COMMUNAUTE FRANCAISE DE BELGIQUE
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Genetic diversity and structure of three Andean tubers: *Oxalis tuberosa* Molina, *Ullucus tuberosus* Caldas and *Tropaeolum tuberosum* Ruiz & Pav.

Marie MALICE

Dissertation originale présentée en vue de l'obtention du grade
de docteur en sciences agronomiques et ingénierie biologique

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Malice Marie (2009). Genetic diversity and structure of three Andean tubers: *Oxalis tuberosa* Molina*, Ullucus tuberosus* Caldas and *Tropaeolum tuberosum* Ruiz & Pav. (Ph.D. Thesis). Belgium, Gembloux Agricultural University, 185 p.

Summary: Andean tubers oca (*Oxalis tuberosa* Molina), ulluco (*Ullucus tuberosus* Caldas) and mashua (*Tropaeolum tuberosum* Ruiz & Pav.) are cultivated tuber-bearing species originating from the high Andes, where they play a major role at the food, agronomic, cultural and economic levels. Genetic diversity within these species is very large but is threatened with genetic erosion.

In this context, we studied samples of oca, ulluco and mashua, maintained in *in situ* and *ex situ* conservation systems in Peru and Bolivia, with the objective to contribute to the effective conservation (*in situ* and *ex situ*) of these neglected species. This study combined Andean indigenous knowledge, agronomic, morphological and molecular data.

We showed that Andean agriculture preserves high diversity, at the inter-specific level but also at the intra-specific one, in terms of number of local varieties. We also pointed out the presence of heterogeneous varieties, the congruence between molecular and morphological data, and a genetic structure influenced by the geographical provenance.

Finally, we summarized our observations in a recapitulative model. We enlightened the importance of intrinsic characteristics of the species (reproductive characteristics) as well as specificities of the Andean agricultural system (sociocultural, economic and environmental factors). This study has contributed significantly to the understanding of the genetic diversity and structure of Andean tubers.

Malice Marie (2009). Diversité génétique et structure de trois tubercules andins: *Oxalis tuberosa* Molina*, Ullucus tuberosus* Caldas and *Tropaeolum tuberosum* Ruiz & Pav. (thèse de doctorat). Belgique, Gembloux, Faculté Universitaire des Sciences Agronomiques, 185 p.

Résumé: Les tubercules andins oca (*Oxalis tuberosa* Molina), ulluco (*Ullucus tuberosus* Caldas) et mashua (*Tropaeolum tuberosum* Ruiz & Pav.) sont des espèces tubéreuses cultivées originaires des régions hautes des Andes, où elles revêtent une importance particulière aux niveaux alimentaire, agronomique, culturel et économique. La diversité génétique au sein de ces espèces est très grande, mais est menacée d'érosion génétique. Dans ce contexte, notre étude s'est basée sur des échantillons de oca, ulluco et mashua, maintenus dans des système de conservation *in situ* et *ex situ* au Pérou et en Bolivie, dans l'objectif de contribuer à la conservation efficace (*in situ* et *ex situ*) de ces espèces négligées. Cette étude a combiné les connaissances autochtones andines, ainsi que des données agronomiques, morphologiques et moléculaires. Nous avons montré que l'agriculture andine conserve une grande diversité au niveau inter-spécifique, mais aussi au niveau intra-spécifique, en terme de nombre de variétés locales. Nous avons également mis en évidence de la présence de variétés hétérogènes, la congruence entre les données moléculaires et morphologiques, et une structure génétique influencée par la provenance géographique. Enfin, nous avons compilé l'ensemble de nos résultats dans un modèle récapitulatif. Nous avons montré l'importance des caractéristiques intrinsèques de l'espèce (mode de reproduction), ainsi que les spécificités du système agricole andin (socioculturels, économiques et environnementales). Cette étude a contribué de manière significative à la compréhension de la diversité génétique et de la structure des tubercules andins.

Malice Marie (2009). Diversidad genética y estrucutura de tres tubérculos andinos *Oxalis tuberosa* Molina, *Ullucus tuberosus* Caldas y *Tropaeolum tuberosum* Ruiz & Pav. (Ph.D. Thesis). Belgium, Gembloux Agricultural University, 185 p.

Resumen: Los tubérculos andinos oca (*Oxalis tuberosa* Molina), ulluco (*Ullucus tuberosus* Caldas) y mashua (*Tropaeolum tuberosum* Ruiz & Pav.) son especies tuberosas cultivadas originarias de las regiones altas de los andes, donde revisten una importancia particular a niveles alimentario, agronómico, cultural y agronómico. La diversidad genética de estas especies es muy grande, pero está amenazada por la erosión genética. En este contexto y con el objetivo de contribuir a la conservación eficaz (*in situ* y *ex situ*) de estas especies olvidadas, nuestro estudio está basado en muestras de oca, ulluco y mashua, mantenidas bajo los sistemas de conservación *in situ* y *ex situ* en el Perú y en Bolivia. Este estudio ha combinado los conocimientos autóctonos de los Andes, así como con datos agronómicos, morfológicos y moleculares. Hemos mostrado que la agricultura andina conserva una gran diversidad a nivel inter-específico, pero también a nivel intra-específico, en términos de número de variedades locales. De la misma manera, hemos puesto en evidencia la presencia de variedades heterogéneas, la congruencia entre los datos moleculares y morfológicos, y una estructura genética influenciada por la precedencia geográfica. Finalmente, hemos compilado el conjunto de nuestros resultados en un modelo recapitulativo. Hemos mostrado la importancia de las características intrínsecas de la especie (tipo de reproducción), así como las especificidades del sistema agrícola andino (socioculturales, económicas y medio ambientales). Este estudio contribuye de manera significativa a la comprensión de la diversidad genética y de la estructura de los tubérculos andinos.

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CHAPTER I: General introduction

General context

The Andean region is one of the most important areas of domestication and diversification of crop plants, acting for a high percentage of crop genetic resources in the world (40% according to Castillo, 1995). Many crop species were domesticated and are still cultivated in the Andean region, ranging from grains, tubers, fruits, and industrial crops to medicinal and ornamental plants (Castillo, 1995). Some have been transferred all over the world and have become widely cropped, while most of the Andean species are still scarcely known outside the Andes (Cárdenas, 1989; Arbizu et al., 1997; Cadima Fuentes, 2006).

Among these are found three species of Andean tuber crop (Figures 1, 2 and 3), adapted to the agroecological conditions of the Andean highlands: oca (*Oxalis tuberosa* Molina), ulluco (*Ullucus tuberosus* Caldas) and mashua (*Tropaeolum tuberosum* Ruiz & Pav.).

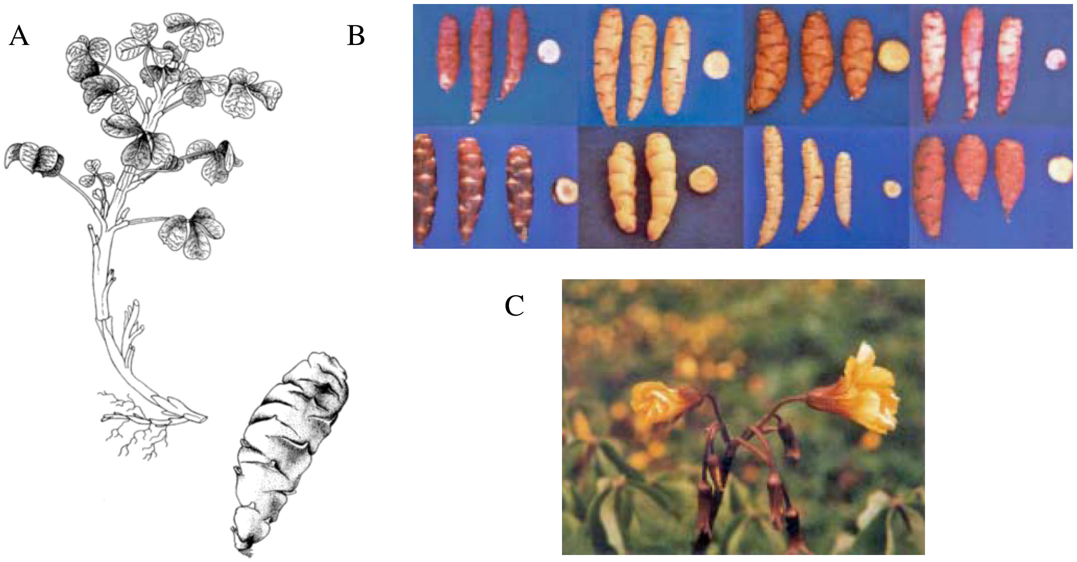

Figure 1: *Oxalis tuberosa* Molina, oca. A: plant and tuber – B: tubers – C: flowers (A: National Research Council, 1989 - B, C: Cadima Fuentes, 2006)

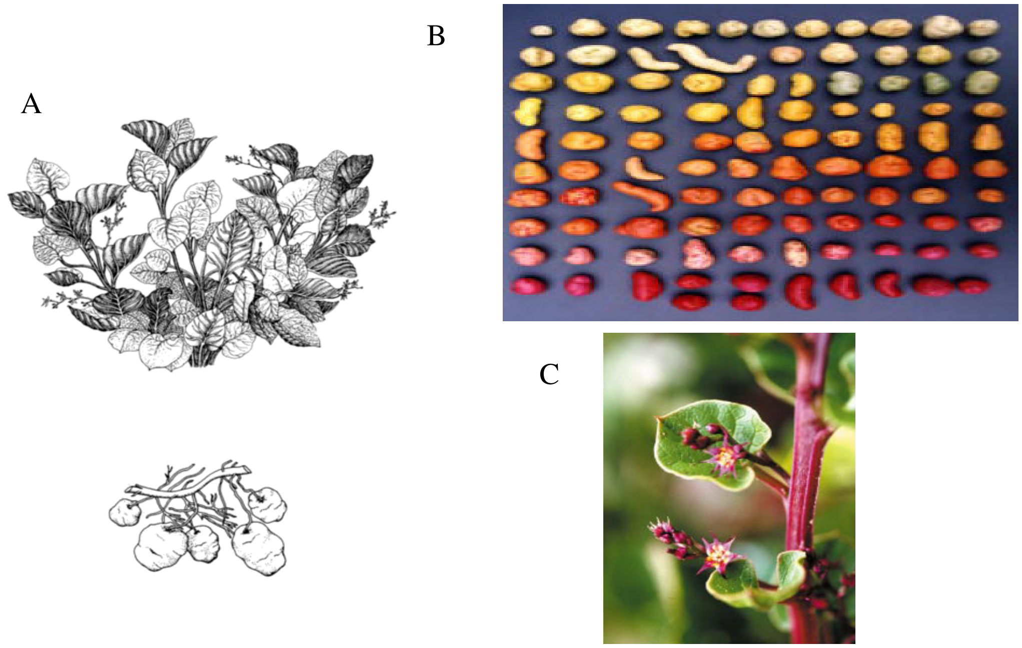

Figure 2: *Ullucus tuberosus* Caldas, ulluco. A: plant and tubers – B: tubers – C: flowers (A: National Research Council, 1989 - B, C: Arbizu *et al.*, 1997)

Andean tubers (oca, ulluco and mashua) are crops from the Andean highlands areas, where they were cultivated for thousands of years (Rousi *et al*. 1989; Trognitz *et al.,* 2000; Grau *et al.*, 2003). Pottery vestiges are evidence of their early domestication, cultivation and use in different Andean communities (Sperling & King, 1990).

They are cultivated at high altitude (2800-4100m), primarily in small plots by traditional Andean farmers, and associated with several tuber-bearing *Solanum* species. They are nearly exclusively propagated via vegetative reproduction through tubers, and there is no evidence of seed propagation under cultural conditions (Trognitz et *al.*, 1998).

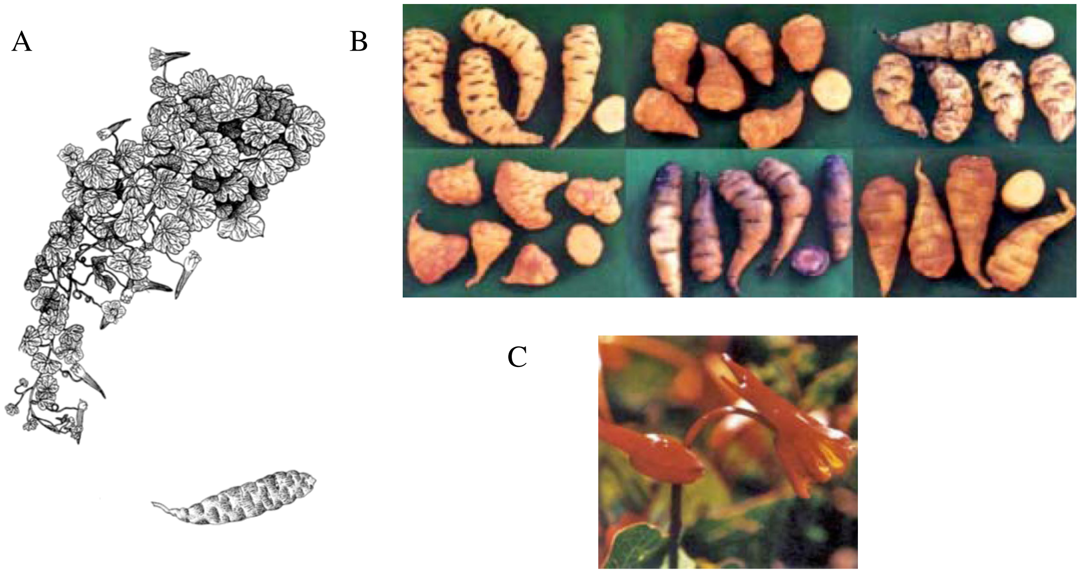

Figure 3: *Tropaeolum tuberosum* Ruiz & Pav., mashua. A: plant and tuber – B: tubers – C: flowers (A: National Research Council, 1989 - B, C: Cadima Fuentes, 2006)

With other indigenous crops, these three Andean tubers are the basis of agricultural production systems of the Andes, essential for the survival of small rural Andean communities. They supply a secondary staple food in communities depending primarily on the potato and provide economic income to the majority of Andean farmers (Sperling & King, 1990). They are present in many cultural rituals and customs (León, 1964; Cárdenas, 1989). The three Andean tubers (oca, ulluco and mashua) have been during long time marginalized, their consumption and production remain limited to rural communities, in the framework of subsistence agriculture. They are considered as secondary crops, little known in the world and therefore not valued. Marginalization of these species originates from numerous factors as difficulties in the product marketing, low social prestige (they constitute the staple food of poor populations), laborious cooking processes and low economic return in a marginal agriculture (Hernández Bermejo & León, 1992).

Marginalization plays a crucial role in **genetic erosion** and biodiversity loss observed within the Andean tuber crop species (Altieri & Merrick 1987; Tapia & Estrella, 2001). In addition, according to Iriondo *et al.* (2008), small farmers maintaining significant amounts of crop genetic diversity through local cultivars face several growing pressures, such as increased population, poverty, land degradation, environmental change, introduction of modern crop varieties and integration into national market economies. As a consequence of these pressures, an alarming loss of genetic variability of the three Andean tubers is observed.

During generations, farmers in Andean communities have developed complex farming systems. Traditionally, they maintained a remarkable amount of native and introduced crops adapted to the microclimates of the highlands, providing a various diet for the local population. Moreover, they used diverse agricultural practices and planting procedures to cultivate crop populations in environments that are heterogeneous in space and often unpredictable in time. Reason to plant several crops is to lower the risk of crop failure and increase food security.

Although high genetic erosion continues, many of these local practices have persisted through generations. A relatively high degree of genetic diversity created by the early Andean people and by nature (Castillo, 1995) is still maintained by peasant cultivators (Ortega, 1997; Terrazas & Valdivia, 1998; Terrazas *et al.*, 2007). Farmers continue to maintain pools of Andean tubers varieties; and oca, ulluco and mashua still show a high phenotypic diversity, especially in their tubers (Popenoe, 1989; Cadima Fuentes, 2006). However, as in many areas of the world, a large part of the diversity of the Andes, as well as the associated indigenous knowledge, are being lost. With a growing importance, economic decisions constraint farmers to grow only a few crop varieties, and not to invest in the conservation of the varieties that are less favoured by the market.

On the other hand, an erosion of the Andean tubers' variability could endanger their adaptation to the environmental fluctuating conditions that characterize the Andes. Longer-term adaptation to a fluctuating and heterogeneous environment, or to a rich cultural context, may thus be compromised (Brush, 2000).

In order to conserve genetic resources of oca, ulluco and mashua, germplasm is currently maintained *in situ* (in farmers' fields) and *ex situ* (mainly in field genebanks). These three Andean tuber species have a promising economic potential (National Research Council, 1989) because of their agro-industrial and medicinal properties, and their adaptability to difficult environmental conditions (Castillo, 1995). Therefore, conservation of plant genetic resources (*in situ* and *ex situ*) and indirect studies of the genetic diversity of these species are now of paramount importance.

High genetic diversity is one of the features of Andean agriculture, but this raises numerous questions. Why is there so much intraspecific diversity, and why is this concentrated in certain regions (biodiversity microcentres)? What are the contribution of natural, farming and human dynamics to the creation and the conservation of this diversity? What is the most suitable approach to describe this diversity? What are factors that could affect and reduce this diversity? What are the most appropriate actions to maintain this diversity? …

The three Andean tuber species (oca, ulluco and mashua) could constitute a relevant model to answer these questions, with the emphasis on neglected vegetatively propagated crop species.

Why the three Andean tubers species oca, ulluco and mashua have been chosen to be studied during this thesis?

Genetic diversity and structure of these crops have not been investigated on a very large scale until now, contrary to the potato species. Implications of such a study could be important in two further issues.

Firstly, at a fundamental level, these species can constitute a potential genetic model for traditional crop species vegetatively reproduced, whose evolution were mainly guided by selection processes imposed by farmers and farming environment. Indeed, unlike potato species, Andean tubers oca, ulluco and mashua were largely neglected by researchers, extension works, and politics. They were not subjected to plant breeding programs and deep genetic studies.

In addition, at a more applied level, studying genetic diversity and structure is indispensable for the exploitation of the genetic potential of these species and for the establishment of appropriate strategies of genetic resources conservation and improvement.

Objectives of the thesis

In this context, the objective of the present thesis is to study genetic diversity and structure of Andean tuber crop species oca, ulluco and mashua; through cultural, morphological and molecular (ISSR marker system) approaches.

Various study levels were considered to achieve the main objective of the thesis. Genetic diversity and structure were considered at the species, morphotype and variety levels. Moreover, genetic diversity maintained *in situ* and *ex situ* were compared.

A ***variety*** can be defined as a set of tubers identified by a vernacular name related to agromorphological features and traditional uses, while an ***accession*** is a plant sample held in an *ex situ* setting for conservation and use. A ***morphotype*** has been defined as a set of accessions with identical morphological characters.

Methods

Morphological descriptors have been published for oca and ulluco (IPGRI/CIP, 2001; 2003), and are under elaboration for mashua. They are the results of collaborations between Andean countries, with support from national and international institutes.

Inter simple sequence repeat (ISSR) technique (Zietkiewics *et al.*, 1994), based on the PCR method, amplifies DNA segments present at an amplifiable distance between two identical microsatellite regions oriented in opposite direction. Inter-microsatellite fragments of different sizes are amplified simultaneously in a single PCR reaction. Primers, of 16-25 bp long, are constituted of microsatellites sequences, and are usually anchored at 3'- or 5'-end with 1 to 4 degenerate bases. ISSR technique is highly reproducible, because of the long primers used, leading to higher annealing temperatures and higher stringency (Bornet & Branchard, 2001). The technique also reveals a high degree of polymorphism (Wolfe & Liston, 1998; Pradeep Reddy *et al*., 2002). ISSRs segregate as dominant markers.

Passport data concern the documentation of crop diversity collections. Each accession's passport data may include basic information on what was collected, where and when; and may supply data on other information like local names and uses, geographic range, agroecological parameters (including altitude, slope, aspect, etc.), and agronomic information. All of this data must be easily accessible, and ideally stored on computer databases and incorporated into genebank management systems. This is particularly important for planning future collecting missions and determining duplications in collections, as well as to establish species distribution maps.

Description of the thesis and the choice of the study sites

After this general introduction (Chapter I), the second chapter of this thesis (Chapter II) is constituted of a bibliographic review, accepted for publication in the revue *BASE* as: Malice M. & Baudoin J.P. Genetic diversity and germplasm conservation of three minor Andean tuber crop species.

The purpose of this bibliographic introduction is to present the three neglected Andean tuber species (oca, ulluco and mashua), to document their genetic diversity and to present the possibilities to conserve their resources in *in situ* and *ex situ* conditions. A case study is presented at the end of this review, i.e. the *in situ* conservation of Andean tubers in the microcentre of Candelaria (Bolivia). This emphasizes on the role of Andean farmers, and of their actions, in the maintenance and the creation of Andean tubers genetic variability in traditional Andean agroecosystems.

Chapters III to VI present results and discussions and rely on the analysis of samples of oca, ulluco and mashua, maintained in *in situ* and *ex situ* conservation systems, in Peru and Bolivia (Figure 4).

