192 for morphological and molecular markers, respectively). This observation is probably linked to the vegetative propagation system of ulluco. Similar values of mean genetic distance have been found, for instance, by PISSARD *et al.* (2006) in oca. In contrast, our results confirm the high morphological variation of the ulluco crop, and reveal a high clonal diversity based on ISSRs (high number of genotypes). These findings are rather astonishing, since in a vegetatively propagating plant species, genetic diversity is not increased by sexual reproduction and a decrease

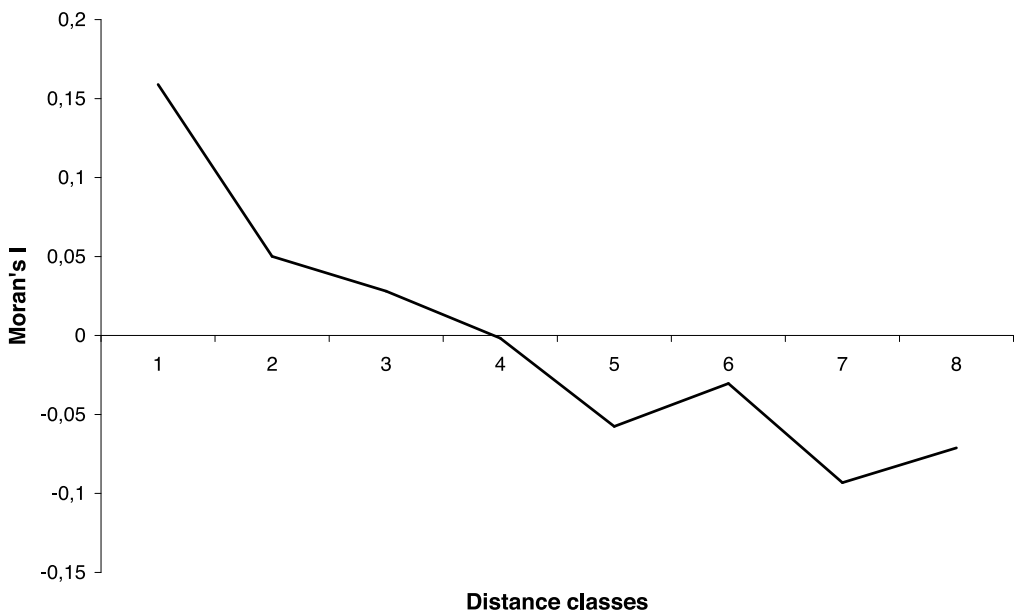

Fig. 4. Correlogram of Moran's *I* per distance class obtained from ISSR data.

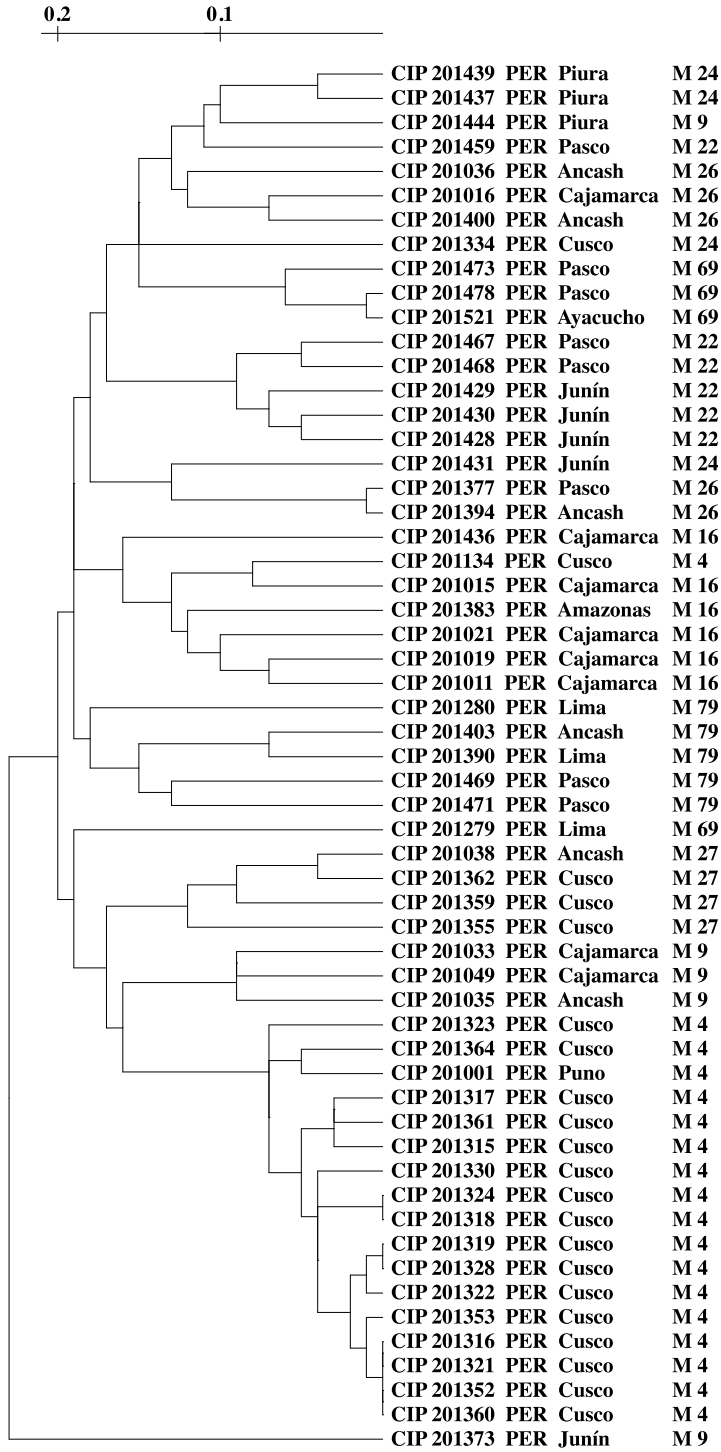

Fig. 5. Dendrogram based on ISSR polymorphism and Jaccard's distance. The only nine morphotypes represented by four or more accessions are taken into account.

of genetic diversity within populations is expected over time, as a result of drift or selection (AUGE *et al.* 2001). Despite its long history of asexual propagation it is possible that ulluco has maintained variation from past sexual processes (PIETILÄ & JOKELA 1990). Although sexual reproduction is uncommon in ulluco today, it may have been the dominating breeding system in the past. Diversity detected nowadays would then be largely relictual.

Andean farmers recognise all their varieties on the basis of morphological characters and vernacular names. They maintain genetic diversity, replanting their varieties from year to year. In addition to centuries of clonal propagation, somatic mutations may have occurred periodically and have increased diversity. New forms, selected by farmers, were fixed by vegetative propagation. In addition to somatic mutations and past sexual events, at least four features of Andean agricultural system can be considered as key elements in creating and conserving diversity in vegetatively propagated crop species.

Firstly, diversity in ulluco can be explained by the large ecological and geographic distribution of the varieties in the Andes, which requires adaptation of plant material to specific climatic and edaphic conditions. Additionally, Andean history and the farmers themselves could also have played an important role in generating diversity now encountered in ulluco. Because of the wide array of climatic and edaphic conditions found in the Andes, farmers selected and conserved the highest number of varieties possible. All of these, each with its particular traits, are cultivated in a large number of microenvironments, in small plots located at different altitudes. This type of farming, with vertical control of ecological zones, represents for the farmers an insurance against crop failure.

Secondly, seeds of ulluco are viable: they can germinate in the laboratory but also probably in the field (LEMPIÄINEN 1989). Concomitantly with the conservation of existing varieties through the traditional vegetative multiplication, new forms originating from seedlings can be conserved, unconsciously or not, by Andean farmers. This situation, i.e. the introduction of plants originating from seeds in vegetatively propagated varieties, has been noticed in crops such as cassava (ELIAS *et al.* 2001) and potato (BRUSH *et al.* 1981, QUIROS *et al.* 1992). This unmanaged sexual reproduction contributes to an increased diversity. ORTEGA (1997) showed that, in potato, vegetative propagation and outcrossing provide stability and variability, respectively, and lead to an increase in genetic diversity after very long periods of time. For ulluco, the importance of seedlings in field conditions is not well known, but the species has been shown to produce viable seeds (LEMPIÄINEN 1989). The hypothesis that incorporation of seed-derived tubers could act as a source of genetic variation in ulluco cannot be completely eliminated but should be confirmed by field studies.