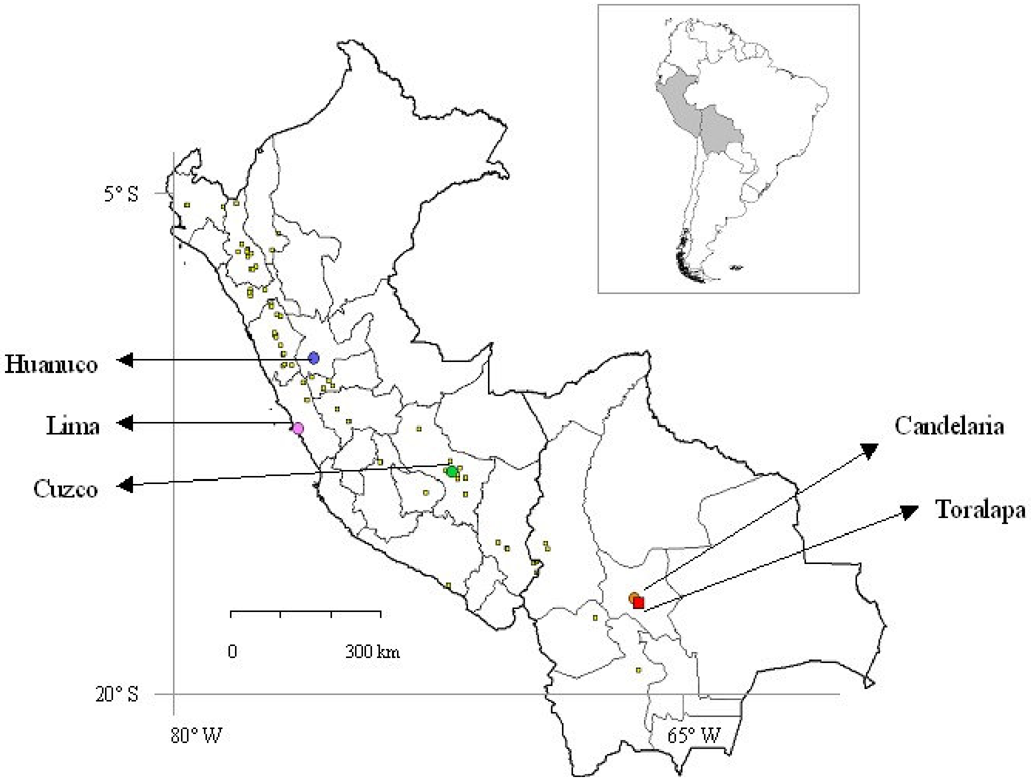

Figure 4: Map showing the location of the Andean tubers' samples used in the present thesis.
Chapter III: 6 oca varieties maintained *in situ* in Candelaria ● and *ex situ* in Toralapa ■ (Foundation PROINPA, Cochabamba, Bolivia)
Chapter IV: 187 ulluco accessions (collecting source: □) from the *ex situ* collection maintained by the CIP (Centro Internacional de la Papa, Lima, Peru)
Chapter V: 15 oca, 15 ulluco and 26 mashua varieties maintained *in situ* in Huanuco ● (Peru)
Chapter VI: 101 oca varieties maintained *in situ* in Cuzco ● (Peru)

In chapter III, we were interested in studying the influence of conservation mode (*in situ* / *ex situ*) on the diversity of oca, at both inter-and intra-varietal levels.

Achieving this goal required the comparison of varieties kept both *in situ* and *ex situ*, and the warranty to have different varieties clearly identified to understand the inter-and intra-varietal levels. To do this, 20 tuber lots, corresponding to 6 oca varieties conserved *in situ* in Candelaria and *ex situ* in Toralapa (sites managed by the foundation PROINPA –Promoción y Investigación de Productos andinos-, Cochabamba, Bolivia) were analyzed using the ISSR molecular markers. The results of this molecular analysis were compared to the current system used to characterize these collections, based primarily on morphological description and folk naming system of oca varieties. Results of this second chapter were published as: Malice M., Martin N., Pissard A., Rojas-Beltran J.A., Gandarillas A., Bertin P., Baudoin J.P. 2008. A preliminary study of the genetic diversity of Bolivian oca (*Oxalis tuberosa* Mol.) varieties maintained *in situ* and *ex situ* through the utilization of ISSR molecular markers. *Genetic Resources and Crop Evolution* **54** : 685-690.

Results were also presented as a poster: Malice M., Martin N., Baudoin J.P. 2004. Etude de la structure et de la diversité génétique de la oca (*Oxalis tuberosa* Mol.) conservée *in situ* à Candelaria - Bolivie. *In :* Actes du 5ème Colloque national du Bureau des Ressources Génétiques (BRG) : « Un dialogue pour la diversité génétique », Lyon (France) : 120-121.

In chapter IV, we have apprehended ulluco's genetic diversity at the intra-specific level. To do this, a sample composed of as many ulluco's accessions as possible was needed, and we analyzed accessions from the *ex situ* collection maintained by the CIP (Centro Internacional de la Papa, Lima, Peru). The *ex situ* collection of the CIP consists of several hundred accessions from various Andean countries, of which 187 ulluco accessions were selected to provide the broadest and most representative sample of geographical diversity and morphotype constitution. Results, discussed according to characteristics of the ulluco species and of the Andean agricultural system, were published as: Malice M., Villarroel Vogt C.L., Pissard A., Arbizu C., Baudoin J.P. 2009. Genetic diversity of the Andean tuber crop species *Ullucus tuberosus* as revealed by molecular (ISSR) and morphological markers. *The Belgian Journal of Botany*. **142** (1).

Results were also presented as oral communications in international congresses:

- Malice M., Villarroel Vogt C.L., Rochez G., Arbizu C., Baudoin J.P. 2006. Etude de la diversité génétique de l'ulluco (*Ullucus tuberosus* Caldas) en conservation *ex situ*. *In* : Actes des Xèmes Journées Scientifiques du Réseau "Biotechnologies végétales : Quelles biotechnologies pour une agriculture durable?", 8-11 mai 2006. Ed. Khelifi D., Agence Universitaire de la Francophonie, Constantine (Algérie) : 21-22.
- Villarroel Vogt C.L., Malice M., Pissard A., Arbizu C., Baudoin J.P. 2007. Estudio de la diversidad genética de la papalisa (*Ullucus tuberosus* Caldas) conservada *ex situ* mediante la utilizacion de marcadores moleculares tipo ISSR. La biotecnologia como herramiento para el dessarrollo del pais, V Reunion Nacional de Biotecnologia, 29 - 31/08/2007, La Paz (Bolivia).

Chapters V and VI are the results of contacts with three Universities in Peru (Lima, Cusco and Huanuco), with the objective to develop *in situ* conservation programs in diversity microcentres in Cusco and Huanuco.

In chapter V, we used 15 varieties of oca, 15 varieties of ulluco and 26 varieties of mashua maintained *in situ* in the diversity microcenter of Huanuco (Peru), in order to estimate the intervarietal diversity and to compare the varieties when identified by folk taxonomy and by molecular ways. We showed a high degree of correspondence between varieties described by the molecular markers and varieties as described by the Andean farmers. Assignment technique divided both oca and ulluco samples into genepools that may be linked to the folk naming system. ISSR molecular markers enlightened intra-varietal genetic variability for most varieties, suggesting an underestimation of the *in situ* genetic variability.

A publication of these results is in preparation and will be submitted to *Crop Science* as: Malice M., Bizoux J.P., Blas Sevillano R.H., Baudoin J.P. Evaluation of genetic diversity of neglected Andean tuber crop species (oca, ulluco, mashua) in the *in situ* microcentre of diversity of Huanuco (Peru) using ISSR markers.

Chapter VI allowed a better understanding of genetic and structure diversity of oca analyzed with the morphological markers. In this purpose, 101 oca varieties maintained in the diversity microcenter of Cusco (Peru) were characterized morphologically. Also a field experiment has been conducted in three rural communities of this microcentre and the oca production system was analyzed. Results highlighted the existence of a very important intra-varietal diversity that can be explained by the selection carried out by farmers.

Results were proposed to publication in *Plant Genetic Resources Newsletter* as: Malice M., Valemblois C., Pérez Huartado I.M., Blas Sevillano R.H., Baudoin J.P. Morphological diversity of the Andean tuber crop species oca (*Oxalis tuberosa* Molina) from the diversity microcentre of Cusco (Peru).

Results of this chapter were also presented as a poster: Malice M., Valemblois C., Pérez Huartado I.M., Blas Sevillano R.H., Baudoin J.P. 2009. Diversité morphologique de la oca (*Oxalis tuberosa* Molina), conservée *in situ* dans le microcentre de Cusco (Pérou). *In :* 5ème Colloque d'écologie des communautés végétales – ECOVEG5, Gembloux (Belgium).

The last part of the thesis (Chapter VII), that constitutes the general discussion, compiles and discusses all results presented in the previous chapters of the thesis, together with those found in the literature and dealing with genetic diversity of Andean tubers and of other vegetatively reproduced crops, with the final purpose of producing a tentative unifying model clarifying genetic diversity of vegetatively reproduced crop species.

Final conclusion and some perspectives for future works end the thesis.

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CHAPTER II: Bibliographic review - Genetic diversity and germplasm conservation of three minor Andean tuber crop species.

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Genetic diversity and germplasm conservation of three minor Andean tuber crop species.

Titre abrégé. Conservation of Andean tuber genetic resources

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Diversité génétique et conservation du germoplasme de trois tubercules andins mineurs.

Résumé. Dans les agrosystèmes traditionnels andins, trois espèces mineures de tubercules cultivés sont d'une importance régionale ou locale: la oca (*Oxalis tuberosa* Molina), l'ulluco (*Ullucus tuberosus* Caldas) et la mashua (*Tropaeolum tuberosum* Ruiz & Pav.). La diversité génétique au sein de ces espèces est très élevée et pourrait résulter de l'importante variabilité écologique et culturelle qui caractérise la région andine. Actuellement, plusieurs facteurs anthropiques ou écologiques entraînent la perte de cette diversité et contribuent au phénomène d'érosion génétique. La mise au point de stratégies de conservation des ressources génétiques des tubercules andins, tant *in situ* que *ex situ*, passe par la connaissance de la diversité ainsi que par l'étude des stratégies paysannes andines à la base de cette diversité.

Mots-clés. tubercules andins, oca, ulluco, mashua, diversité génétique, conservation *in situ*, conservation *ex situ*, ressources phytogénétiques, Bolivie

Genetic diversity and germplasm conservation of three minor Andean tuber crop species.

Abstract. In traditional Andean agrosystems, three minor tuber crop species are of regional or local importance: oca (*Oxalis tuberosa* Molina), ulluco (*Ullucus tuberosus* Caldas) and mashua (*Tropaeolum tuberosum* Ruiz & Pav.). Genetic diversity within these species is very large and could result from the high ecological and cultural variability that characterizes the Andean area. Nowadays, many anthropic or ecological factors cause the loss of diversity and contribute to genetic erosion. The development of conservation strategies for genetic resources of Andean tubers, *in situ* as well as *ex situ*, includes a better knowledge of diversity in addition to the study of Andean farming strategies linked to this genetic diversity.

Keywords. Andean tubers, oca, ulluco, mashua, genetic diversity, *in situ* conservation, *ex situ* conservation, phytogenetic resources, Bolivia

1. Introduction

Several crops, produced by many small scale farms in rural areas of traditional agriculture, have a largely unknown importance for feeding an increasing proportion of the world's population. Andean region, which extends from South Venezuela to North Argentina and Chile, has been recognized as a main area for minor crop development and germplasm conservation, including tuber species like potato (*Solanum* spp.), grain species like quinoa (*Chenopodium quinoa* Willd.), maize (*Zea mays* L.), tarwi (*Lupinus mutabilis* Sweet) and beans (*Phaseolus* spp.), or fruit species like cherimoya (*Annona cherimollia* Mill.). Moreover, Andean region is also a centre of origin for little known, but potentially important crop species: the Andean tubers oca (*Oxalis tuberosa* Molina), ulluco (*Ullucus tuberosus* Caldas) and mashua (*Tropaeolum tuberosum* Ruiz & Pav.). These species have been neglected for a long time in research, extension and breeding programs and scientific interest emerges since barely a decade. Exclusively propagated through tubers, these crops have formed a high number of clonal varieties that can be distinguished by tuber color and shape (Rousi et al., 1986; Grau et al., 2003; Emshwiller, 2006). However an alarming loss of variability is currently observed (Altieri & Merrick, 1987; Tapia & Estrella, 2001), and both *ex situ* and *in situ* conservation methods are developed.

The purpose of this paper is to document genetic diversity of these three neglected Andean tuber species and to present possibilities of *in situ* and *ex situ* conservation. The example of *in situ* conservation of Andean tubers in the microcentre of Candelaria (Bolivia) will be presented as a case study.

2. Andean tubers

Oca, ulluco and mashua belong, together with potato, to the group of edible tuber crops indigenous to the Andean mountains, where they are a food staple in rural communities. Because of its worldwide importance, potato is the most widely studied Andean tuber species. Little information was available on other Andean tubers until just a decade ago. Through research and field projects, knowledge has been generated in different topics from conservation, genetic diversity, production systems and alternative uses of these tubers.

Oca, ulluco and mashua are cultivated for their edible tubers in small areas under traditional agricultural systems and marginal conditions, in all Andean countries, mainly in Peru, Ecuador and Bolivia, and at high altitudes (2800 to 4100 meters). They share the same Andean ecological niches as cultivated potatoes. According to Arbizu et al. (1997), they are grown under short-day conditions of the Andes (11-12 hours), with rainfalls ranging from about 400 to 700 mm across the growing season (an average of 7 months). Andean tubers are exclusively propagated through tubers and there is no evidence of seed propagation by farmers. Oca, ulluco and mashua have remained strictly Andean crops, because their conservation and use are associated with socio-cultural aspects of the Andean people and their traditional production systems (León, 1964). Nevertheless, they are essential to ensure food diversification and livelihood of numerous Andean populations. The production of tubers is mostly devoted to farmer's family food supply. Small volumes are also sent to the market, but demand is limited and temporary (Tapia Vargas, 1994).

2.1. Oxalis tuberosa

The name oca is derived from the Quechua words *okka, oqa* or *uqa*. Other names are *oca* in Peru, Ecuador and Bolivia, *cuiba* or *quiba* in Venezuela, *huasisai* or *ibi* in Colombia, *truffette acide* in French, or *papa extranjera* in Mexico (Arbizu et al., 1997, Cadima Fuentes, 2006).

Oxalis tuberosa belongs to the Oxalidaceae family that includes eight genera. The genus *Oxalis* includes more than 800 species. Some of them set small tubers, but only *O. tuberosa* is cultivated for its edible tubers. Literature denies the existence of wild oca or related tuber bearing species, though wild populations of *Oxalis* bearing small tubers have been found in Bolivia (Emshwiller & Doyle, 1998). Cultivated oca is octoploid (2n = 8x = 64).

After potato, oca is the second more common tuber species in the Andean region. Its cultivated surface does not exceed 10 000 ha (Tapia Vargas, 1994). Reported yields for oca in Ecuador do not exceed 2 t/ha, even if in experimental trials it can reach 15-28 t/ha (Tapia et al., 2004). In Peru, oca has a mean production of 5 t/ha and in Bolivia 3-5 t/ha (Tapia Vargas, 1994; Cadima Fuentes, 2006). Oca is consumed water cooked or baked, always after that tubers have been exposed for several days to the sun in order to acquire sugar taste. They can also be consumed dehydrated, as chuño (Cárdenas, 1989).

Oca is an annual herbaceous plant that is erect in the first stages of its development, then prostrate towards maturity. Stems vary in color from yellow-green to grey. The color of the tuber surface is an important discriminant character. In the standard descriptors (IPGRI/CIP, 2001) up to 12 variations of colors are mentioned. The tubers can also show secondary colors distributed either in or around the eyes (i.e. the axillary buds), or unevenly distributed as bands. Both the tubers as the stems have a tendency to fasciation. Leaves are trifoliate; leaflets are green in the upper face and purple or green on the underside. Tuber shape is ovoid, claviforme or cylindrical (Popenoe, 1989). The tuber eyes vary from horizontal, slightly curved, short or long, close or away to each other, and superficial or deep. Bracts covering eyes can be wide and short, or almost nonexistent (Cárdenas, 1989; Cadima Fuentes, 2006). Most oca accessions flower regularly under field conditions. Inflorescences are made of 4-5 hermaphrodite flowers. However, spontaneous seed set is rare and is only observed when both several genotypes are grown together and natural pollinators (the solitary bumblebees *Bombus funebris* Smith) are present (Trognitz & Hermann, 2001). The oca species possesses a trimorphic system of genetic incompatibility (Gibbs, 1976), associated with the presence of three floral morphs. Each clonal accession presents one of the three flower morphs: long (long-styled, with mid- and short-level stamens/anthers), mid (mid-styled, with long- and short-level stamens/anthers) and short (short-styled, with long- and mid-level stamens/anthers). Legitimate pollinations occur when stigma gets pollen of the corresponding morph; illegitimate pollinations occur when stigma gets pollen of a different morph. Legitimate pollinations usually produce the highest seed set. This fits well with the observations of Emshwiller (1998), showing that cultivated oca is an outbreeder. Trognitz et al. (2000) studied three factors responsible for the poor seed formation in oca (size and fertility of pollen, and pollen tube growth), in order to analyse the relationship between these characters and stylar incompatibility. The reduced number of seeds produced seems to be linked to the oca's stylar incompatibility that may affect the growth of pollen tube (Trognitz et al., 2000). The fruit is a dehiscent capsule with five locules, and 1-3 or more seeds are produced per locule (Cárdenas, 1989).

2.2. Ullucus tuberosus

The name ulluco is derived from the Quechua word *ulluku* (*ullu* means male organ). *Ullucus tuberosus* is also known as *chugua*, *ulluma*, *iliaco* and *chiga* in Colombia; *melloco*, *hubas*, *chuga*, and *ulluco* in Ecuador; *olluco*, *olloco*, *ullush*, *ullucu* or *lisas* in Peru; and *papalisa*, *illoco*, *ulluma* and *lisa* in Bolivia (Arbizu et al., 1997, Cadima Fuentes, 2006).

The genus *Ullucus* of the family Basellaceae is monospecific. *Ullucus tuberosus* comprises two sub-species: *aborigineus* and *tuberosus*. Cultivated ulluco belongs to the sub-species *tuberosus*, and is cultivated for its edible tubers. Within the sub-species *aborigineus* are found the wild forms of ulluco, which form small tubers of 1-1.5 cm diameter, pink, red, brown or sometimes white (Cadima Fuentes, 2006). The basic number of chromosomes of ulluco is $x = 12$. According to Arbizu (2004), wild ullucos (subsp. *aborigineus*) are all triploid ($2n = 3x = 36$), while around 96% of the cultivated ullucos (subsp. *tuberosus*) are diploid ($2n = 2x = 24$), 3% are triploid ($2n = 3x = 36$) and 1% are tetraploid ($2n = 4x = 48$). According to Pietilä (1995), who tested the effect of cross-pollination versus self-pollination on seed set, ulluco is an outbreeder. To our knowledge, mechanisms that promote allogamy in ulluco remain unknown.

In Ecuador, ulluco is the second tuber crop species after potato. In Peru and Bolivia, ulluco has a smaller importance, and is the third species after potato and oca. In Ecuador, Tapia et al. (2004) reported a mean yield of 3.5 t / ha. In Peru, the mean yield is 4-5 t / ha (Cadima Fuentes, 2006). In Bolivia, ulluco is cropped on around 3000 ha, giving a yield of 3-5 t / ha (Tapia Vargas, 1994); it is mainly cultivated in the Departments of Cochabamba and Chuquisaca, with yields of 3 t / ha (Cadima Fuentes, 2006).

Ulluco is an erect, compact and mucilaginous annual plant. Stems are succulent, angular and 30-60 cm height. Stem color varies from clear yellow-green to red-grey. Leaves are simple and can present four shapes. Tuber can be round, cylindrical, elongated or twisted. Tuber eyes (axillary buds) are superficial and without bracts (Popenoe, 1989). The tuber color shows a wide variation, with 12 states ranging from white to red (IPGRI/CIP, 2003). Besides this predominant tuber color, it is also common to find a secondary tuber color. Inflorescences are axillary and abundant with numerous small flowers, magenta (red-purple), green-yellow alone or with red-purple (Cárdenas, 1989; Arbizu, 2004). Fruit formation is rare and seems to depend on the genotype (Pietilä & Jokela, 1990). However ulluco has been shown to set seeds, though the number of seeds produced is low. The fruit is dry and indehiscent and contains one seed, morphologically normal (Rousi et al., 1989) and capable of germination (Lempiäinen, 1989). According to Pietilä & Jokela (1994), morphological abnormalities in the ovules and embryo sacs are responsible for the low seed number. These authors suggested that vegetative way of propagation favoured the accumulation of a genetic load: somatic mutations (especially those affecting sexual reproduction) may have appeared and be maintained during hundreds generations of vegetative reproduction, explaining why ulluco could have partly lost its capacity for female sexual reproduction.

2.3. Tropaeolum tuberosum

The name mashua is derived from the Quechua names *maswa* or *mashwa*. Many names are given for mashua: *mashwa*, *mashua* in Peru and Ecuador; *isaño*, *añu* in Bolivia; and *cubio* in Colombia (Arbizu et al., 1997, Cadima Fuentes, 2006).

Tropaeolum is the largest genus of the Tropaeolaceae family that includes 86 species distributed in all South America (Grau et al., 2003). These authors recognise two sub-species: the cultivated *T. tuberosum* ssp. *tuberosum* and the wild *T. tuberosum* ssp. *silvestre*. The latter does not set tubers. Both wild and cultivated forms extend from Venezuela to northwest Argentina (Cadima Fuentes, 2006). Most cultivated mashua are tetraploid, with a basic chromosome number of x = 13 (2n = 4x = 52). However, cytological studies show conflicting results and other ploidy levels and chromosome numbers have been observed (Cadima Fuentes, 2006; Grau et al., 2003).

Mashua is the Andean tuber covering the smallest crop area in the region and has therefore received the lowest attention. Its culture has been maintained because of its frost tolerance, its resistance to diseases and pests and its stable yields in low-fertile soils of the Andean region (Grau et al., 2003). Mashua is cultivated on small plots around the farms, or close to the fields of more important cultures like potato and oca. According to Grau et al. (2003), mashua is mainly cultivated in Peru (more than 7000 ha). The cultivated surface of mashua is estimated at about only 100 ha in Bolivia and 50 ha in Ecuador. Yield data in farmer's fields vary from 30 to 60 t/ha, which demonstrates the high production potential of mashua (Cadima Fuentes, 2006).

Mashua is an annual herbaceous plant 20-80 cm high. Stems are cylindrical, 3-4 mm thick, branching, and can vary in color from green to purple-grey with varying degrees of pigmentation. Foliage color varies from yellow-green to dark green. Leaves are 5-6 cm width, tri or pentalobate. In the same plant, tri and pentalobate leaves can be observed. Tubers are less variable in shape than those of oca and ulluco (Popenoe, 1989). Tuber color is variable, ranging from yellow-white to purple-grey and black. Tuber eyes (axillary buds) are always deep, wide and narrow, without bracts (Cárdenas, 1989). Unlike oca and ulluco, mashua flowers profusely and sets many viable seeds with high germination rates (Cárdenas, 1989). According to Grau et al. (2003) mashua is self-fertile. Flowers are solitary and zygomorph. Five sepals of intense red color are united at the base; the three higher forming a spur of 1-1.5 cm length. Seeds can be desiccated to low moisture levels at the ambient conditions of the Andean highlands and will germinate after several months of storage. So far, sexual seeds are currently not used for mashua conservation, which relies on clonal maintenance in fields (Grau et al., 2003).