Thirdly, cultivation of several varieties of ulluco in a single field is a normal practice in the Andes. Planting heterogeneous plant material in the same field, including different varieties and species, is a traditional crop husbandry that increases the chance to harvest at least some plants in case of biotic or abiotic constraints.

Finally, tuber flow is a form of germplasm migration and an important mechanism through which diversity appears in Andean agricultural systems. Each year, farmers decide on which varieties and what amounts they want to cultivate. In addition to the selected and stored tubers of their own cropping system, they obtain new tubers from other farmers and from markets (ESPINOZA 2001). The agrobiodiversity fairs favour the exchange of varieties between the participating farmers (of a particular community or of various communities), making the dispersion of a high genetic diversity possible. Tuber transportation to markets gathers varieties from different regions.

Comparative analyses showed congruence between molecular and morphological variation, but also pointed out variability within morphotypes. Such variability has also been observed in another Andean tuber crop, oca (MALICE *et al.* 2007), and has been attributed to mutations or to confusion between morphologically similar but genetically distinct individuals. Mixed cropping and tuber flow represent situations that favour intra-morphotype variability.

Multivariate analyses (MCA and PCA) showed no clear separation between accessions collected from different locations. In fact, in Andean agrosystems, ulluco is a staple food and is cultivated by the great majority of farmers from 3 000 to 4 000 m a.s.l. Hence, it is probable that farmers have selected similar varieties, based on morphological and agronomical traits, as well as culinary, medicinal and commercial properties. However, our results revealed a geographical influence on the diversity pattern. Generally, accessions geographically closer are more genetically similar. A similar correlation, though higher, was also observed in oca (Pissard *et al.* 2006). For ulluco, the geographical distribution of varieties is not yet known, but different geographical groups of accessions have been recorded for chromosome numbers (Cardenas & Hawkes 1948), morphological characters (Rousi *et al.* 1989) and seed set (Pietilä & Jokela 1990). In the Andean highlands, where ulluco is commonly cultivated, the social status of a farmer is notably determined by the number of varieties he owns and grows in his fields (Ortega 1997). The diversity found at a small scale (in a farmer's field, a community or a department) is thus expected to be high. In the Andean area, farmers of different zones exchange genetic material and acquire tubers sometimes from distant places (Espinoza 2001). Combined with the high number of varieties owned by each farmer, these practices could explain our results indicating higher and lower genetic differentiation at small and large scales, respectively.

From a conservation viewpoint, our findings raise important questions about genetic resources management of vegetatively propagated crops like ulluco. Genetic diversity of ulluco maintained *in situ* is expressed as the number of varieties, identified by morphological and agronomic characters. In *ex situ* conservation systems, diversity is usually represented by the number of accessions or the number of morphotypes. The main goal of *ex situ* collections is to maintain the genetic diversity of the crop during a very long time, without modifications and with minimal redundancies. However, many germplasm collections contain redundant accessions, which are usually identified through passport data and morphological characterisation. As PCR-based techniques are becoming more available, molecular markers are increasingly used to identify redundant material (Dean *et al.* 1999, van Treuren *et al.* 2004). In our experiment, we found 41.7% and 1.6% redundancy for morphological and molecular data, respectively. However, considering an accession as redundant is often all but easy, and the question raised by curators is not to ascertain identity between two accessions but rather to establish whether they are sufficiently different to be considered as distinct (Le Clerc *et al.* 2005). More in-depth investigations to check duplicates should be encouraged in ulluco. Moreover, as intra-morphotype variability has been shown for most of them, more knowledge is needed of the congruence between morphological and molecular data. If it does not allow a reduction in germplasm collection size, at least it could help to improve management procedures.

In summary, our results showed that diversity in ulluco is rather found at small scales. Genetic structure of ulluco can be explained by the characteristics of *Ullucus tuberosus* and the Andean agricultural system: (1) high clonal diversity is potentially due to past sexual reproduction and has been maintained for centuries by Andean farmers; (2) in the Andes, at altitudes between 3 000 and 4 000 m a.s.l., ulluco is a staple food cultivated by the majority of farmers who selected similar varieties; (3) diversity originated from geographical differentiation (presence of indigenous germplasm), (4) diversity has later been modified by cultural practices, at small scale (centres of diversity) but also at larger scale (biodiversity fairs). In conclusion, these findings are essential to improve genetic resources conservation of ulluco, as well as other vegetatively propagated crop species.

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