3. Andean tubers genetic diversity: from genetic erosion to resources conservation

Farmers' actions are without any doubt essential for the creation and the maintenance of Andean tubers diversity (Quiros et al., 1992). Marginalization of these species originates from numerous factors: difficulties in the product marketing, low social prestige (they constitute the staple foods of poor populations), laborious cooking processes, low economic return in a marginal agriculture (Hernández Bermejo & León, 1992). In addition, according to Iriondo et al. (2008), small farmers maintaining significant amounts of crop genetic diversity through local cultivars face several growing pressures, such as increased population, poverty, land degradation, environmental change, introduction of modern crop varieties and integration into national market economies. These factors contribute to the genetic erosion of Andean tubers. On the basis of field (passport data, morphological descriptors, socio-economical questionnaires) and laboratory (molecular analysis) studies, Tapia & Estrella (2001) showed that genetic erosion in the three Andean tuber species varies from 25% to 46,5% in Ecuador. Nevertheless, the extent of genetic erosion of Andean tubers remains controversial. In fact, even if large genetic variability is exposed to erosion problems, many of the rare genotypes are still used and subsist thanks to indigenous population, who adopt strategies to cope with biotic and abiotic risks through a wide range of traditional farming techniques (Brush, 1995). The conservation of crop diversity against genetic erosion is now recognized as a worldwide challenge. In this context, two fundamental approaches exist for the conservation of plant genetic resources: (i) *ex situ* conservation, in which genetic variation is maintained away from its original location, and (ii) *in situ* conservation, in which genetic variation is maintained at the location where it is encountered, either in the wild or in traditional farming systems (Brush, 2000; Iriondo et al., 2008). Compared with *ex situ* conservation approach, *in situ* has an important advantage in that it maintains natural genetic interactions between crops, their wild relatives and the local environment; while *ex situ* techniques freeze adaptive evolutionary development, especially in the context of pest and disease resistance (Hawkes et al., 2000; Maxted et al., 1997).

First actions to conserve diversity were the creation of *ex situ* genebanks around the world (Sperling & King, 1990). Most recently, *in situ* conservation has been developed as a complementary method to *ex situ* conservation (Terrazas & Valdivia, 1998; Tapia et al., 2004). Complementarity between *in situ* and *ex situ* is commonly presented as essential to secure a sustainable conservation of plant genetic resources. Adopting complementary strategies for resources conservation is useful to overcome the advantages and impediments of both techniques (Hawkes et al., 2000; Maxted et al., 1997). Numerous accessions maintained, *in situ* as *ex situ*, are not well characterized and evaluated, leading to an underutilization and an insufficient exploitation of collections. *In situ* and *ex situ* constraint is the presence of a high number of redundant accessions, highlighting the need to characterize accessions and to define core collections.

3.1. *Ex situ* conservation of Andean tubers

As Andean tubers are vegetatively propagated species, the most appropriate method for *ex situ* conservation remains the establishment of genebanks in the field. Management of field collections and conservation costs are important in the case of vegetatively propagated species, mainly because of the need to regenerate varieties in the field and to maintain them as tubers. Alternative conservation of oca as botanical seed was experienced by Trognitz et al.

(1998). They showed that botanical seed production is possible under controlled pollination conditions, and that the tristylus system of incompatibility seems to control the amount of seed produced. Cross-pollinations allowed the production of a higher seed quantity, which implicates that a botanical seed maintained in a genebank does not conserve genes of a unique oca accession, but genes of at least two different accessions. Moreover, it is known that oca rarely sets seeds in natural conditions, and that sterile clones have been reported (Trognitz et al., 2000). Therefore, accessions should be tested prior to their use for botanical seed production, in order to detect the possibility of sterility. So far, oca's germplasm conservation as seed is currently not applied.

3.2. *In situ* conservation of Andean tubers

On-farm conservation of plant genetic resources can be defined as the continued cultivation and management of a diverse set of crop populations by farmers in the agroecosystems where a crop has evolved (Jarvis et al., 2000). This dynamic method aims at maintaining the evolutionary processes that shape the diversity. In this context, the role of the farmers is essential for two reasons: (1) crop diversity is not only the result of natural factors, such as mutation and natural selection, but also and particularly, of human selection and management; and (2) in the last instance, farmer's decisions define whether these populations are maintained or will disappear. The maintenance of crop intra-specific diversity by farmers requires a better understanding of what, how and when they do it (Brush, 1991; Castillo, 1995). *In situ* conservation by farmers is not only related to the set of varieties they keep, but also to the management processes of these varieties and the knowledge guiding these processes, i.e. "farmers' management of diversity" (Castillo, 1995).

Farmers' management of diversity refers to the cultivation of a diverse set of crop populations that are named and recognized as farmers' varieties. The set of varieties is formed through a constant process of experimentation, evaluation and selection of old and more recent varieties. There are two levels of selection: (a) the choice of the varieties to maintain; and then (b) for each one, the choice of the seed stock that will be planted the next season. This selection process is dynamic and is influenced by the supply of varieties from other farmers, villages, regions or even countries. Four components of farmers' management of diversity can be identified: (1) seed exchanges between farmers, (2) variety selection, (3) variety adaptation, and (4) seed selection and storage (Bellon, 1997). Sites for on-farm *in situ* conservation of Andean tubers have been identified and are under study in many Andean countries, e.g. in Peru (Huánuco, Cusco, Cuyo Cuyo, Huancavelica; Tapia, 2000), Bolivia (Candelaria; Terrazas & Valdivia, 1998) and Ecuador (Las Huaconas; Tapia et al., 2004).

4. A case study from Bolivia: on-farm in situ conservation of Andean tubers in the microcenter of Candelaria

The area of Candelaria, located in the Department of Cochabamba (Bolivia), is known for its traditional farming system of Andean tubers. It presents a high concentration of native oca, ulluco and mashua varieties and has been recognized as a microcentre of diversity for Andean tubers conservation (Terrazas & Garcia, 2003). This site of on-farm *in situ* conservation is currently managed by the PROINPA Foundation (Promoción e Investigación de Productos Andinos, Cochabamba, Bolivia – www.proinpa.org), whose main goal is to maintain and promote Andean crops genetic diversity. The microcentre of Candelaria is located 63 km northeast of the city of Cochabamba (17°20’ S 65°50’ W). Mean annual temperature is 8-10°C, relative humidity ranges form 70-90% and annual rainfall is 900-1000 mm (Gonzáles et al., 2003). The mean cultivated surface for a family is 18 ares for oca, 14 ares for ulluco and 1 are for mashua. Mean tuber yields are 15-20 t/ha for the three Andean tuber species (Terrazas & Garcia, 2003).

In 2002, the total diversity identified in the microcentre of Candelaria included 32 varieties for oca, 16 for mashua and 8 for ulluco (Terrazas et al., 2003). Each variety is characterized by agromorphological traits, such as tuber's color and shape, food value, culinary uses and medicinal properties, all used by farmers to identify them (Cadima et al., 2003b). Unfortunately, this valuable germplasm seems to be under threat of genetic erosion. In fact, Terrazas et al. (2003) pointed out a decrease in the average number of cultivars used by a family in Candelaria. This reduction in cultivar number is mainly observed for oca.

Cropping system in the microcentre is influenced by ecological and topographic conditions. Soil heterogeneity, altitudinal gradient, humidity and temperature determine crop management system according to altitude. Farmers distinguish three altitude levels within the microcentre (Terrazas & Valdivia, 1998): (i) the lower stage or *pampa* (3000 - 3350 m); (ii) the hillside or *ladera* (3350 - 3650) and (iii) the step hillock or *punta* (> 3600 m). To each level correspond particular climatic, biotic and soil conditions requiring judicious choice of species and varieties and agricultural techniques (Terrazas et al., 2003). The way germplasm is handled by the farmers in Candelaria constitutes a model based on time and space. Varieties are distributed in a wide range of environmental (soil and climate heterogeneity, altitude levels), geographical (localisation of the family in the microcentre) and social (migration, market pressure) conditions. In such a system, species and varieties are dispersed in the germplasm of several families, on numerous plots localised across the three altitude levels (Terrazas & Valdivia, 1998). Mosaic system is all but static and is continually modified through time by several mechanisms generating a 'dynamic mosaic system' (Terrazas &

Valdivia, 1998; Terrazas & Garcia, 2003) where tubers of each variety are frequently transported from place to place. Farmers generally own plots of land located at different altitude levels. When seed is impoverished (due to viral accumulation and vegetative reproduction) and does not thrive in a plot, farmers move them to a plot located at another altitude level to revitalize their production. Varieties are consequently subjected to different environmental conditions and pressure selections, with the consequence to stop viral accumulation (Terrazas & Valdivia, 1998).

Diversity in Candelaria consists of the familial germplasm, i.e. a lot of tubers from several species and varieties of Andean tubers usually inherited from parents (Terrazas & Valdivia, 1998). Composition and structure of these familial lots is not static: quantity and quality of tuber-seed vary in time. Biotic and abiotic conditions (i.e. diseases, climatic, or other damages leading to the loss of the harvest or the disappearance of the variety) and exchanges between farmers (within or between communities) influence germplasm composition of each family. These processes create dynamic intra- and inter-communities tuber flow (Terrazas & Garcia, 2003). In addition, markets and biodiversity fairs are important sources of new germplasm, gathering tubers from different families, farmers or communities (Espinoza, 2001). These practices are known to be very good sources of genetic diversity in the case of vegetatively reproduced crop species, like Andean tubers. In addition, Terrazas & Garcia (2007) analyzed the main factors that influence the farmers' decisions for Andean tubers diversity management in Candelaria. Favouring the direct training of farmers is an effective way to promote *in situ* conservation of Andean tubers in farmers' fields. However, it is necessary to expand research on the influence of other factors of socio-economic and ecological importance. Terrazas & Garcia (2007) showed that access to “Field Schools” and other direct training methods implemented in Candelaria, positively impacted on the conservation of a greater number of varieties per family. By contrast, other socio-economic factors, such as land area and animals owned by the family, exert a weak or no influence on the conservation of varieties in farmers' family.

To ensure the conservation of native varieties maintained in the *in situ* microcentre of Candelaria, tubers have also been maintained in an *ex situ* conservation centre located in Toralapa (Department of Cochabamba), at 3430 meters altitude (Cadima et al., 2003a). This germplasm, recognized as the national germplasm bank for roots and tubers in Bolivia, consists of about 500 accessions of oca, 200 accessions of ulluco and 80 accessions of mashua from the whole country. This *ex situ* collection is also managed by the PROINPA Foundation in a complementary way to *in situ* conservation in Candelaria.

5. Conclusion

Andean tubers are neglected tuber-bearing crop species, native to the Andean highlands. They have been cultivated for thousand of years for their edible tubers, through which they are propagated. For many centuries, they have continuously contributed to the food security of the Andean populations and are part of their culture and social expression. Phenotypic diversity of Andean tubers is very high. Biotic and abiotic pressures of the Andes, coupled with anthropic selection for food purpose and crop husbandry, have resulted in a large morphological variation. Andean tubers are exclusively vegetatively propagated and consequently have formed clonal varieties, with particular phenotypic characters and vernacular names given by the local people. But nowadays this valuable germplasm is subject to genetic erosion and the number of cultivated varieties is decreasing. Both *in situ* and *ex situ* conservation programs are developed. For a better conservation of Andean tuber genetic diversity under both systems, it would be extremely important to obtain additional information on the "farmers' management processes". Among the numerous data still needed to better understand the evolution of clonal crops in traditional agricultural systems, the following factors can be pointed out: the role of the cultural, economic and ecological environment; the impact of tubers' exchanges via markets, barters, biodiversity fairs; the influence of sexual reproduction in successive generations; the levels of diversity maintained by *in situ* and *ex situ* conservation methods.

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CHAPTER III: A preliminary study of the genetic diversity of Bolivian oca (*Oxalis tuberosa* Mol.) varieties maintained *in situ* and *ex situ* through the utilization of ISSR molecular markers

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A preliminary study of the genetic diversity of Bolivian oca (Oxalis tuberosa Mol.) varieties maintained *in situ* and *ex situ* through the utilization of ISSR molecular markers

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Abstract ISSR molecular markers have been used to investigate genetic diversity of oca (*Oxalis tuberosa* Mol.), an Andean neglected tuber crop species. Sampling procedure allowed a preliminary study of the genetic diversity at the intra- and intervarietal levels. Twenty tuber lots conserved *in situ* in the microcentre of Candelaria and *ex situ* in the Toralapa Centre (Bolivia) were identified. Four ISSR primers amplified a total of 25 fragments of which 17 (68%) were polymorphic. These experiments show that the structure of oca varieties is mainly based upon vernacular names with a greater differentiation among tuber lots than within them, supporting agromorphological data. ISSR technique enlightened the existence of heterogeneous varieties in oca and divergence between *in situ* and *ex situ* conservation strategies. These observations are potentially linked to the different ways of management of tubers in these two conservation systems.

Keywords Andean tubers - *Ex situ* conservation - Genetic diversity - *In situ* conservation - ISSR - *Oxalis tuberosa* Mol.

1. Introduction

Oca (*Oxalis tuberosa* Mol.) is one of the eight neglected species found in marginal Andean farming systems, whose starchy tubers constitute a basic component of the staple food for millions of people in rural communities. Morphological variation within this species is large (Cardenas 1989). Oca could represent an interesting model species for the study of genetic diversity of neglected and vegetatively propagated plants. Its genetic diversity is in fact mainly determined by its breeding system, the prevalence of traditional varieties in subsistence agricultural systems and the lack of improved cultural practices. Schemes *for in situ* or *ex situ* conservation of oca have already been proposed, to cope with genetic erosion and to conserve valuable resources of this species. In Bolivia, the Foundation PROINPA maintains accessions of oca *ex situ* at the Centre of Toralapa whereas varieties of the same species are preserved in situ in microcentres of diversity, such as Candelaria. The area of Candelaria is known for its traditional Andean tuber production and its high concentration of native varieties (Terrazas and Valdivia 1998). The inter-simple sequence repeats (ISSR) technique, developed by Zietkiewicz et al. (1994), has been successfully used to reveal molecular polymorphism in oca (Pissard et al. 2006). The present survey was initiated to produce preliminary data in order to establish conservation strategies of oca. The sampling procedure was designed to provide a preliminary molecular characterization of the Bolivian oca materials maintained *in situ* in Candelaria and *ex situ* in Toralapa, in comparison to the currently agromorphological description of varieties.

2. Materials and methods

The oca accessions sampled in Toralapa are originating from Candelaria and were introduced in the genebank in 1995. For the present study, plant materials have been collected in 2003 on several varieties, in order to study the diversity between them. A variety was defined as a set of tubers identified by a vernacular name related to agromorphological features and traditional uses. The entity sampled in the field was called a tuber lot; if varieties were cultivated *in situ* by different farmers or *ex situ* as distinct accessions, they were sampled as different lots to verify their genetic integrity. Individual samples, basic units for molecular analysis, were randomly taken on three plants of each tuber lot, to verify the presence of heterogeneous lots (i.e., composed of several genotypes) and to have a look at the diversity within varieties. As listed in Table 1, 20 lots of oca corresponding to six varieties were investigated and ISSR analysis was conducted on 51 oca samples.

DNA was extracted from fresh young leaves. ISSR reactions were performed with four primers selected by Pissard et al. (2006) (primers 3, 8, 11 and 12) with a slight modification in MgCl2 concentration (2 mM). After electrophoresis in a 2.5% agarose gel in a 1· TAE buffer stained with ethidium bromide, ISSR fragments were scored for presence or absence. Binary matrix was subjected to analysis of molecular variance (Amova) (Schneider et al. 2000), cluster analysis (Van de Peer and de Wachter 1994) and principal component analysis (PCA) (SAS 8e for windows).

Table 1 List of 51 oca samples used for molecular analysis, collected on 20 tuber lots corresponding to six varieties conserved in situ and ex situ

Six varieties	51 samples		
	In situ	Ex situ	
Yurac piliruntu	C1a–C1b–C1c	T9a–T9b–T9c	BOL 4398
		T10a–T10b–T10c	BOL 4405
Kellu kayara	C2a–C2b–C2c	T11a–T11b–T11c	BOL 4428
	C3b–C3c	T12a–T12b	BOL 4430
		T13a–T13b–13c	BOL 4434
Kamusa	C4a	T14a–T14b–T14c	BOL 4422
		T15a–T15b–T15c	BOL 4426
Titicoma	C5a–C5b	T16a–T16b	BOL 4366
		T17a	BOL 4372
Señora oca	C6a–C6b–C6c	T18a–T18b–T18c	BOL 4357
		T19a–T19b–T19c	BOL 4360
Lluchu oca	C7a–C7b	T20a–T20b–T20c	BOL 4359
	C8a–C8b–C8c		
Total 20 tuber lots = 8 conserved in situ + 12 conserved ex situ			

Samples are identified by origin (C = Candelaria, T = Toralapa), collection number (1–20) of tuber lot, an index (a, b and c; corresponding to one of three samples collected by tuber lot) and vernacular name. For accessions maintained ex situ, initial number is specified (BOL xxxx)

3. Results

Results reflect the ability of ISSRs in revealing genetic variability within a limited sample of Bolivian oca germplasm. Analysis performed with four primers revealed 25 fragments, of which 17 were polymorphic. To estimate the variance components, three different Amova's were performed. A first 2-levels Amova applied to the total 20 tuber lots studied showed, at very highly significance level, a higher genetic variation among tuber lots (82.93%) than within tuber lots (17.07%, $P < 0.001$), which is in agreement with the vegetative reproduction of oca. To assess the influence of the conservation system, we conducted Amova for the materials conserved *in situ* and *ex situ*. Variation was higher within tuber lots conserved *in situ* (24.19%, P < 0.001) compared to those conserved *ex situ* (15.04%, P < 0.001). In an UPGMA dendrogram (Fig. 1) most of the 51 tested samples were grouped according to varietal names, into two major groups (A and B), regrouping respectively clusters a–e and f–h. Genetic distances of Dice ranges from 0 to 0.51. This cluster analysis also confirmed that genetic differentiation is higher among than within tuber lots. ISSR data allowed to discriminate all lots of oca, even some identified by the same vernacular name. When 2 or 3 samples were available for a tuber lot, heterogeneity was noticed within varieties. By comparing the two conservation strategies (*in situ* and *ex situ*), we observed that four clusters (c, e, g and h) were formed by tuber lots having the same vernacular name and conserved in Candelaria and Toralapa. Molecular study also showed divergence among many varieties conserved *in situ* and *ex situ*. This is well illustrated by Kellu kayara, for which lots T12, T13 and C3 were grouped in cluster e while the lot T11 and C2 were found respectively in cluster a and d.

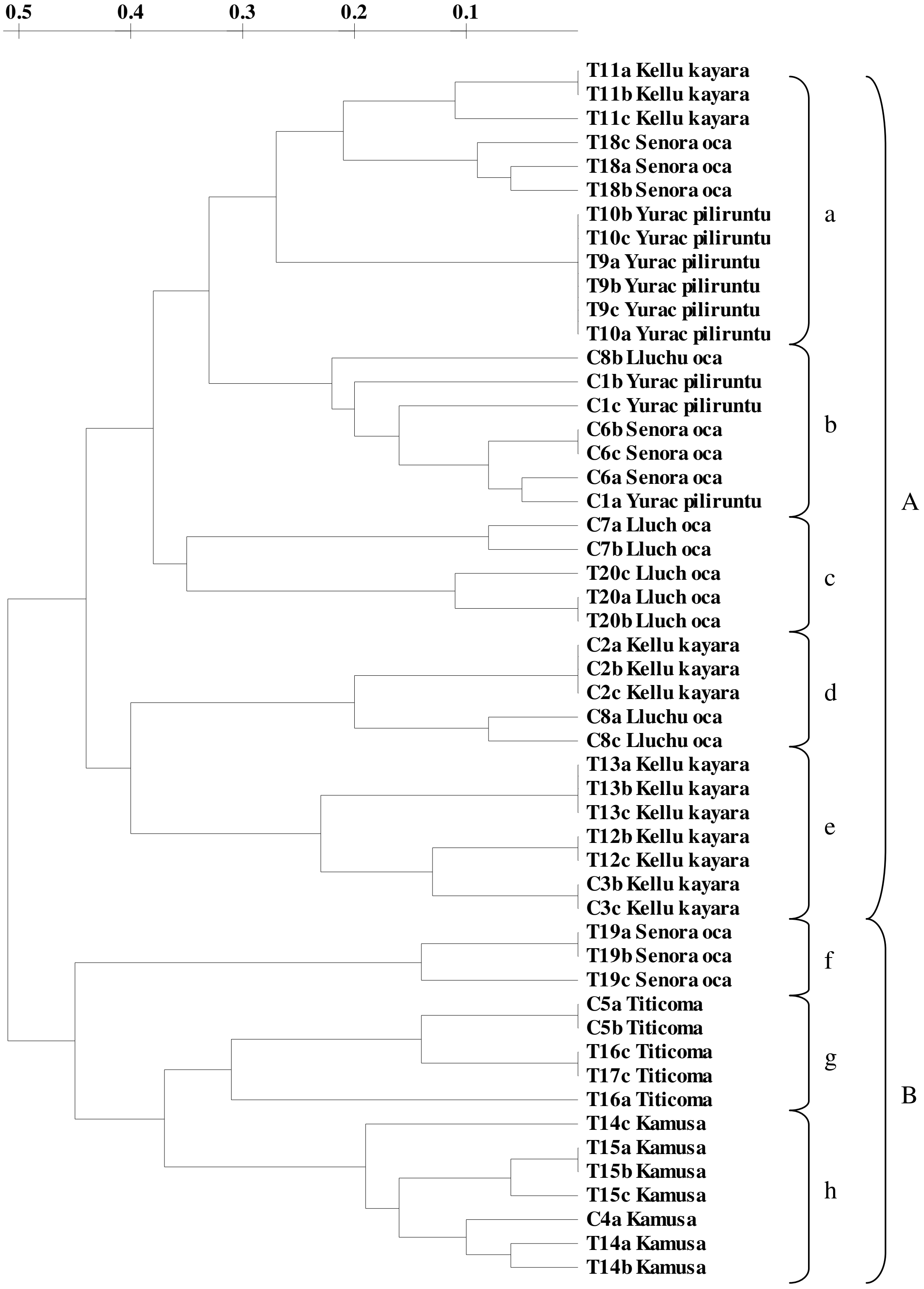

Fig. 1 Dendrogram based on ISSR polymorphism of the 51 oca's individuals representing 20 tuber lots conserved *in situ* and *ex situ*: application of unweighted paired group method algorithm (UPGMA) and distance of Dice

Figure 2 presents the first two axes of the Principal Components Analysis. Tuber lot Señora oca T19 is clearly isolated from the others and forms group I. According to the axis 2, group III is well separated from group I, which was not shown with the dendrogram. Oca's individuals in group III of PCA belong to the group B of the dendrogram while the individuals in group II of PCA belong to the group A. The overall structure of the studied Bolivian oca material is related to the tuber lots, except for Señora oca T19.

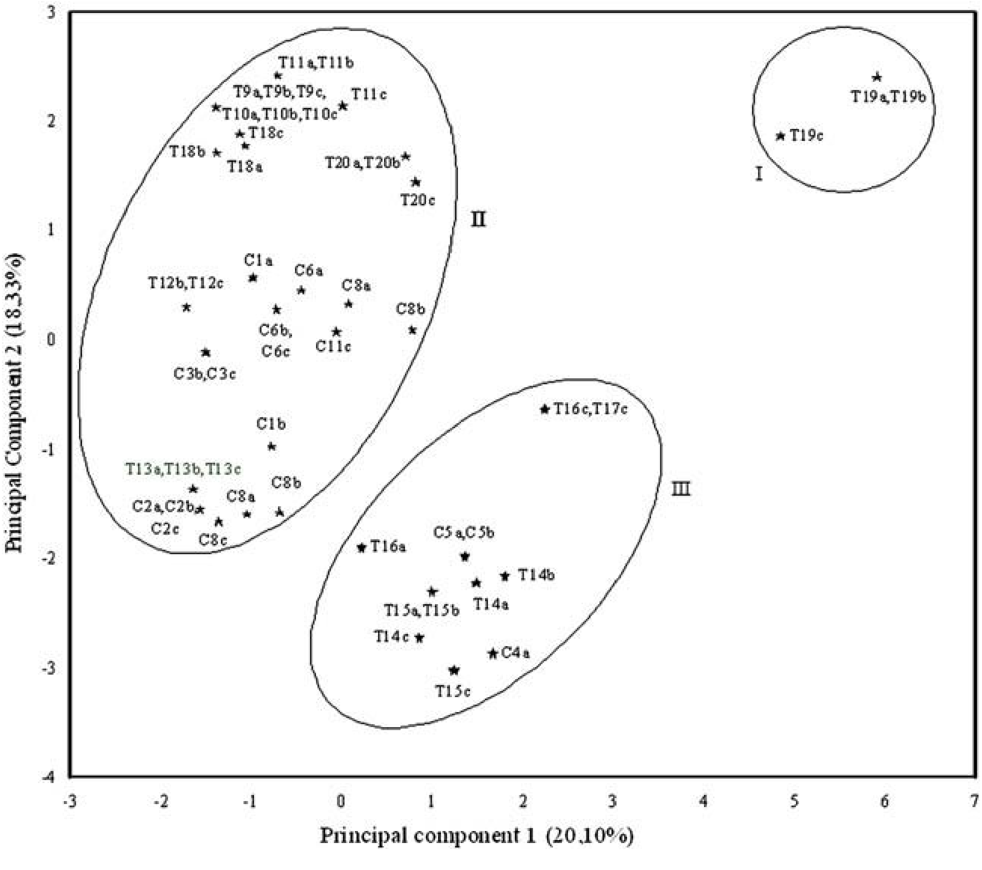

Fig. 2 Principal Component Analysis based on ISSR polymorphism of 51 oca's individuals representing 20 tuber lots conserved *in situ* and *ex situ*

4. Discussion

Efforts are needed to characterize and conserve genetic diversity of oca, an essential component of Andean farmers' communities. Genetic diversity data using molecular markers were until now relatively scarce. In this preliminary study, genetic diversity of Bolivian oca maintained *in situ* and *ex situ* was assessed. ISSR technique gave additional information that contributes to explore genetic resources of oca and to develop long-term conservation strategies. ISSR markers showed a great genetic differentiation among tuber lots of oca as well as a tendency for a higher similarity among varieties having the same vernacular name. A major part of the variation was observed between lots, supporting data from the present way of classification based on agromorphological description of varieties. Original considerations can be pointed out with ISSRs. Our results revealed intravarietal diversity for some tuber lots, which are heterogeneous even if collected from a single farmer or accession. However, more individual samples by tuber lot are needed to conclude about intravarietal diversity for genetics and conservation purposes. As a strict vegetative propagation is observed in the oca species, due to a stylar incompatibility, such intravarietal variability could be ascribed to mutations or to confusion of genetically distinct but morphologically similar individuals, as observed by Elias et al. (2001) on cassava. Until now, efforts made by the PROINPA Foundation for a better complementarity of the two conservation systems focus on the *in situ* and *ex situ* preservation of varieties identified by a vernacular name and morphological features. However, molecular data illustrated divergences among some varieties maintained in both conservation systems. Moreover, intravarietal diversity seems to be higher *in situ*. At present time, due to the relatively short *ex situ* conservation history of oca in Toralapa, explanation can only be credited by different way of tubers' management in the two systems and their specific characteristics. In *ex situ* system on the one hand, the use of a restricted number of tubers for the establishment of the collection and for its annual regeneration could lead to a bottleneck effect. In the *in situ* system, on the other hand, larger populations of oca in farmers' fields are subjected to various cultural practices, including rotations, mixed cropping, planting at different altitudes, as well as to gene flow in agrobiodiversity fairs (exchange of tubers, barter), which allows a diversification of oca varieties. These particular characteristics could lead to a divergence between oca materials maintained in the two systems, as reported by Del Rio et al. (1997) in potato, in common bean by Gomez et al. (2005) or in oca, ulluco and isaño in Equator by Tapia et al. (2004). Complementarity between the two conservation strategies (*in situ*/*ex situ*) needs therefore to be adapted accordingly. In order to preserve genetic resources of oca *in situ* and *ex situ*, we need to redefine biological unit of conservation.

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Complement to chapter III

Assignment tests applied to data of the publication Malice *et al.*, 2007

After publication of the previous results, binary matrix was reused to analyze the genetic structure of the sample studied. The software Structure 2.2 (Pritchard *et al.*, 2000) was used to perform assignment tests and to assign individual accessions to gene pools or genetic clusters (K). We followed the method described in Chapter V of the thesis.

Based on Figure 5, we chose k=3 as the most likely number of distinct genetic clusters in this sample of oca. The highest value of ΔK at k=3 was 43.9.

Results of assignment tests (Figure 6) confirm the results presented in Malice et al. (2007). Our study addressed the pattern of genetic diversity of oca varieties at small scale. We have shown great diversity and demonstrated the dynamism of this diversity in relation to both space and time, at the scale of a single microcentre.

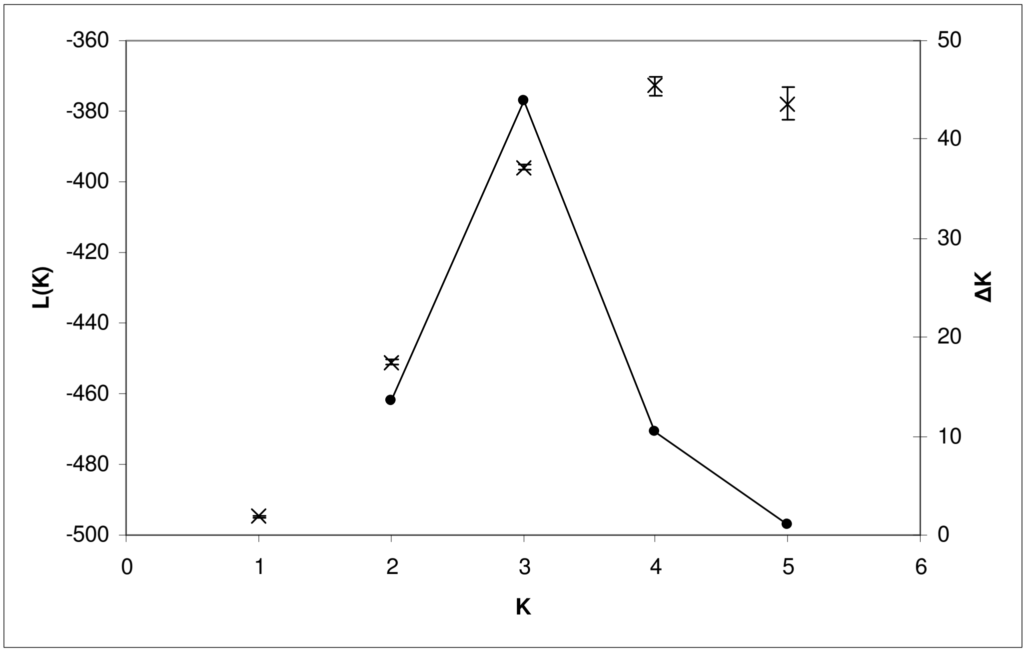

Figure 5: Inference of genetic clusters (*K*) using the model-based clustering method of Pritchard *et al.* (2000).

Mean (±standard deviation) of log probability of data [L(K)] based on five independent runs (x) as a function of the value of K and the rate of change in the log probability of data ΔK between successive values of K (●) for the whole data set (N = 51).

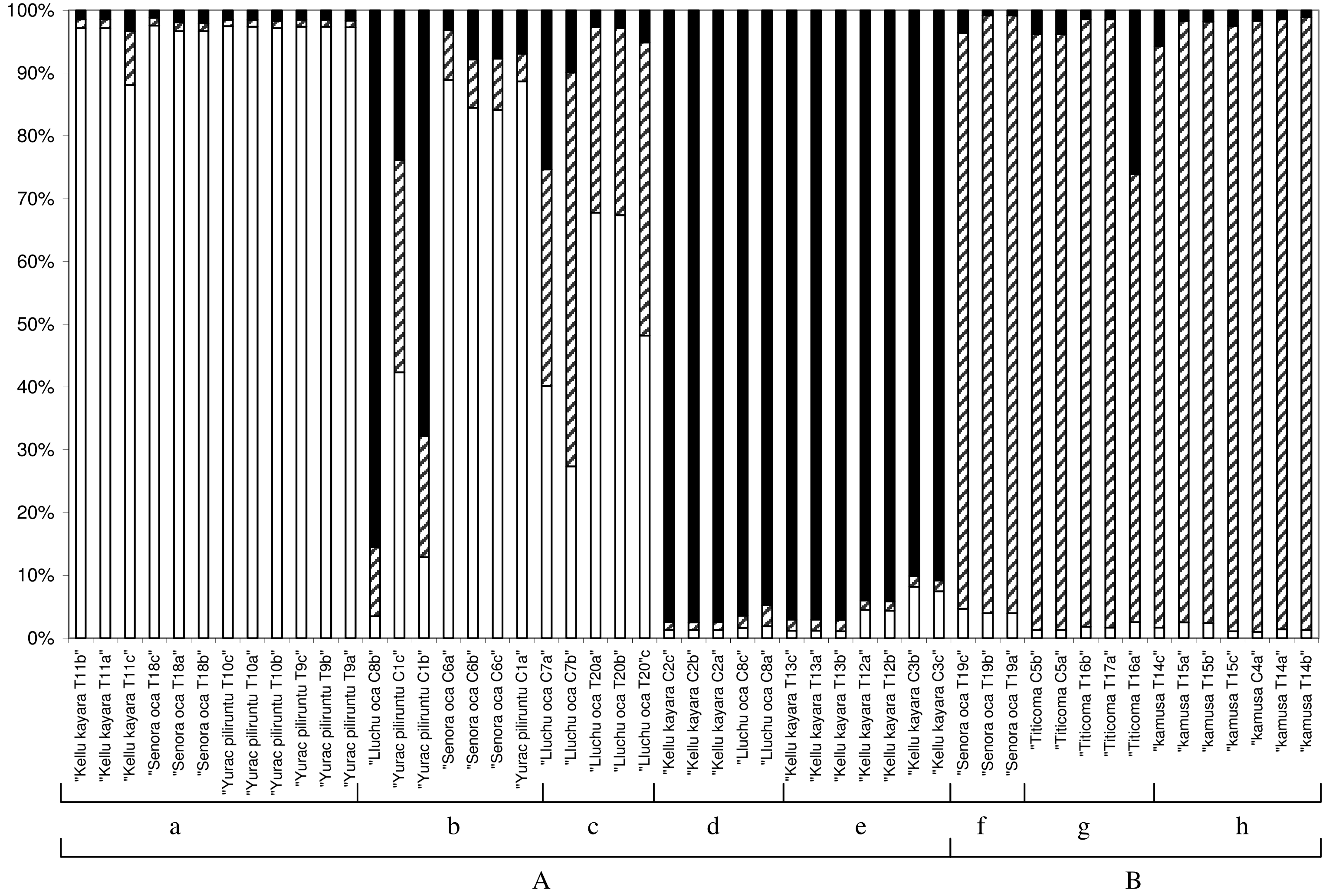

Figure 6: Structure of the genetic diversity of the 51 oca accessions as assigned into 3 genetic clusters (K) detected using the model-based clustering method of Pritchard *et al.* (2000).

Clusters presented in the dendrogram (Chapter III, Fig.1) have been reported in Figure 6. F_{ST} calculations conducted on the clusters defined by Structure analysis (for k = 3) showed that genetic differentiation between clusters was very highly significant (F_{ST} = 0.22, $P<0.001$). The AMOVA analysis revealed that the total variation was mainly due to variability within clusters (77.7%, $P<0.001$) rather than among clusters (22.3%, $P<0.001$).

The results of the assignment test and the definition of 3 genetic clusters could be related to the "uses categories", as defined by Emshwiller (2006) on oca or Zimmerer (1991) on potato. During her research, Emshwiller (2006) showed that oca, like potato (Zimmerer, 1991), is classified into "uses categories" and that the molecular data (AFLP) agree with the classification of oca by Quechua farmers in Pisac District into two use categories: (1) sweet varieties, called *wayk'u* (boiled) oca, exposed to sunlight for a few fays to sweeten them and (2) the varieties preserved by processing into dried oca tubers called *kaya*, prepared by exposing tubers to several alternating days of hot sun and nights of frost until they are completely dry, similarly to the process of making *chuño* from Andean potatoes.

It is known that farmer's practices and preferences influence genetic diversity and structure of their crops. Concerning oca, our results suggest the interesting hypothesis that these use categories may have different evolutionary histories, revealed by the distinct genetic clusters we found. Anyway, these presumptions should be confirmed by other data.

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CHAPTER IV: Genetic diversity of the Andean tuber crop species *Ullucus tuberosus* as revealed by molecular (ISSR) and morphological markers

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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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Running title: Genetic diversity of *Ullucus tuberosus*

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.

Keywords: *Ullucus tuberosus*, Andean tuber, genetic diversity, ISSR, molecular markers, morphological markers.

1. 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 conservation 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 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.

2. Material and Methods

2.1. Plant materials

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).

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

Morphotype	Accessions	Morphotype	Accessions	Morphotype	Accessions
M4	CIP 201323, CIP 201364, CIP 201330,	M37	CIP 201007	M99	CIP 201115
	CIP 201319, CIP 201134, CIP 201316,	M38	CIP 201009	M100	CIP 201116
	CIP 201321, CIP 201352, CIP 201360,	M39	CIP 201010	M101	CIP 201120
	CIP 201322, CIP 201328, CIP 201353,	M40	CIP 201062	M102	CIP 201122
	CIP 201317, CIP 201001, CIP 201315,	M41	CIP 201063	M109	CIP 201147
	CIP 201361, CIP 201324, CIP 201318	M42	CIP 201064	M110	CIP 201148
M5	CIP 201002	M44	CIP 201294	M111	CIP 201149
M6	CIP 201003	M45	CIP 201112, CIP 201066, CIP 201111	M112	CIP 201150
M7	CIP 201005	M46	CIP 201067	M113	CIP 201418, CIP 201153
M8	CIP 201443	M47	CIP 201072	M120	CIP 201448
M9	CIP 201033, CIP 201049, CIP 201373,	M48	CIP 201074, CIP 201073	M122	CIP 201075
	CIP 201035, CIP 201444	M49	CIP 201432, CIP 201461, CIP 201472	M124	CIP 201277
M11	CIP 201008, CIP 201441, CIP 201446	M50	CIP 201433	M125	CIP 201375
M12	CIP 201025	M51	CIP 201434	M126	CIP 201376
M13	CIP 201026	M53	CIP 201310	M127	CIP 201380
M14	CIP 201387, CIP 201054, CIP 201029	M54	CIP 201311	M128	CIP 201393, CIP 201382
M16	CIP 201021, CIP 201019, CIP 201015,	M55	CIP 201312	M129	CIP 201385
	CIP 201383, CIP 201011, CIP 201436	M56	CIP 201329	M130	CIP 201389
M17	CIP 201057, CIP 201386, CIP 201017	M57	CIP 201331	M131	CIP 201396
M18	CIP 201051	M58	CIP 201121, CIP 201332, CIP 201123	M134	CIP 201397
M19	CIP 201052	M64	CIP 201420, CIP 201379, CIP 201388	M135	CIP 201398
M20	CIP 201381, CIP 201053	M65	CIP 201365	M137	CIP 201283
M21	CIP 201058	M67	CIP 201466, CIP 201451, CIP 201460	M138	CIP 201401
M22	CIP 201430, CIP 201428, CIP 201429,	M68	CIP 201452	M139	CIP 201402
	CIP 201459, CIP 201467, CIP 201468	M69	CIP 201279, CIP 201453, CIP 201473,	M140	CIP 201404
M23	CIP 201427		CIP 201478, CIP 201521	M141	CIP 201405
M24	CIP 201334, CIP 201431, CIP 201439,	M70	CIP 201454	M144	CIP 201406
	CIP 201437	M71	CIP 201455	M145	CIP 201408
M25	CIP 201023, CIP 201034	M72	CIP 201391	M146	CIP 201409
M26	CIP 201016, CIP 201377, CIP 201394,	M73	CIP 201458	M147	CIP 201410

	CIP 201400, CIP 201036	M74	CIP 201475	M148	CIP 201419, CIP 201415, CIP 201412
M27	CIP 201359, CIP 201038, CIP 201362, CIP 201355	M75	CIP 201462, CIP 201476	M149	CIP 201414, CIP 201413
M28	CIP 201041	M76	CIP 201463	M150	CIP 201417, CIP 201422
M29	CIP 201449, CIP 201438	M77	CIP 201464	M152	CIP 201421
M30	CIP 201440	M78	CIP 201465	M154	CIP 201285
M31	CIP 201384, CIP 201407, CIP 201442	M79	CIP 201403, CIP 201280, CIP 201390, CIP 201469, CIP 201471	M155	CIP 201293
M32	CIP 201012	M80	CIP 201470	M156	CIP 201289
M33	CIP 201013	M82	CIP 201477	M157	CIP 201292
M34	CIP 201014, CIP 201024	M87	CIP 201411	M159	CIP 201416
M36	CIP 201022			M162	CIP 201378

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

Country	Collecting 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

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

2.2. 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 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).

2.3. 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 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.

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.

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).

3. Results

3.1. Morphological and molecular diversity

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

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		

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.

3.2. 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).

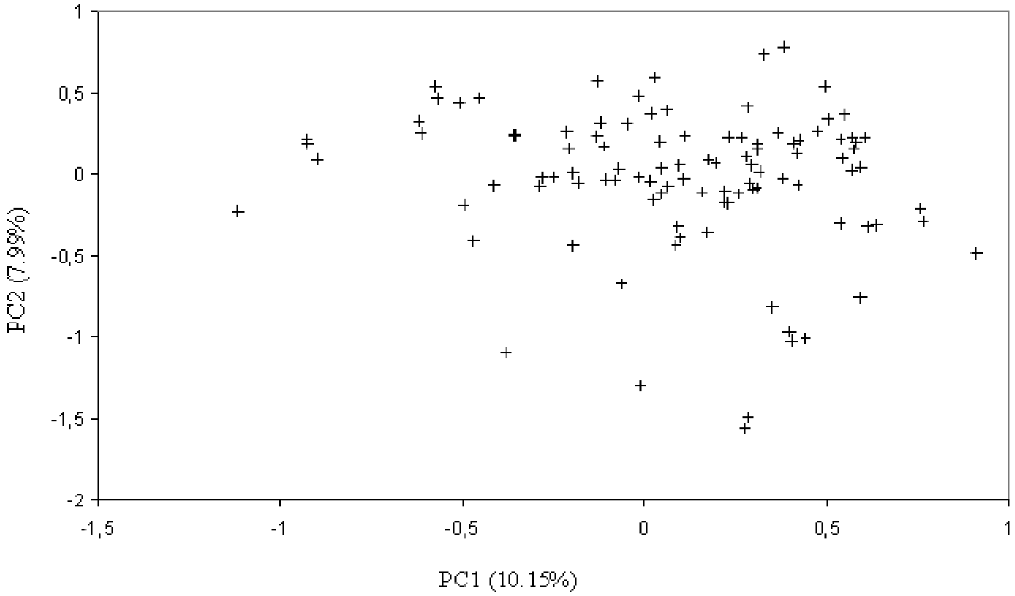

Fig. 2: MCA plot based on morphological characterisation.

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.

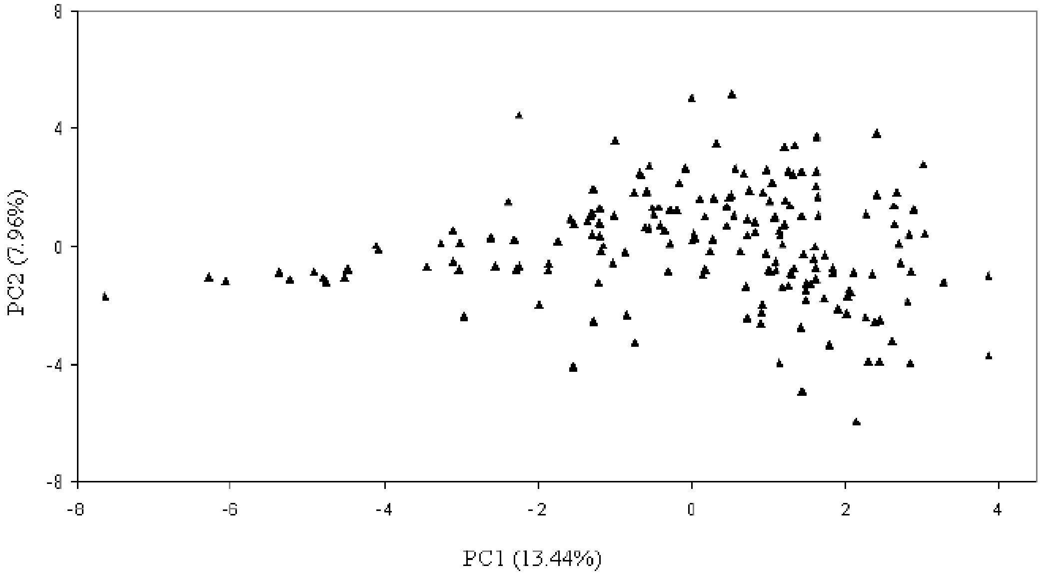

Fig. 3: PCA plot based on molecular (ISSR) characterisation.

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 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).

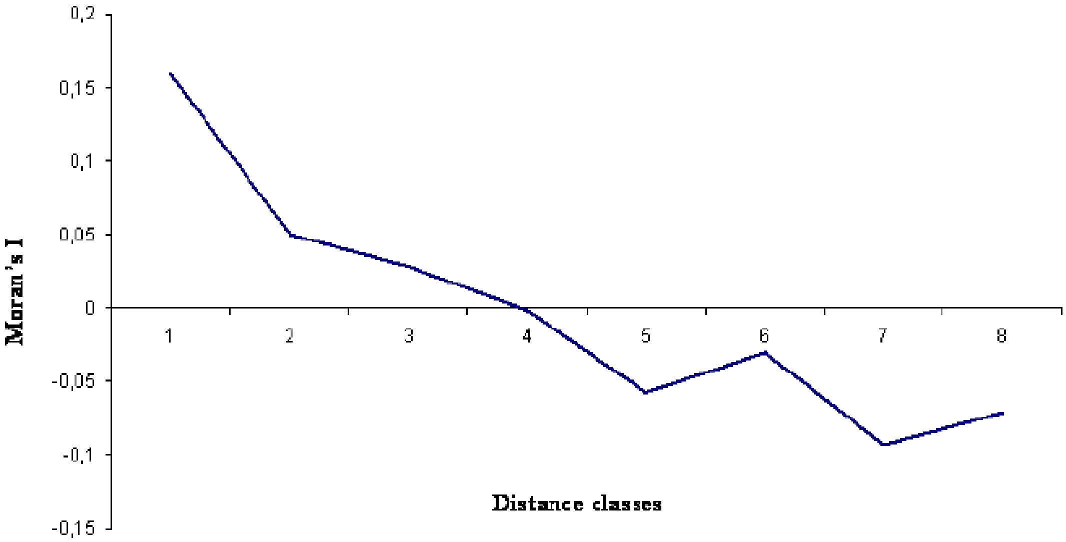

Fig. 4: Correlogram of Moran's *I* per distance class obtained from ISSR data.

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 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).

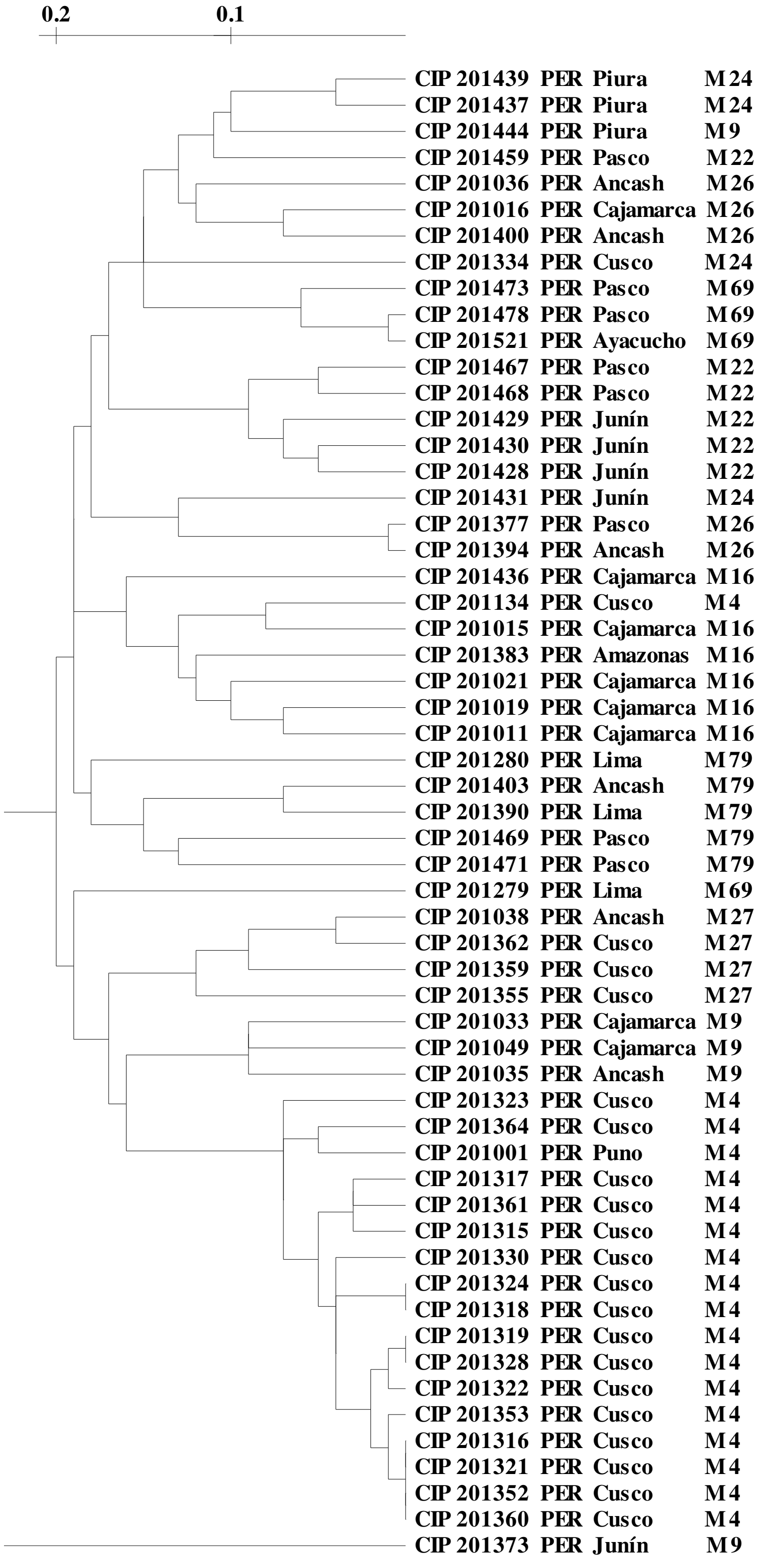

Fig. 5. Dendrogram based on ISSR polymorphism and Jaccard's distance. The only nine morphotypes represented by four or more accessions are taken into account.

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).

4. 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 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 a 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.

Acknowledgements

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Complement to chapter IV

Assignment tests applied to data of the publication

Malice *et al.*, 2009

As for the chapter III, we completed the data analysis of chapter IV with the analysis of the genetic structure with the software Structure 2.2 (Pritchard *et al*., 2000) and assigned ulluco accessions to genetic clusters (K). We followed the method described in Chapter V of the thesis.

Based on Figure 7, we chose k=2 as the number of distinct genetic clusters in this sample of ulluco. Results of the assignment test (Figure 8) were congruent with those presented in Malice et al. (Under press). F_{ST} calculations conducted on the clusters defined by Structure analysis (for k = 2) showed that genetic differentiation between clusters was very highly significant ($F_{ST} = 0.35$, $P<0.001$). The AMOVA analysis revealed that the total variation was mainly due to variability within clusters (65.4%, $P<0.001$) rather than among clusters (34.6%, $P<0.001$).

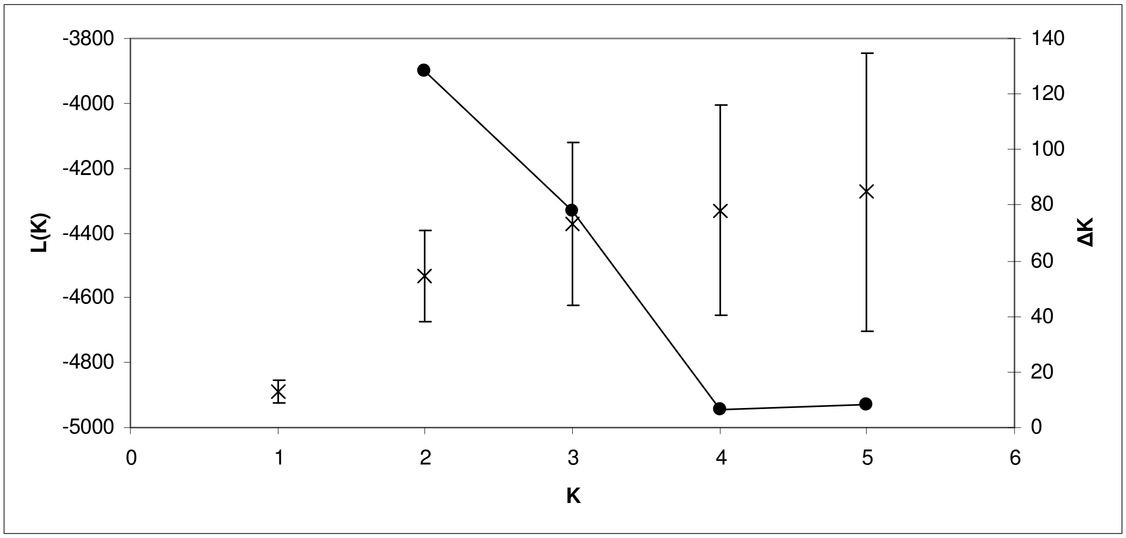

Figure 7: Inference of genetic clusters (k) using the model-based clustering method of Pritchard *et al*. (2000).

Mean (±standard deviation) of log probability of data [L(k)] based on five independent runs (x) as a function of the value of k and the rate of change in the log probability of data Δk between successive values of k (●) for the whole data set (N = 187).

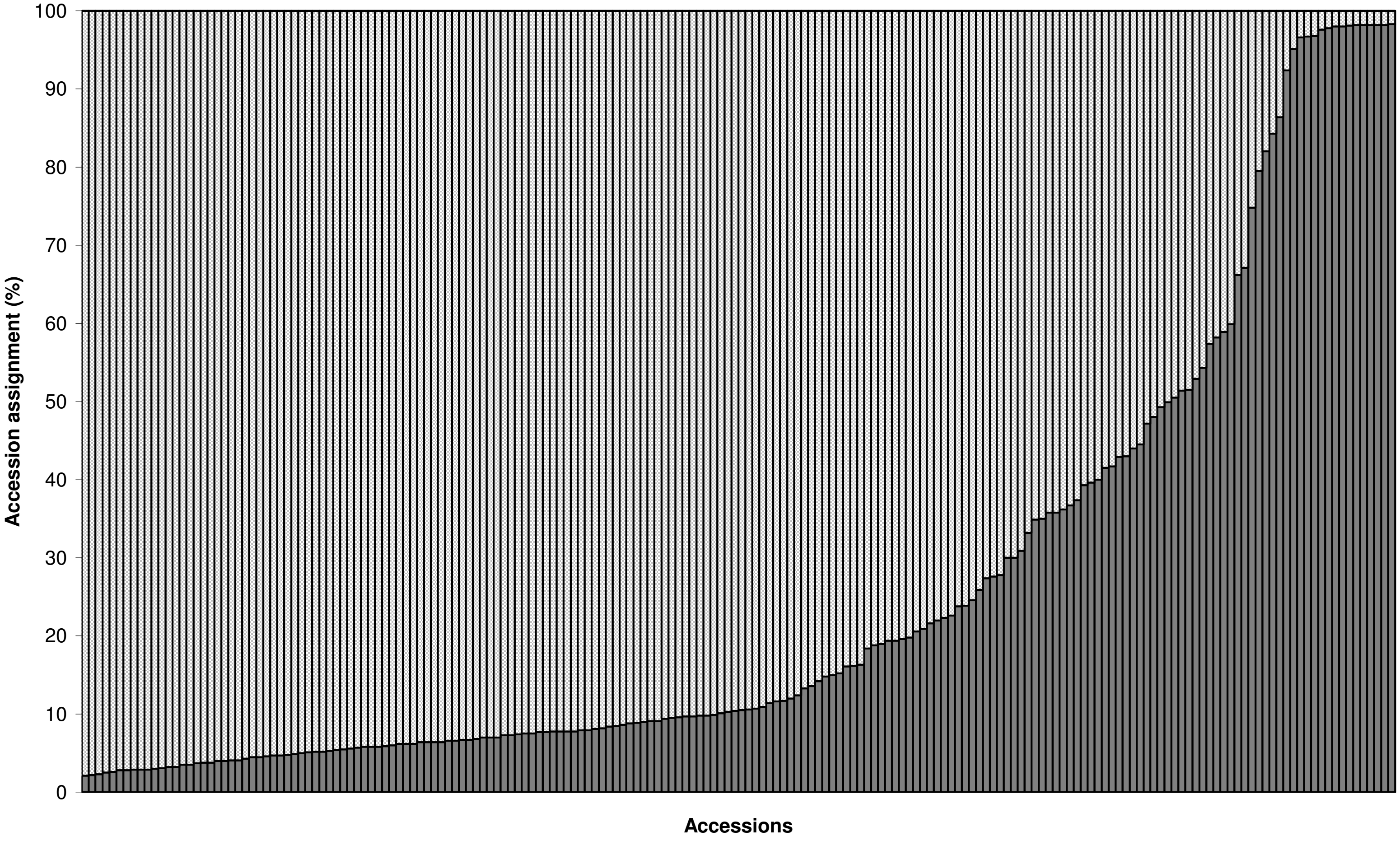

Figure 8: Structure of the genetic diversity of the 51 oca accessions as assigned into 3 genetic clusters (k) detected using the model-based clustering method of Pritchard *et al*. (2000).

The Structure-assigned individual accessions were plotted on the Principal Component Analysis (PCA) graph. Figure 9 shows the position of the 187 ulluco accessions, defined by the first two principal coordinates PC1 and PC2. Globally, the PCA confirmed our previous analyses. This PCA plot clearly shows that PC1 separates accessions according to their Structure assignment [from left to right: △ accessions of the first genetic cluster (q_{mean} > 80%) ; ■ : accessions intermediary between first and second clusters (q^{mean} < 80%) ;

⬣ accessions of the second genetic clusters (q_{mean} > 80%)].

The same method was followed to build a collection map (Figure 10), with regard to the genetic cluster the accessions belong to. Interestingly, Figure 10 shows that accessions representative of the second genetic cluster ⬣ originate from North Peru, while accessions representative of the first genetic cluster △ originate from South Peru and North Bolivia. Accessions intermediary between first and second clusters ■ are distributed all along the North-South gradient.

Many studies have indicated that the organisation of ulluco diversity is linked to geographic classifications (Cardenas & Hawkes, 1948; Rousi *et al.*, 1989; Pietilä & Jokela, 1990). Our results also reflect such geographical structure.

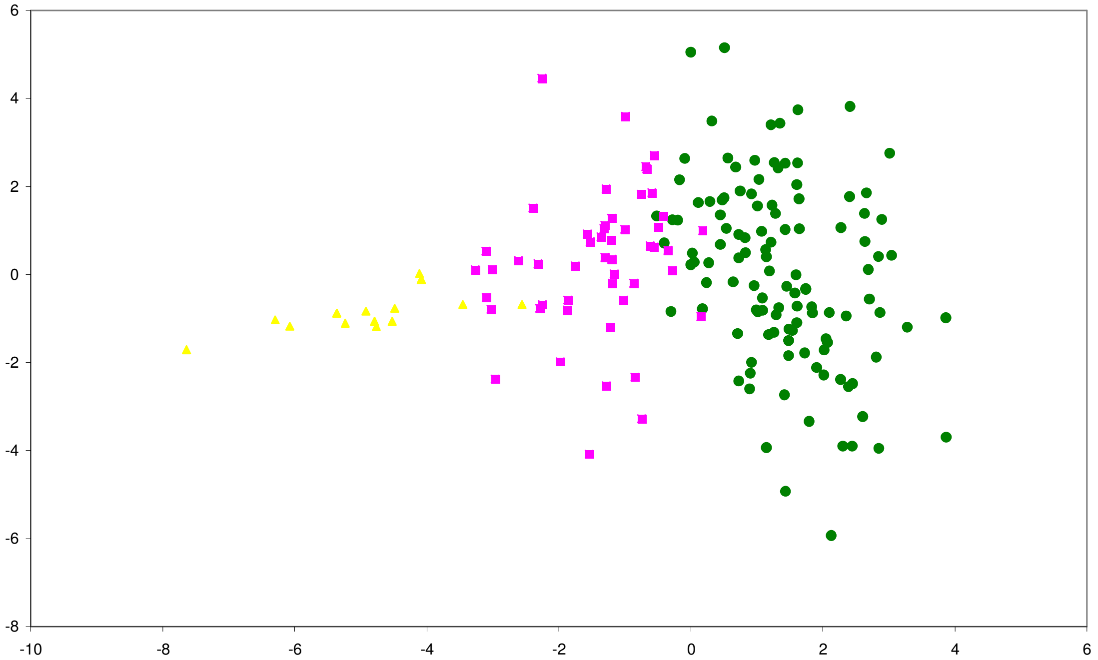

Figure 9: PCA plot based on molecular (ISSR) characterization of the 187 ulluco accessions analyzed in Malice et al. (Under press). Symbols indicate genetic clusters defined by the Structure progam (△ and ⬣ : accessions of the first and second genetic clusters – q_{mean} > 80% / ■ : accessions intermediary between first and second clusters – q_{mean} < 80%)

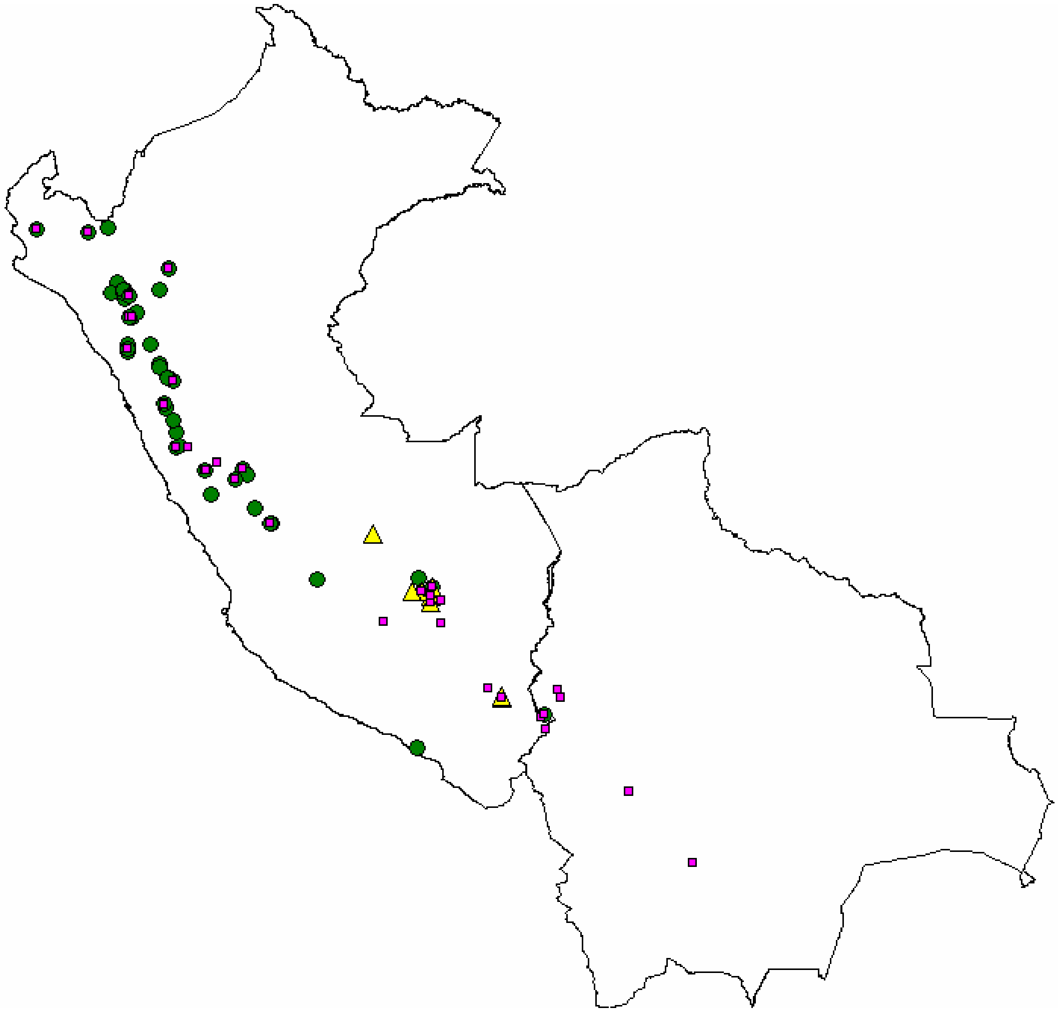

Figure 10: Collection map of the 187 ulluco accessions analyzed in Malice et al. (Under press). Symbols indicate genetic clusters defined by the Structure progam (△ and ● : accessions of the first and second genetic clusters – q_{mean} > 80% / ■ : accessions intermediary between first and second clusters – q_{mean} < 80%)

A probable explanation of our results (Figures 8 to 10) could be that ulluco may have developed from two main genepools, predominantly found in North Peru and the region encompassing South Peru and North Bolivia. According to Arbizu (2004), the area of the Lake Titicaca (South Peru - North Bolivia) is the origin centre of the ulluco.

The hypothesis that ulluco has evolved from two regions in the Andes (North Peru and the region of the Lake Titicaca), and radiated along the Andean axis, is suggested by patterns of genetic structuration we found, and is certainly linked to traditional farming practices and tubers' exchanges via markets, barters, biodiversity fairs…

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CHAPTER V: Evaluation of genetic diversity of Andean tuber crop species (oca, ulluco, mashua) in the *in situ* microcentre of diversity of Huanuco (Peru) using ISSR markers

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Evaluation of genetic diversity of neglected Andean tuber crop species (oca, ulluco, mashua) in the *in situ* microcentre of diversity of Huanuco (Peru) using ISSR markers

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Keywords: Andean tubers, ISSR, molecular markers, morphological markers, genetic diversity, Peru, assignment test

Abstract

Genetic diversity of three species of Andean tubers, oca (*Oxalis tuberosa* Molina), ulluco (*Ullucus tuberosus* Caldas) and mashua (*Tropaeolum tuberosum* Ruiz & Pav.) has been studied in a diversity microcentre (Huanuco, Peru) using the ISSR molecular markers. We compared the genetic diversity as given by the ISSR technique and the farmer's description of the varieties. A high degree of correspondence was found between the two types of classification. Assignment technique divided both oca and ulluco samples into two genetic clusters (K=2), while the mashua sample probably belongs to a single genetic cluster (K=1). For oca and ulluco, each genetic cluster regroups numerous varieties and ISSR technique enlightened intra-varietal genetic variability for most varieties, suggesting an underestimation of the *in situ* genetic variability. We attempted to explain how variation in breeding systems and farmers' practices influence patterns of genetic diversity. These findings confirm the intuition that a considerable amount of variability remains unexploited in neglected Andean tuber crop species.

1. Introduction

Oca (*Oxalis tuberosa* Molina), ulluco (*Ullucus tuberosus* Caldas) and mashua (*Tropaeolum tuberosum* Ruiz & Pav.) are tuber-bearing crop species endemic in the Andes. They are sporadically cultivated in marginal farming systems of high altitudes, from 2500 to 4100 meters (Cárdenas, 1964; Cadima Fuentes, 2006) in all Andean countries, with the highest concentration of diversity in the central Andes (southern Peru and northern Bolivia, Arbizu *et al.*, 1997). They are cultivated for their edible tubers and constitute, together with the potato species (*Solanum* sp.), Andean farmers' staple crops (Hernández Bermejo & León, 1992). Even if they flower in farming conditions, fruit and seed production are marginal for oca and ulluco, respectively due to a trimorphic system of genetic incompatibility (Gibbs, 1976; Trognitz *et al.*, 1998), and to morphological abnormalities in the ovules and embryo sacs combined with genetic loads (Pietilä & Jokela, 1994). In contrast, mashua flowers profusely and sets many viable seeds with high germination rates (Grau *et al.*, 2003). In farming conditions, propagation of these three species is exclusively realized through tubers, and botanical seed propagation by farmers has never been observed (Hernández Bermejo & León, 1992). Nonetheless, Andean tubers still display high levels of phenotypic diversity, especially in their tubers (Arbizu *et al.*, 1997). Unfortunately, Andean tubers diversity tends to decrease because of genetic erosion (Altieri & Merrick, 1987; Tapia & Estrella, 2001; Iriondo *et al.*, 2008), leading to the urgent need to establish appropriate conservation strategies. The first step to define genetic resources conservation programs for on farm *in situ* conservation consists in the evaluation of genetic diversity and structure of the species. Former studies of Andean tubers genetic diversity described variation between cultivars reflected by morphological traits (Rousi *et al.*, 1989; Terrazas & Valdivia, 1998; Grau *et al.*, 2003). More recently, molecular markers have been used to study this diversity and the ISSR technique has been successfully applied to evaluate genetic diversity in cultivated accessions of the three Andean tubers species (Malice *et al.*, 2007; Pissard *et al.*, 2008a-b; Malice *et al.*, under press). Main results brought by this approach have shown the presence of heterogeneous varieties, the congruence between molecular and morphological data, and a genetic structure influenced by the geographical provenance. All these findings were explained by the ways of tubers management and by the characteristics of the species and of the Andean agricultural system. Also, vernacular names assign by farmers to identify cultivars are numerous and reflect the high diversity of the crops (Arbizu *et al.*, 1997; Cadima Fuentes, 2006; Emshwiller, 2006). It is known that varieties are identified according to a set of traits related to tuber and sometimes plant characteristics (Terrazas & Valdivia, 1998). Previous results on oca (Malice *et al.*, 2007) showed that varieties structure is overall based upon vernacular names, supporting peasant classification.

In the present study, ISSR markers were used to assess the genetic variability of Andean tubers accessions in an *in situ* germplasm collection located in the microcentre of Huanuco (Peru). The diversity organization obtained by the molecular markers were compared with the classification of the Andean tuber varieties by folk taxonomy.

2. Materials and Methods

2.1. Study site and plant materials

This work was carried out in the microcentre of diversity of Huanuco (Fig.1), located in Peru, in the district of Kichki, province of Huanuco (09°54'06" latitude South, 76°26'28" longitude East). This area, that covers a surface of approximately 160 km², is located at an altitude ranging from 2500 to 4176 meters. Mean temperature is 10 °C with an annual rainfall of

1100 mm/year and a relative humidity of 83%. Soils are sandy and shallow with organic matter content lower than 2%.

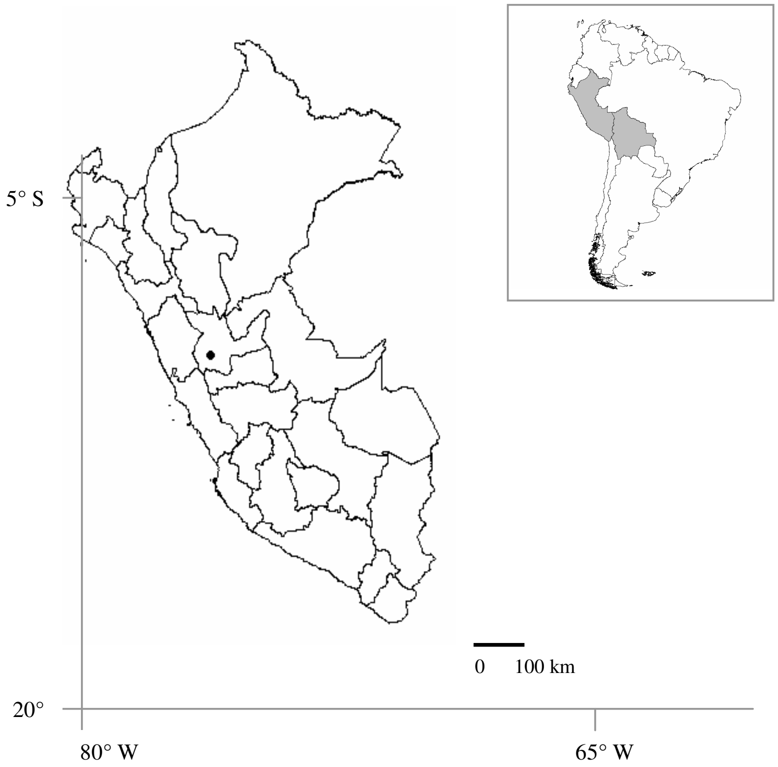

Figure 1. Map of Peru showing the location of the microcentre of Huanuco where the study was conducted.

The plants were obtained from the germplasm collection constituted in 2005 from collecting missions in peasant fields throughout the microcentre and maintained in a conservation garden in Shircapata-Huayllacallán. According to Maxted *et al.* (1997), conservation gardens are one form on the *in situ* conservation, where are maintained the varieties found in the microcentre. This garden has been installed and is managed with the collaboration of the farmers.

Sampling strategy was designed to study the inter- and intra-varietal diversities conserved in the peasant communities of the microcentre. Passport data and varieties names were obtained from farmers during the collecting missions. The samples consisted in 15 varieties of oca (Table 1A), 15 of ulluco (Table 1B) and 26 of mashua (Table 1C) collected within the conservation garden in year 2006. Hereafter we have considered a `variety' as a set of tubers originating from various locations and grown by various farmers, identified by a single varietal name using folk taxonomy (Malice *et al.*, 2007). For each variety, 1 to 3 individual plants, identified by an ID number, were collected from one or several farmers maintaining this variety (Table 1).

Table 1. List of the oca (A), ulluco (B) and mashua (C) samples used for molecular analysis.

A

Variety	Individual plant (ID number)	Peasant community	Farmer
Anaranjado oga	MTPA-079	San Juan deTingo	Feliciano Hilario
	MTPA-105	Tres Mayo de Huayllacayan	Gerardo Sanchez
	MTPA-134	Tres Mayo de Huayllacayan	Gerardo Sanchez
Cera oga	MTPA-023	Santa Rosa de Monte Azul	Teodoro Rosado
	MTPA-081	San Juan deTingo	Feliciano Hilario
Garwaricra	MTPA-040	Santa Rosa de Monte Azul	Teodoro Rosado
	MTPA-090	San Juan deTingo	Gerardo Sanchez
Garwash pillao	MTPA-109	Tres Mayo de Huayllacayan	Gerardo Sanchez
	MTPA-121	Tres Mayo de Huayllacayan	Gerardo Sanchez
Garwuash piña	MTPA-013	Santa Rosa de Monte Azul	Teodoro Rosado
	MTPA-041	Santa Rosa de Monte Azul	Teodoro Rosado
Huayta oga	MTPA-017	Santa Rosa de Monte Azul	Teodoro Rosado
	MTPA-083	San Juan deTingo	Feliciano Hilario
Jilguero	MTPA-014	Santa Rosa de Monte Azul	Teodoro Rosado
	MTPA-104	Tres Mayo de Huayllacayan	Gerardo Sanchez
Leche con café	MTPA-022	Santa Rosa de Monte Azul	Teodoro Rosado
	MTPA-052	Santa Rosa de Monte Azul	Teodoro Rosado
Mio mio	MTPA-008	Santa Rosa de Monte Azul	Teodoro Rosado
	MTPA-012	Santa Rosa de Monte Azul	Teodoro Rosado
Muru tornillo	MTPA-066	Santa Rosa de Monte Azul	Teodoro Rosado
	MTPA-088	San Juan deTingo	Feliciano Hilario
Ogllag papa oga	MTPA-070	San Juan deTingo	Feliciano Hilario
	MTPA-113	Tres Mayo de Huayllacayan	Gerardo Sanchez
Pillahuina	MTPA-027	Santa Rosa de Monte Azul	Teodoro Rosado
	MTPA-040	Santa Rosa de Monte Azul	Teodoro Rosado
Rosado oga	MTPA-005	Santa Rosa de Monte Azul	Teodoro Rosado
	MTPA-011	Santa Rosa de Monte Azul	Teodoro Rosado
Yana pillao	MTPA 039	Santa Rosa de Monte Azul	Teodoro Rosado
	MTPA 084	San Juan deTingo	Feliciano Hilario
	MTPA 086	San Juan deTingo	Feliciano Hilario
Yuraj pillao	MTPA 044	Santa Rosa de Monte Azul	Teodoro Rosado
	MTPA 095	Tres Mayo de Huayllacayan	Gerardo Sanchez

B

Variety	**Individual plant (ID number)**	**Peasant community**	**Farmer**
Anaranjado redondo	MTBM-288	Tres Mayo de Huayllacayan	Gerardo Sánchez
	MTBM-264	San Alejandro de Calláncas	Moises Nolasco
	MTBM-222	Santa Rosa de Monte Azul	Teodoro Rosado
Anaranjado Wickllush	MTBM-279	Tres Mayo de Huayllacayan	Gerardo Sánchez
	MTBM-211	Santa Rosa de Monte Azul	Teodoro Rosado
Garwash manca ñawi	MTBM-246	San Juan de Tingo	Feliciano Hilario
	MTBM-201	Santa Rosa de Monte Azul	Teodoro Rosado
Garwash pikllush	MTBM-284	Tres Mayo de Huayllacayan	Gerardo Sánchez
	MTBM-203	Santa Rosa de Monte Azul	Teodoro Rosado
Garwash shaipy	MTBM-255	San Alejandro de Calláncas	Moises Nolasco
	MTBM-215	Santa Rosa de Monte Azul	Teodoro Rosado
Garwash wikllush	MTBM-239	San Juan de Tingo	Feliciano Hilario
	MTBM-213	Santa Rosa de Monte Azul	Teodoro Rosado
Juyto sarampión	MTBM-274	Tres Mayo de Huayllacayan	Gerardo Sánchez
	MTBM-257	San Alejandro de Calláncas	Moises Nolasco
	MTBM-231	Santa Rosa de Monte Azul	Teodoro Rosado
Olluima olluco	MTBM-223	Santa Rosa de Monte Azul	Teodoro Rosado
	MTBM-243	San Juan de Tingo	Feliciano Hilario
Rosado manca ñawi	MTBM-236	San Juan de Tingo	Feliciano Hilario
	MTBM-271	Tres Mayo de Huayllacayan	Gerardo Sánchez
	MTBM-206	Santa Rosa de Monte Azul	Teodoro Rosado
Shactay olluco	MTBM-238	San Juan de Tingo	Feliciano Hilario
	MTBM-273	Tres Mayo de Huayllacayan	Gerardo Sánchez
Shulash olloco	MTBM-251	San Alejandro de Calláncas	Moises Nolasco
	MTBM-202	Santa Rosa de Monte Azul	Teodoro Rosado
	MTBM-241	San Juan de Tingo	Feliciano Hilario
	MTBM-277	Tres Mayo de Huayllacayan	Gerardo Sánchez
Tarma olluco	MTBM-252	San Alejandro de Calláncas	Moises Nolasco
	MTBM-226	Santa Rosa de Monte Azul	Teodoro Rosado
Tarmeña olluco	MTBM-234	San Juan de Tingo	Feliciano Hilario
	MTBM-209	Santa Rosa de Monte Azul	Teodoro Rosado
Tarmeñita	MTBM-237	San Juan de Tingo	Feliciano Hilario
	MTBM-289	Tres Mayo de Huayllacayan	Gerardo Sánchez
	MTBM-259	San Alejandro de Calláncas	Moises Nolasco
Verde juyto	MTBM-276	Tres Mayo de Huayllacayan	Gerardo Sánchez
	MTBM-227	Santa Rosa de Monte Azul	Teodoro Rosado

C

Variety	Individual plant (ID number)	Peasant community	Farmer
Ataguepsha	MTHP-075	Santa Rosa de Monte Azul	Teodoro Rosado
Cansha shagapa	MTHP-273	San Alejandro de Calláncas	Moises Nolasco
Chaucha rosado	MTHP-207	San Juan de Tingo	Feliciano Hilario
Chicchicsa mashua	MTHP-082	Santa Rosa de Monte Azul	Teodoro Rosado
Gallopa tristan	MTHP-119	Santa Rosa de Monte Azul	Teodoro Rosado
Garwash warina	MTHP-275	San Alejandro de Calláncas	Moises Nolasco
Garwash yana ñawi	MTHP-254	San Alejandro de Calláncas	Moises Nolasco
Huaytash	MTHP-267	San Alejandro de Calláncas	Moises Nolasco
Jirca yatash	MTHP-202	San Juan de Tingo	Feliciano Hilario
Limon mashua	MTHP-060	Santa Rosa de Monte Azul	Teodoro Rosado
Manca ñawi	MTHP-057	Santa Rosa de Monte Azul	Teodoro Rosado
Misha trompash	MTHP-247	San Alejandro de Calláncas	Moises Nolasco
Mishipa shapran	MTHP-219	San Juan de Tingo	Feliciano Hilario
Mono mashua	MTHP-197	San Juan de Tingo	Feliciano Hilario
Puca cinta	MTHP-109	Santa Rosa de Monte Azul	Teodoro Rosado
Shagopa	MTHP-199	San Juan de Tingo	Feliciano Hilario
Shulash mashua	MTHP-193	San Juan de Tingo	Feliciano Hilario
	MTHP-255	San Alejandro de Calláncas	Moises Nolasco
Unknown 1	MTHP-229	San Juan de Tingo	Feliciano Hilario
Unknown 2	MTHP-231	San Juan de Tingo	Feliciano Hilario
Unknown 3	MTHP-232	San Juan de Tingo	Feliciano Hilario
Trompash	MTHP-206	San Juan de Tingo	Feliciano Hilario
Wacapa huagran	MTHP-203	San Juan de Tingo	Feliciano Hilario
Yana ñawi	MTHP-190	San Juan de Tingo	Feliciano Hilario
	MTHP-239	San Alejandro de Calláncas	Moises Nolasco
Yana ulto	MTHP-089	Santa Rosa de Monte Azul	Teodoro Rosado
Yuraj mashua	MTH-P248	San Alejandro de Calláncas	Moises Nolasco
Zapallo mashua	MTHP-237	San Alejandro de Calláncas	Moises Nolasco

2.2. DNA extraction and molecular characterization

DNA extraction of oca, ulluco and mashua accessions was performed on fresh leaves, using the protocol described by Blas (2005).

DNA amplifications of oca samples were performed in 25 µl containing 5 ng DNA, 1x buffer (New England BioLabs), 3 mM $MgCl_2$, 0.08 mM dNTPs, 0.40 µM primers (Malice *et al.*, 2007; Pissard *et al.*, 2008c), and 2 U *Taq* polymerase. The thermal cycler (PTC-200 MJ Research Inc.) was programmed for an initial denaturation step of 10 min at 95°C followed by 35 cycles of 30 sec at 95°C, 45 sec at optimized annealing temperature (Table 2) and 2 min at 72°C, and a final extension step of 5 min at 72°C.

DNA amplifications of ulluco samples were performed according to Malice *et al.* (under press). ISSR amplifications were performed in 25 µl volumes containing 5 ng DNA, 1x buffer, 2.5 mM $MgCl_2$, 400 µM dNTPs, 0.25 µM primers (Malice *et al.*, under press), 0.2 µg/µl 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 specific annealing temperature (Table 2) and 4 min at 72°C, and a final extension step of 7 min at 72°C.

DNA amplifications of mashua samples were performed in 25 µl volumes containing 5 ng DNA, 1x buffer, 2 mM $MgCl_2$, 400 µM dNTPs, 0.40 µM primers (Pissard et al, 2008a), 0.2 µg/µl BSA, and 1 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 40 cycles of 1 min at 95°C, 1 min at specific annealing temperature (Table 2) and 2 min at 72°C, and a final extension step of 5 min at 72°C.

Amplification products were separated on 1.8% TAE agarose gels, stained with SybrSafe and visualized under UV light.

Table 2. ISSR primers used for DNA amplification of Oca **(A)**, Ulluco **(B)** and Mashua **(C)**, amplified fragments number, primers annealing temperature, polymorphic fragments number and polymorphic information content (PIC values).

	Primer	Sequence 5' → 3'	Annealing temperature (°C)	Amplified fragments	Polymorphic fragments	Polymorphism (%)	PIC value
A	Oca-1	DHB-$(CGA)_5$	53,0	8	6	75	0,23
	Oca-2	$(AG)_8$-YT	47,0	7	6	86	0,23
	Oca-3	$(GA)_8$–YC	51,0	4	4	100	0,31
	Oca-4	$(AG)_8$-T	50,0	9	8	89	0,36
	Oca-5	$(AG)_8$-A	50,0	9	9	100	0,40
	Oca-6	$(GA)_8$-C	50,0	9	8	89	0,35
	Oca-7	$(TG)_8$-YC	47,0	9	7	78	0,24
B	Ulluco-1	BDB-$(ACA)_5$	46,0	9	5	56	0,22
	Ullcuo-2	DD-$(CCA)_5$	54,3	11	8	73	0,23
	Ulluco-3	VHV-$(GT)_7$	53,5	7	4	57	0,18
	Ulluco-4	BDB-$(CAC)_5$	55,4	5	4	80	0,27
	Ulluco-5	$(AG)_8$-YT	47,4	5	4	80	0,29
	Ulluco-6	$(AG)_8$-C	47,9	13	8	62	0,15
	Ullcuo-7	$(GA)_8$-C	49,4	8	6	75	0,26
C	Mashua-1	$(GA)_8$-YC	51,0	5	5	100	0,40
	Mashua-2	$(AG)_8$-C	50,0	9	8	89	0,35
	Mashua-3	$(AG)_8$-T	50,0,0	5	4	80	0,22
	Mashua -4	$(GA)_8$-A	47,0	4	3	75	0,27
	Mashua -5	VHV-$(GT)_7$-G	51,0	5	4	80	0,30

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

Reproducible ISSR markers were scored for the presence (1) or absence (0) of each band. Binary matrices were, independently for each of the 3 species, subjected to data analysis. A polymorphic index (PIC) was calculated as $PIC_{(i)} = 1 - (F_{p(i)}{}^2 + F_{a(i)}{}^2)$, where $F_{p(i)}$ is the presence frequency of the i^{th} band and $F_{a(i)}$ is the absence frequency of the i^{th} band. PIC values were then calculated to evaluate primers effectiveness to differentiate accessions by adding the PIC values of all the bands amplified by the same primer.

Genetic distances between accessions were calculated with Jaccard's coefficient, using the TreeCon software (Van de Peer & de Wachter, 1994). Principal Component Analysis (PCA) was performed using SAS 9.1 (SAS Institute Inc.).

We also used the Bayesian model-based clustering method of Pritchard *et al.* (2000), software Structure 2.2., to perform assignment tests and to assign individual accessions to gene pools or genetic clusters (K). We used the basic admixture model, with the assumed number of populations (K) varying from 1 to 5, 5 replicate runs per K value, a burning period length of $5x10^4$, and a post-burning simulation length of 10^5. No a priori population information was used. Log likelihood of data [L(K)] and Δ K statistic (rate of change between successive K values) were calculated to infer the optimal number of clusters (K) in the data set. For each individual accession, mean percentage of membership (q_{mean}) was assessed to each of the K genetic clusters. The degree of genetic differentiation among genetic clusters defined by Structure analysis was further tested, considering the individual accessions with a cluster membership probability (q_{mean}) higher than 80% (23 oca accessions and 31 ulluco accessions analyzed). F_{ST} estimates and AMOVA analysis were performed using the Arlequin 2.0 software (Schneider *et al.*, 2000).

3. Results and discussions

3.1. ISSR variation

In order to assess genetic diversity, the number of amplified fragments, the number of polymorphic fragments and polymorphic information content (PIC values) were calculated for each primer (Table 2). Overall, our results demonstrate the ability of the ISSR technique to assess genetic diversity of Andean tubers oca, ulluco and mashua. DNA amplifications using 7, 7 and 5 primers respectively for oca, ulluco and mashua, generated a total of respectively 55, 58 and 28 reproducible bands, from which 48 (87%), 39 (67%) and 24 (85%) were polymorphic. High percentages of polymorphism are commonly observed with ISSR markers. Mean PIC values per ISSR primer ranged from 0.15 for primer Ulluco-6 to 0.40 for primers Oca-5 and Mashua-1 (Table 2). In the present work, 11 of the 22 primers showed high capacity to reveal polymorphism within the studied accessions, as their PIC values were relatively high (≥ 0.25). These primers distinguished almost all accessions from each other (with the exception of 4 accessions for oca and 2 for ulluco).

3.2. Genetic diversity

Concerning oca, the mean genetic distance between individuals was 0.41 ± 0.16, with a range from 0 (between MTPA-023 and MTPA-081, and between MTPA-109 and MTPA-121) to 0.77 (between MTPA-008 and MTPA-104). Ulluco individuals displayed a mean genetic distance of 0.24 ± 0.09, with a range from 0 (between MTBM-209 and MTBM-234) to 0.42 (between MTBM-237 and MTBM-255). Finally, the mean genetic distance between individuals of mashua was 0.50 ± 0.18 with a range from 0.07 (between MTHP-190 and MTHP-239) to 0.82 (between MTHP-082 and MTHP-219).

Concerning intravarietal variability, the genetic distance varied from 0 (Cera oga and Garwash pillao) to 0.74 (Jilguero) in oca, from 0 (Tarmeña olluco) to 0.22 (Shactay olluco and Tarmeñita) in ulluco and from 0.07 (Yana ñawi) to 0.20 (Shulash mashua) in mashua. This analysis showed that individual plants from the same variety tended to share identical or similar genotypes (mean genetic distances within varieties were overall slight). However individual plants from some others varieties presented very different genotypes, and intra-varieties genetic distances varied widely for these varieties, reflecting heterogeneity within them.

3.3. Population structure

As shown on Figures 2A and 2B based on Structure outputs, the highest value of ΔK (oca: 71.28 - ulluco: 51.09) was observed at K=2 for both species. The rate of change in the likelihood L(K) was maximum at K=2 for ulluco (-672.9) and at K=3 for oca (-780.8). So we chose K=2 as the most likely number of distinct genetic clusters in both samples of oca and ulluco. Conversely, the assignment test performed on mashua revealed that the mashua sample we studied most probably belong to a single genetic cluster.

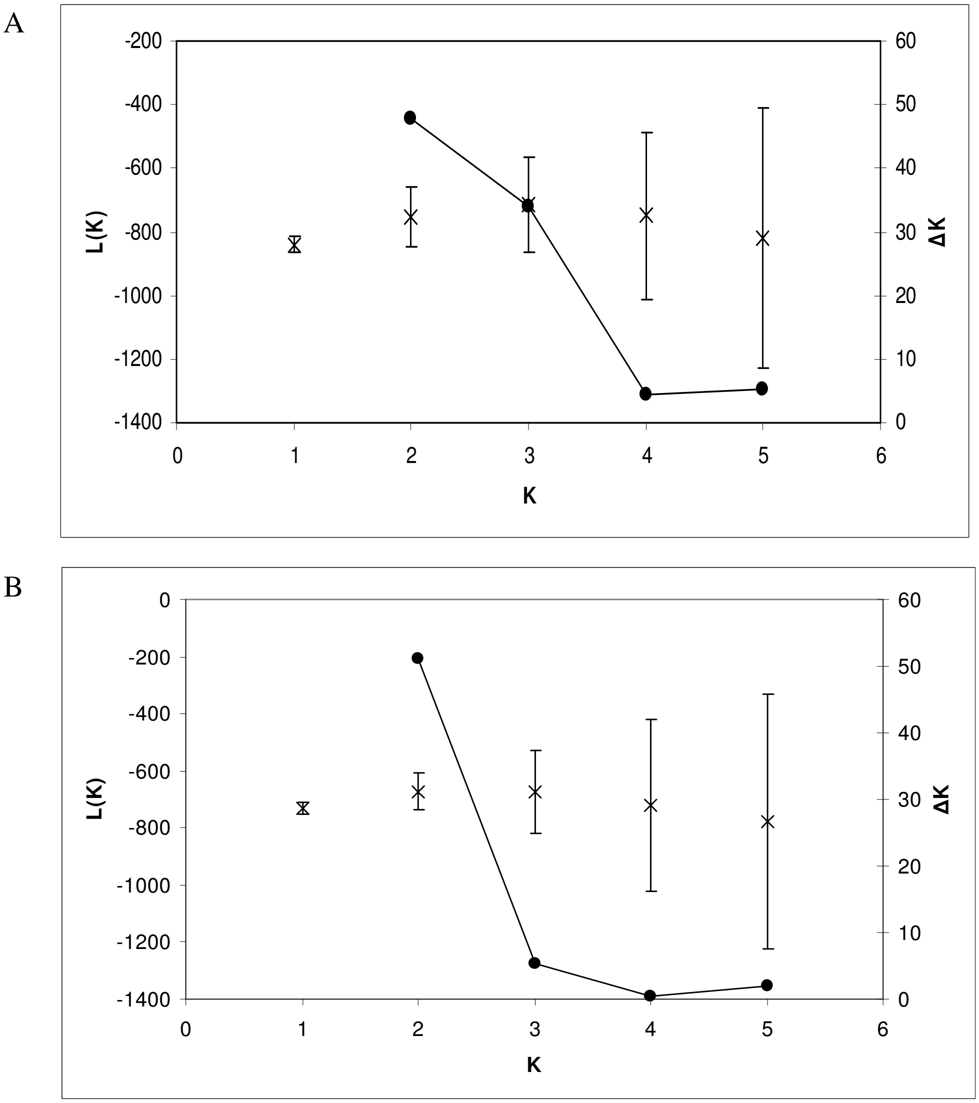

Figure 2. Inference of genetic clusters (K) of oca (A) and ulluco (B) using the model-based clustering method of Pritchard *et al.* (2000). Mean (±standard deviation) of log probability of data [L(K)] based on five independent runs (x) as a function of the value of K and the rate of change in the log probability of data DK between successive values of K (●) for the whole data set (A: N = 32 – B: N = 36).

Results of assignment tests and pattern of genetic structure for oca (Figure 3A) and ulluco (Figure 3B) indicate that the individual accessions of the same variety did not group unambiguously by name, but there was a tendency for them to do so. Accessions of a given variety were gathered together in the same genetic cluster, except for 2 oca varieties (Mio mio, Yana pillao) and 3 ulluco varieties (Rosado manca ñawi, Garwash pikllush, Juyto sarampión), in which individual accessions clustered in two different groups.

F_{ST} calculations conducted on the clusters defined by Structure analysis (for K = 2), on both oca and ulluco data sets, showed that genetic differentiation between clusters was very highly significant (oca: $F_{ST} = 0.40$ – ulluco: $F_{ST} = 0.25$, $P<0.001$). Results of the AMOVA analysis showed that all variance components were also very highly significant ($P<0.001$) and that the total variation was mainly due to variability within clusters (oca: 59.3% - ulluco: 73.6%) rather than among clusters (oca: 40.7% - ulluco: 26.4%).

Next step to judge the inferred number of genetic clusters consisted in plotting the Structure-assigned individual accessions on the Principal Component Analysis (PCA) plot. Figure 4 shows the position of the 32 oca (A), 36 ulluco (B) and 28 mashua accessions (C), defined by the first two principal coordinates PC1 and PC2 (these axes accounted for 9.83% and 6.59%, 5.82% and 4.86%, 3.84% and 3.50% of the total variation, respectively for oca, ulluco and mashua). Globally, the PCA confirmed our previous analyses. The first component (PC1 axis) clearly divides the accessions according to the Structure-defined genetic clusters they belong to. From the mashua PCA analysis (Figure 3C), neither discontinuity nor clear clustering appears. Same observation has already been done in other Andean tubers diversity studies (Malice *et al.*, 2007; Malice *et al.*, under press). Anyway, Pissard *et al.* (2008a) showed 3 very distinct genetic clusters in mashua, which were defined according to geographical provenance of accessions across Peru.

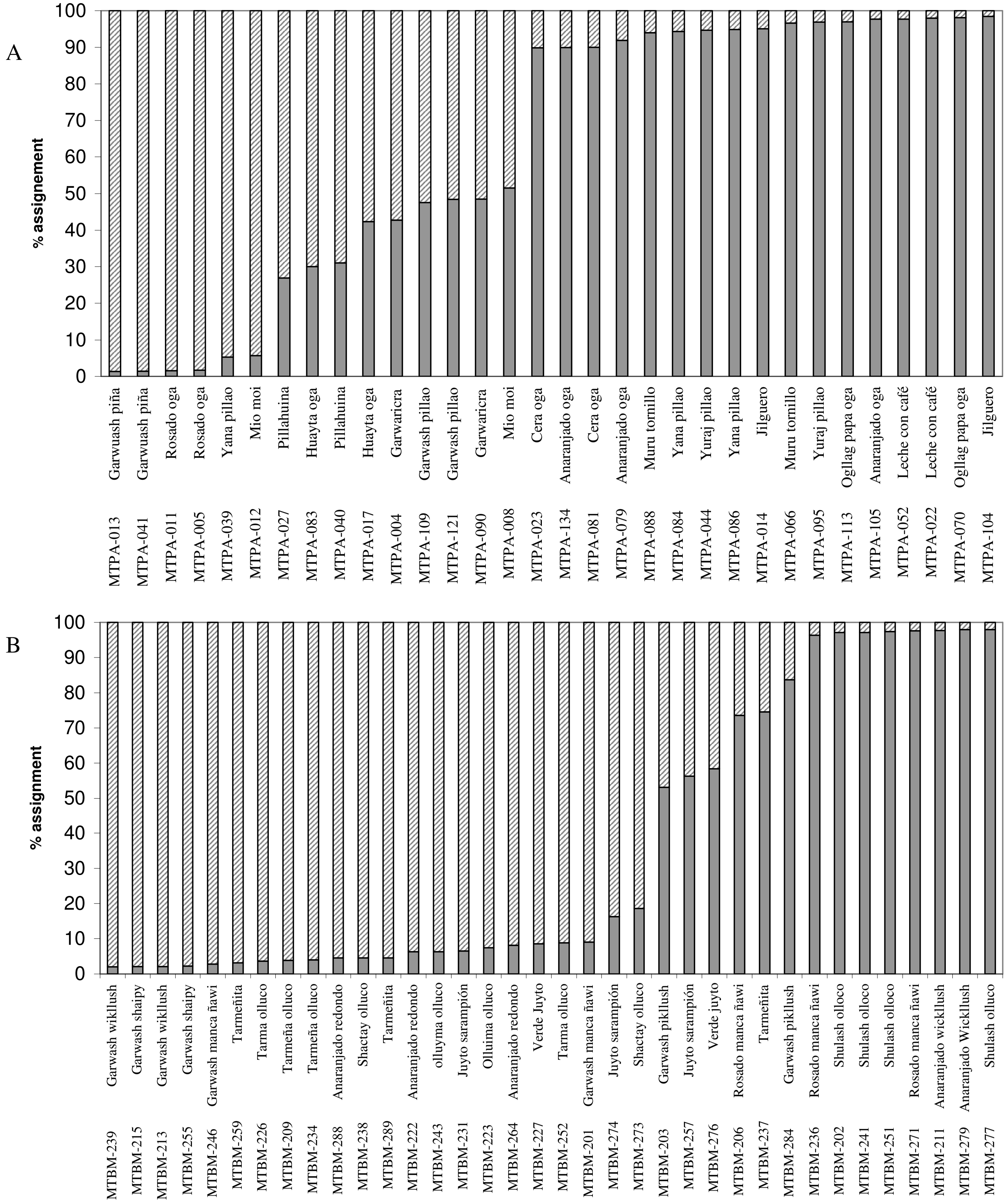

Figure 3. Structure of the genetic diversity of the 32 oca (A) and the 36 ulluco accessions (B) as assigned into 2 genetic clusters (K) detected using the model-based clustering method of Pritchard *et al.* (2000). Accessions are indicated by their identification number and their variety name (Table 1).

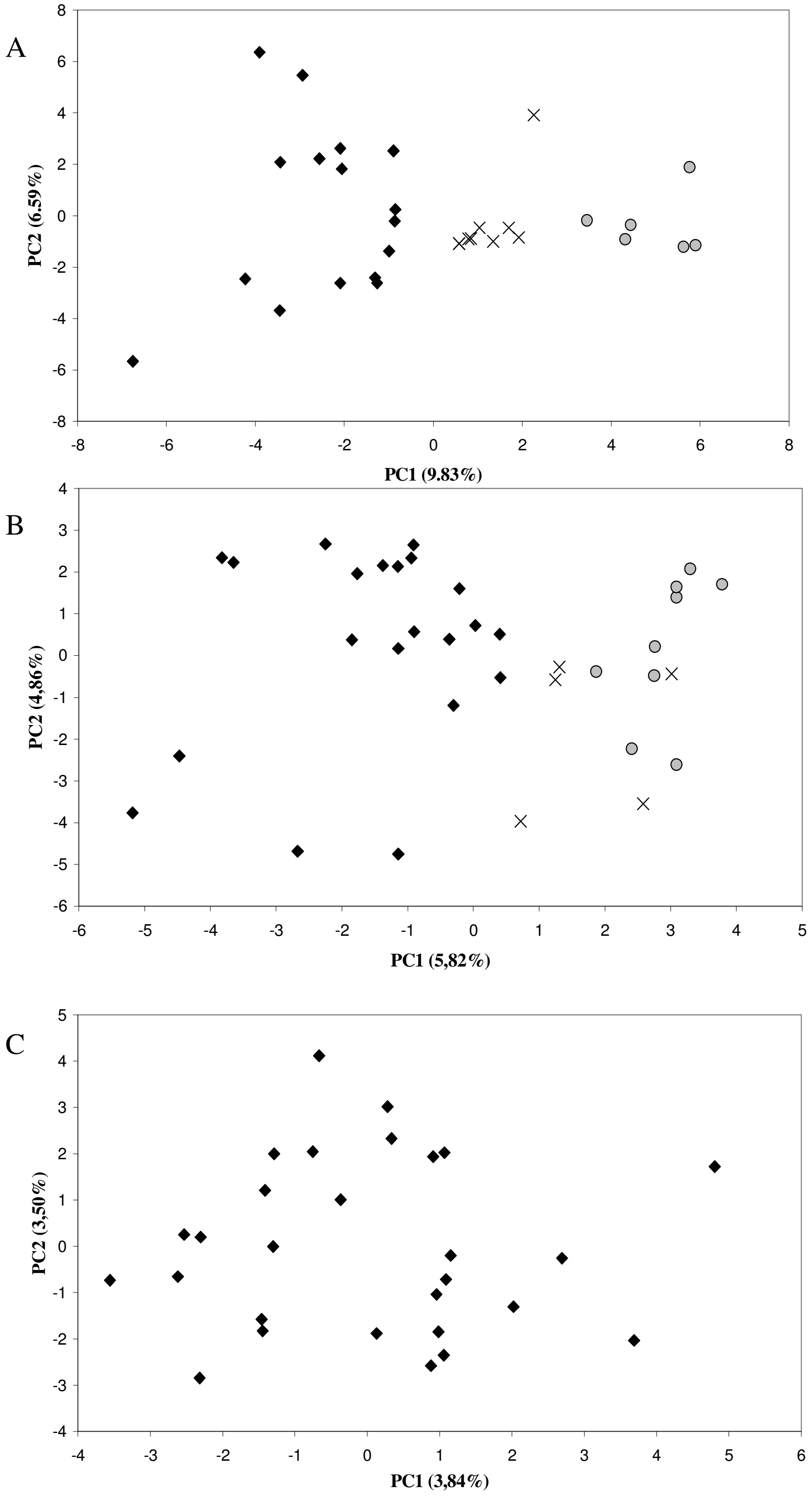

Figure 4. PCA plot based on molecular (ISSR) characterization of (A) 32 oca, (B) 36 ulluco and (C) 28 mashua accessions. Symbols indicate genetic clusters defined by the Structure progam (◆ and ● : accessions of the first and second genetic clusters – $q_{mean} > 80\%$ / × : accessions intermediary between first and second clusters – $q_{mean} < 80\%$)

4. Discussion

This work demonstrated that the ISSR technique is a reproducible and polymorphic tool to study genetic diversity of Andean tuber crop species. These molecular markers allowed a successful identification of genetic variation in the three species of Andean tubers (oca, ulluco and mashua), maintained in a conservation garden localized in the Peruvian diversity microcentre of Huanuco.

The mean values of genetic distance (oca: 0.41 - ulluco: 0.24 - mashua: 0.50) taken as indicators of diversity are high; this high diversity is also reflected by the great number of folk varietal names given to these three species. These observations are probably related to the balanced action of both (i) conservation method of the germplasm (conservation garden in traditional farming system) and (ii) evolution forces acting on Andean tubers genetic diversity during generations of farmers in traditional Andean agroecosystems (Brush *et al*., 1981). There is no doubt that traditional cultivation practices do not just maintain genetic diversity but can also act as an important source of diversity (Terrazas & Garcia 2003; Terrazas & Valdivia, 1998; Quiros *et al*., 1992; Espinoza, 2001). Our samples have been cultivated by farmers of the microcentre of Huanuco, and have been put into the conservation garden one year before our sample recollection. This period of time being relatively short, we can assume that genetic diversity and structure now encountered in the conservation garden faithfully represents the situation in farmers' fields. In an *in situ* conservation system, populations in farmers’ fields are influenced by farming practices characterizing Andean farming system (mixed cropping, planting at different altitudes), as well as by Andean practices like the participation to agrobiodiversity fairs or the exchange of tubers, and this may result in an enhancement of genetic diversity (Ramirez, 2002; Ortega, 1997). Mashua presents a higher genetic diversity than the 2 other Andean tuber species. This observation can be linked to its relatively high capacity to set seed in traditional farming conditions and to perform both allo- and autogamy (Grau *et al.*, 2003), favouring genetic exchanges between varieties.

ISSR analysis revealed the presence of heterogeneous varieties. Similar observations have also been reported in other studies, for Andean tubers (Malice *et al*., 2007; Pissard *et al*., 2008c, Emshwiller, 2006) or in other vegetatively reproduced crops (Quiros *et al*., 1990; Zimmerer, 1991; Elias *et al*., 2001a,b). This type of incongruity between folk naming and ISSR genotyping systems (i.e. varietal heterogeneity) indicates that genetic variability is generally underestimated in the fields. If we had only built our study on the varietal names to describe a "variety", the conclusion would have been that number of conservation units are 15 in oca, 15 in ulluco, and 26 in mashua. But, using ISSR molecular markers, we found a larger genetic diversity within each of these three species (oca: 30 – ulluco: 35 – mashua: 28). However, by comparing varieties that are named by farmers and genotypes that are distinguished by the ISSR molecular markers, we noticed a general congruence between these two modes of classification. In fact, all individual accessions with the same name present similar ISSR data (with the exception of 2 oca and 3 ulluco varieties). Quiros *et al*. (1990) on potato and Emshwiller (2006) on oca also observed this consistency between folk taxonomy and isozyme and AFLP variation, respectively.

Our assignment of individual accessions into genetic clusters, with the Structure program, revealed the presence of one genetic cluster for mashua, while both oca and ulluco can be divided in two major groups. The AMOVA analysis and F_{st} values both revealed a high level of population differentiation in oca and ulluco. In addition, the PCA analysis supported all these observations. Different seed set rates (oca and ulluco *versus* mashua) and traditional farming practices in the Andes may contribute to the genetic diversity patterns observed in these three species. Andean tubers are exclusively vegetatively reproduced by tubers and it seems very unlikely that farmers use seeds. Furthermore, seed production in oca and ulluco remains limited due to different factors inhibiting this process (Gibbs, 1976; Trognitz *et al.* 1998; Pietilä & Jokela, 1994). Concerning mashua, Grau *et al.* (2003) described both allo- and autogamy and a great botanical seed production has been reported (Popenoe *et al*., 1998). Nevertheless, in traditional Andean agriculture, mashua is also exclusively propagated through tubers. This situation may be explained by different factors: true-to-type breeding, reduced crop period and prevalence of vegetative propagated crops in Andean agriculture (Grau *et al*., 2003). Nevertheless, we suggest that despite vegetative reproduction of these Andean species, by tubers, specific mechanisms for seed production influence the pattern of genetic diversity and structure found (oca and ulluco *versus* mashua). Oca and ulluco samples were both divided into two genepools. In the particular case of mashua, a possible large seed production, the introduction of spontaneous seedlings coming from sexual reproduction and their integration into the germplasm used for vegetative propagation could explain why different genepools could not be differentiated in our sample. Another factor explaining the absence of genepool definition in the mashua sample from the microcentre of Huanuco is inter-varieties gene flow due to allogamy.

In addition to the reproduction system, traditional Andean farming practices, agromorphological selection by the Andean farmers and spatial arrangement of the fields also affect the structure of Andean tubers. Two features are significant in the management of tubers diversity: Andean farmers cultivate many varieties of each of the three Andean tuber species (oca, ulluco and mashua) and these varieties are sown simultaneously in each field. Such mixed planting could influence the genetic structure and diversity of these crops, particularly those that are still able to reproduced sexually. For these species, and particularly for mashua, it is not excluded that farmers maintain genotypes from sexual reproduction and use them for vegetative propagation, like in cassava (Elias *et al*., 2001a) or Andean potatoes (Quiros *et al*., 1992). As a result, new genotypes and phenotypes may be produced and selected by farmers according to interesting agromorphological characteristics of the varieties. Another key element of Andean agricultural systems that influences genetic diversity and structure of Andean tubers is the exchange of tubers between farmers and communities. In fact, farmers can give or receive seeds at small scale (within a diversity microcentre) but also at larger scale (biodiversity fairs and markets). Such exchanges have also been reported for Andean tubers (Terrazas & Valdivia, 1998).

In summary, our results revealed a high genetic diversity at the range of a single microcentre, confirming the high genetic diversity within the three Andean neglected species oca, ulluco and mashua, and the presence of heterogeneous varieties within these species. Understanding these complex processes of creation and maintenance of genetic diversity in Andean tuber crop species is essential to improve collection management and genetic resources conservation of neglected Andean tuber crop species threatened by genetic erosion.

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CHAPTER VI: Morphological diversity of the Andean tuber crop species oca (*Oxalis tuberosa* Molina) from the diversity microcentre of Cusco (Peru)

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Morphological variation and on-farm management of the neglected tuber crop oca (*Oxalis tuberosa* Molina) in the peasant communities of Cusco, Peru.

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Abstract

The oca (*Oxalis tuberosa* Molina) is an endemic tuber crop species from the high Andes, which presents local economic and nutritional importance. Three rural communities of the region of Cusco (Peru) have been identified as diversity microcentres for the oca and as an *in situ* on-farm conservation site. Field work was conducted in the three rural communities and oca production systems were analyzed. Sociocultural, economic and natural factors affect the pattern of genetic variation. Genetic diversity of 101 oca varieties was also investigated using 18 morphological descriptors. These analyses highlighted the existence of a very important intra-varietal diversity that can be explained by the selection carried out by farmers.

Key words: *Oxalis tuberosa* Molina, Cusco, Peru, morphological diversity, varietal names

1. Introduction

Until recently, genetic diversity studies focused on food crops of worldwide importance. Nevertheless, numerous neglected crops could play an important role concerning the improvement of nutrition and welfare of millions of people in the world (Padulosi *et al.*, 2002). Although considered as minor on a global level, these crops have often a significant local importance for the subsistence of many agricultural communities (Tapia, 2000). Adapted to marginal environments and preserved in their origin areas by socio-cultural preferences, traditional agricultural and use practices, neglected crops harbour a large genetic diversity that has to be preserved. These crops are often threatened with extinction by multiple agronomic, genetic, economic, social and cultural factors (Tapia & Estrella, 2001; Iriondo *et al.*, 2008).

Nine neglected species are found in the Andean farming systems: *Oxalis tuberosa* Molina (oca), *Ullucus tuberosus* Caldas (ulluco), *Tropaeolum tuberosum* Ruiz & Pav. (mashua), *Arracacia xanthorrhiza* Bancroft (arracacha), *Smallanthus sonchifolia* Poepp. & Endl. (yacón), *Canna edulis* Ker-Gawler (achira), *Mirabilis expansa* Ruiz & Pavón (mauka), *Pachyrhizus ahipa* Weddell-Parodi (ahipa) and *Lepidium meyenii* Walpers (maca). Their edible underground roots and tubers are of great economic and nutritional importance for Andean farmers' subsistence (León, 1964; Tapia Vargas, 1994). Among these, oca (*Oxalis tuberosa*) is the most important Andean tuber crop after potato (Hernandez Bermejo & León, 1992; Arbizu *et al.*, 1997). Oca is endemic in the high Andes, growing at high altitudes (from 2800 to 4100 m). Oca is vegetatively propagated exclusively through tubers. Under agricultural conditions, the species flowers abundantly but set seeds only exceptionally (Trognitz & Hermann, 1998). Oca has been identified as allo-octoploid (2n=8x=64 chromosomes - Emshwiller & Doyle, 2002).

Even if molecular markers have recently been developed (Malice *et al*., 2007; Pissard *et al*., 2008), the descriptions of oca's diversity remain commonly based on morphological traits. Despite its vegetative mode of propagation, oca maintains large phenotypic diversity (Arbizu *et al*., 1997; Cadima Fuentes, 2006). This is illustrated by the tuber pigmentation, varying from nearly white to nearly black, with ranges of pink, red, purple, yellow, and orange (IPGRI/CIP, 2001; Emshwiller, 2006). The numerous vernacular names reflect also the high diversity of cultivars (Emshwiller, 2006).

The genetic model of oca is linked to its breeding system, the prevalence of traditional varieties in subsistence agricultural system and the lack of improved cultural practices. Studying genetic diversity of oca is essential to improve conservation of these valuable resources. We examined the morphological diversity of oca's varieties, collected in three rural communities of the diversity microcentre of Cusco. In the present study, the farmer's concept of a variety is considered. We defined the variety as the set of tubers that share the same name and are considered by farmers as the same entity. To better explain this concept, we attempted to describe the correspondence between the name given to a variety and its morphological characteristics.

2. Materials and methods

2.1. Study area and field study

Three farming communities of the diversity microcentre of Cusco were included in this work: Amaru, Paru Paru and Viacha. They are located in the District of Pisac, Province of Calca, and Department of Cusco (Figure 1). A conservation garden is found in each community, where oca varieties collected by the farmers of the microcentre are maintained.

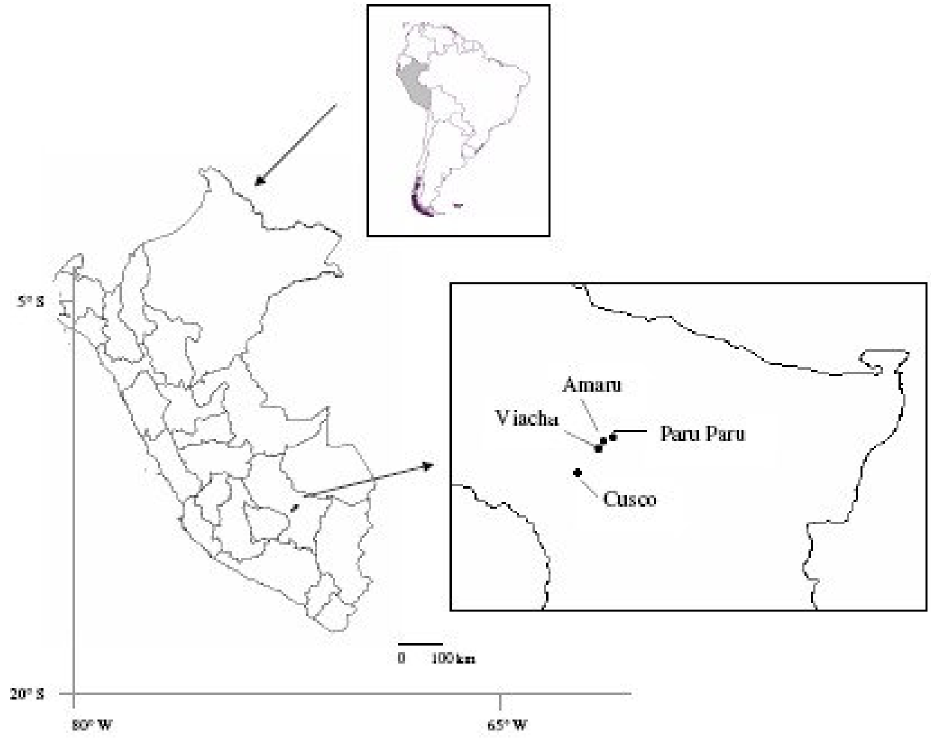

Figure 1: Map of Peru showing the localisation of the city of Cusco, and of the three rural communities (Amaru, Paru Paru and Viacha) where the study was conducted.

During the field study, interviews were conducted with the help of a Peruvian interpreter. We interrogated 12, 15 and 14 farmers of the Amaru, Paru Paru and Viacha community, respectively. For each farmer, we recorded the number of cultivated oca varieties, as an indication of the oca genetic diversity. Three main factors, known to affect farmer's behaviour in the maintenance of Andean tubers' diversity, were recorded during the field essay: (i) *sociocultural factors* (tubers exchanges and participation to biodiversity fairs), (ii) *economic factors* (destination of the harvest), and (iii) *natural factors* (climatic conditions, pest and disease pressures).

2.2. Plant materials and morphological characterization

A collection of 101 oca varieties was studied for agromorphological variability. For each variety, 1 to 29 accessions were morphologically described. The total number of accessions studied was 260 (Table 1). An accession is here defined as a plant sample held in the conservation garden, and identified by an accession number (Table 1).

Morphological data were recorded in 2007 and 2008 using a set of 18 qualitative variables, scored as categorical characters. The descriptors and their states are listed in Table 2. Calculations of Gower's distance between accessions were performed using SAS 9.1 (SAS Institute Inc.).

Table 1: List of the 101 oca varieties from the microcentre of Cusco used for morphological analysis

Varietal name	Accession number	Intravarietal diversity
Chachapia	PA587, PA596, VI212, VI216, VI257, VI263, VI318, VI335, VI507, VI528	0.31
Checche misitu	VI296	
Checche oqa	VI176	
Cheche misitu	VI545	
Chorispi yana	VI269	
Chusco misistu	VI343, VI345	0.12
Damazo oqa	VI182, VI298, VI317, VI351, VI367, VI388, VI411, VI485, VI527, VI542	0.38
Haco misitu	VI494	
Hanco misitu	VI232	
Hanco qello	VI311, VI430, VI435, VI482, VI517	0.34
Huajanquillas	VI264	
Huajanquillay	PA603	
Huayco apiña	AM552, PA650	0.61
Kaspishcha	VI274	
Kishpincha	VI529	
Kishuar	AM556, AM566	0
Kishuar oqa	VI271, VI308	0.52
Kisyhuar	VI357	
Kulli oqa	VI354	
Kusipata	AM558, AM560, AM573, AM577, PA606, PA611, PA622, VI191, VI213, VI230, VI231, VI258, VI282, VI297, VI316, VI338, VI350, VI368, VI386, VI399, VI408, VI421, VI428, VI444, VI483, VI491, VI503, VI525, VI540	0.18
Legitimo misitu	VI320	
Llagas ñucchu	VI265	
Puka yana ñawi	VI371	
Pusi	AM549	
Pusqo	AM559, AM572, VI222, VI330, VI360, VI398, VI439, VI453, VI489, VI513, VI523, VI532	0.06
Qako masitu	VI374	
Qaku oqa	VI177	
Qanco qullo	VI186	
Qanco ushpacha	VI185	
Qello	VI499	
Qello apiña	AM553	
Qello chachapia	VI190	
Qello hanco	VI281	
Qello haytu	AM569, VI187, VI349, VI361, VI381, VI425, VI524	0.24
Qello oqa	VI362	
Qello panti	VI224, VI276, VI277, VI403, VI459, VI478	0.19
Qello pusqo	VI324	
Qeqra misitu	VI413	
Quello panti	VI252, VI332	0.39
Royac	VI356	
Royac panti	VI379	
Royac ushpacha	VI293, VI295, VI326	0.25
Royaq yuraq panti	VI422	
Sacacha	VI233	
Sambucha oqa	VI183	
Sawaysiray oqa	VI248	
Ttica misitu	VI473	
Tullu misitu	VI178, VI262, VI301, VI319, VI373, VI375, VI414, VI493, VI519	0.22

Lluchu misitu	VI544	
Machasqa oqa	VI548	
Maqtacha oqa	VI346	
Misitu	AM571, AM574, VI189, VI193, VI235, VI244, VI261, VI283, VI286, VI306, VI355, VI392, VI415, VI450, VI451, VI488, VI495, VI520, VI521	0.36
Misti	VI490	
Muru haytu	VI378	
Oqe misitu	VI272	
Oqe misitu	VI272	
Panti	AM557, AM562, AM565, PA599, PA601, PA604, PA616, PA658, PA688, VI180, VI192, VI234, VI307, VI369, VI387, VI429, VI445, VI460, VI484, VI492, VI504, VI526, VI541	0.34
Pikacha	VI370	
Pisqobamba oqa	VI305, VI443	0
Puka	PA665	
Puka apiña	AM554	
Puka haytu	VI300	
Puka ishuarani	VI402	
Puka kusipata	PA624	
Puka misitu	VI322, VI427, VI546	0.28
Puka ñawi oqa	VI331, VI358	0.67
Puka oqa	VI175	
Puka panti	VI210, VI259, VI315, VI339, VI340, VI409	0.01
Puka pusqo	VI328	
Puka ttika	PA645	
Puka ushpa	VI260	
Puka ushpacha	VI211, VI341	0.50
Ushapa huayqa	VI394	
Ushpa	VI309	
Ushpa huayqacha	VI431	
Ushpa oqa	AM550	
Ushpa saca	VI287	
Ushpa sacacha	VI446	
Ushpa taqe	PA581, PA613, PA668	0.01
Ushpacha	VI194, VI418, VI447, VI476	0.08
Yana haytu	VI303	
Yana kusipati	AM579	
Yana misitu	VI448, VI452, VI474, VI547	0.25
Yana ñawi pisqobamba	VI406	
Yana oqa	VI179	
Yana oqacha	VI417	
Yana panti	VI266, VI323, VI433	0.21
Yana pisqobamba	VI535	
Yana pusqo	VI419	
Yana ushpa	VI242	
Yana ushpacha	VI270, VI390	0.58
Yurac panti	VI380	
Yurac panti	VI498	
Yuraq kishuarcha	VI420	
Yuraq kusipata	VI251	
Yuraq misitu	VI400, VI437, VI456	0.20
Yuraq oqa	VI170, VI184, VI347	0.26
Yuraq panti	VI223, VI250, VI310, VI438, VI454, VI475, VI512	0.14
Yuraq pusqo	AM575	
Yuraq ushpa	VI221, VI514	0
Yuraq ushpacha	VI249	

Accessions are identified by community of origin (AM = Amaru, PA = Paru Paru, VI = Viacha), collection number (XXX) and vernacular name. For varieties with more than one accession, intravarietal morphological diversity has been calculated.

Table 2. Descriptors used to morphologically describe the 101 oca accessions from the microcentre of Cusco, and their descriptor states.

1. Stem color	1.1. Yellowish green 1.2. Greyish green with greyish red 1.3. Greyish red 1.4. Reddish purple 1.5. Greyish purple
2. Stem wing pigmentation	2.0. Absent 2.1. Present
3. Foliage color	3.1.Yellowish green 3.2. Dark yellowish green 3.3. Dark yellowish green with greyish purple 3.4. Greyish purple with dark yellowish green
4. Abaxial leaf color	4.1. Yellowish green 4.2. Yellowish green with greyish red 4.3. Yellowish green with greyish purple 4.4. Reddish purple with yellowish green 4.99. Other (specify)
5. Petiole color	5.1. Green with white stipules 5.2. Green with clear greyish purple stipules 5.3. Green with greyish purple stipules 5.4. Greyish purple with dark greyish purple stipules 5.5. Greyish red with dark greyish purple stipules
6. Flowering habit	6.0. Absent 6.3. Low 6.5. Moderate 6.7. Abundant
7. Flower color	7.1.Yellow 7.2. Yellowish orange
8. Flower heterostyly	8.1. Brevistyly
13. Secondary tuber surface color	13.0. Absent 13.1. White 13.2. Yellowish white 13.3. Yellow 13.4. Yellowish orange 13.5. Red orange 13.6. Clear red (pink) 13.7. Pale red 13.8. Red 13.9. Greyish red 13.10. Reddish purple 13.11. Greyish purple
14. Distribution of secondary tuber surface color	14.0. Absent 14.1. Eyes 14.2. Around eyes 14.3. On tuberisations 14.4. Eyes and irregularly distributed 14.5. Irregularly distributed 14.6. Irregularly distributed, on tuberisations
15. Predominant tuber flesh color	15.1. White 15.2. Yellowish white 15.3. Yellow 15.4. Yellowish orange 15.5. Red orange 15.6. Red 15.7. Greyish red 15.8. Reddish purple 15.9. Greyish purple
16. Secondary tuber flesh color	16.0. Absent 16.1. White 16.2. Yellowish white 16.3. Yellow 16.4. Yellowish orange 16.5. Red orange 16.6. Clear red (pink)

	8.2. Mesostyly 8.3. Longistyly 8.4. Semi homostyly 8.5. Strong longistyly
9. Corolla shape	9.1. Rotated 9.2. Semi strella 9.3. Pentagonal
10. Sepals color	10.1. Green 10.2. Green with greyish purple 10.3. Greyish purple 10.99. Other (specify)
11. Peduncle and petiole color	11.1. Peduncle and petiole yellowish green 11.2. Peduncle yellowish green and petiole greyish purple 11.3. Peduncle and petiole greyish purple 11.4. Peduncle greyish purple and petiole yellowish green
12. Predominant tuber surface color	12.1. White 12.2. Yellowish white 12.3. Yellow 12.4. Yellowish orange 12.5. Red orange 12.6. Dark red orange 12.7. Clear red (pink) 12.8. Pale red 12.9. Red 12.10. Reddish purple 12.11. Clear greyish purple 12.12. Dark greyish purple

	16.7. Pale red 16.8. Red 16.9. Greyish red 16.10. Reddish purple 16.11. Greyish purple
17. Distribution of secondary flesh tuber color	17.0. Absent 17.1. Cortex 17.2. Vascular ring 17.3. Medulla 17.4. Vascular ring and cortex 17.5. Medulla and cortex
18. Tuber shape	18.1. Ovoid 18.2. Claviform 18.3. Elongated 18.4. Cylindrical

3. Results

3.1. Description of the agricultural system

1. Number and distribution of oca varieties

The number of oca varieties maintained by farmers' families in each community of the microcentre of Cusco is listed in Table 3A.

Most farmers (50%) maintain a relatively reduced number of oca varieties (1 to 3), while a few farmers (10 out of 41) maintain a high number of varieties (7 to 10). Only 7 out of the 91 varieties studied are found in all of the 3 communities; each community maintaining "unique" varieties (i.e. found only in this community): 7 in Amaru, 11 in Paru Paru and 11 in Viacha.

Such a dispersion of the varieties within the communities is consistent with Quiros *et al.* (1990) in the case of potato or with Terrazas & Valdivia (1998) in their study on the diversity microcentre of Candelaria (Bolivia). These latter observed an average of 3 oca varieties cultivated by farmer, and a great majority of the varieties (39/60) being cultivated by less than 10% of the farmers of Candelaria.

2. Sociocultural factors

According to Table 3B, 75% of the farmers received their oca varieties from their parents (heritage) and 25% purchased all their varieties. However, the quantity and quality of tubers of a farmer's family varying over the time, the structure of these oca varieties is not static (Terrazas & Valdivia, 1998). In fact, farmers are continuously looking for tubers of better quality and new varieties. In this context, the market or the local biodiversity fairs are frequent sources of supply. These tubers exchanges play an important role both within and between communities, by maintaining on-farm diversity (Espinoza, 2001; Terrazas & Garcia, 2003).

In the case of the Cusco microcentre, less than 25% of the farmers participate in the biodiversity fairs (Table 3C). These farmers are exclusively distributed in the communities of Paru Paru and Viacha, and go to the fairs with the objective to sell their varieties (respectively 33% and 40%) or to buy new varieties (44% and 40%).

A particular use of a variety is also a key factor for conserving this variety. As an example, the oca variety called *Pusqo* produces a bitter tuber, which is commonly consumed as "*kaya*", similar to the "*chuño*" made from potato. This type of transformation allows long tuber conservation, ensuring food security to the farmer and its family. This *Pusqo* variety was cultivated by the majority of the farmers in our 3 communities, and even by all the farmers from Viacha community.

Table 3: Repartition of the farmers according to the number of oca varieties they maintain (1-3, 4-6, 7-9 and ≥10) and (A) their community, (B) their acquiring mode of the tubers (inheritance and/or purchase), (C) their participation to biodiversity fairs and (D) the destination of their tubers' production.

		Number of oca varieties			
		1-3	4-6	7-9	≥10
A	**Community**				
	Amaru	7	2	1	2
	Paru Paru	11	3	0	1
	Viacha	2	6	2	4
B	**Acquiring mode**				
	Inheritance	8	9	3	4
	Purchase	7	1	0	2
	Inheritance and purchase	5	1	0	1
C	**Participation to biodiversity fairs**				
	Participate	6	2	1	3
	Do not participate	14	9	2	4
D	**Destination of the production**				
	Autoconsumption	13	5	1	5
	Autoconsumption and sale	7	5	2	2
	Autoconsumption, sale and barter	0	1	0	0

3. Economic factors

The main objective of the farmers in the Cusco microcentre is to produce tubers for their own consumption. However, almost 40% of them also produce oca to sell it, especially during the periods of high productivity; and only 3% produce oca to sell or exchange a part of their production against maize (Table 3D).

Agriculture represents the principal financial resource for the farmers of the microcentre and their families. Currently, the interest of young people for farming activities seems to decline. Several factors are likely to contribute to this situation: severe climatic conditions impairing stable and high yields on one hand, and attractiveness of cities on the other hand, prompting young people to leave their communities. In addition other activities, such as ecotourism, decrease the time devoted to agriculture in the 3 visited communities, forcing farmers to reduce activities in their exploitation.

4. Natural factors

In the microcentre of Cusco, as in the whole Andean region, microclimatic conditions vary according to the altitude and hillside slope. Farmers exploit their knowledge of these conditions to avoid crop failure. They distinguish three areas of altitude (3200 to 3800 m, 3800 to 4200 m and 4200 to 4800 m). Each level displays particular environmental characteristics, cropping and cultural practices (Vargas Escalante *et al.*, 2007). These conditions are very similar to those found in the microcentre of Candelaria, Bolivia (Terrazas & Valdivia, 1998; Terrazas & Garcia, 2003) or in other Andean systems (Ortega, 1997). In this kind of system, species and varieties are dispersed in the germplasm of several families, on numerous plots localised across the altitude levels (Terrazas & Valdivia, 1998). Varieties and species are distributed in a wide range of environmental (soil and climate heterogeneity, altitude levels), geographical (localisation of the family in the microcentre) and social (migration, market pressure) conditions, in a mosaic system. Mosaic system is all but static and is continually modified through time by several mechanisms generating a ‘dynamic mosaic system’ (Terrazas & Valdivia, 1998; Terrazas & Garcia, 2003) where tubers of each variety are frequently transported from place to place and across altitudinal levels. In the fluctuating environmental conditions of the Andes, such farming practices is essential to reduce the risk of crops and varieties loss, because of frost, hail, drought or other climatic and biotic stresses.

3.2. Morphological characterization and varieties' names

The mean morphological distance between the 260 accessions was 0.53 ± 0.18, with a range from 0 to 0.92. The average morphological distance within the varieties composed of 2 or more accessions was 0.25 ± 0.19, with distance ranging from 0 to 0.67 (Table 1). These results confirmed the high morphological diversity found within the oca species, but also highlighted a high intravarietal variability.

According to Brush *et al.* (1981) and Zimmerer (1991), farmers classify their potatoes varieties according to 4 taxonomic levels: (i) the generic level (potatoes are distinguished from the other Andean tubers like oca, ulluco and mashua), (ii) the species (according to criteria of cultivation, edibility, processing and frost resistance), (iii) the locally named varieties (mainly based on tuber characteristics) and (iv) the sub-variety (based on secondary characteristics, principally variations in tuber color). In the ethnotaxonomy of oca Emshwiller (2006) also observed a subdivision made by farmers between varieties on the basis of their use. The local varieties are mainly distinguished by their primary tuber shapes and colors. Within some varieties, variability is also observed in secondary tuber characteristics (mainly secondary tuber color), leading to the definition of sub-varieties. In our case, similar observation can be made for 4 varieties (*Pusqo*, *Misitu*, *Panti*, *Ushpacha*), and varietal names reflect a range of colors from white to black.

During the inquiries, we asked the farmers which characters they preferentially use to identify and name their oca varieties. Tuber color, tuber shape and stem color are, in this order of importance, the three most important characters reported by the farmers. These characters can be linked to the concept of the subdivision into sub-varieties. Table 4 illustrates the subdivision of the *Pusqo* varieties.

Table 4. Subdivision of the *Pusqo* varieties according to morphological characterization. Descriptors and their states are those presented in Table 2.

Varietal name	**Identification number**	**Morphological characterization**																	
		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18
Pusqo	AM559	1	0	2	3	1	5	1	2	3	1	2	3	0	0	3	0	0	2
Pusqo	AM572	1	0	2	3	1	5	1	2	3	1	2	3	0	0	3	0	0	2
Pusqo	VI222	1	0	2	3	1	5	1	2	3	1	2	3	0	0	3	0	0	2
Pusqo	VI330	1	0	2	3	1	5	1	2	3	1	2	3	0	0	3	0	0	2
Pusqo	VI360	1	0	2	3	1	5	1	2	3	1	2	3	0	0	3	0	0	2
Pusqo	VI398	1	0	2	3	1	5	1	2	3	1	2	3	0	0	3	0	0	2
Pusqo	VI439	1	0	2	3	1	5	1	2	3	1	2	3	0	0	3	0	0	2
Pusqo	VI453	1	0	2	3	1	5	1	2	3	1	2	3	0	0	3	0	0	2
Pusqo	VI489	1	0	2	3	1	5	1	2	3	1	2	3	0	0	3	0	0	2
Pusqo	VI513	1	0	2	3	1	5	1	2	3	1	2	3	0	0	3	0	0	2
Pusqo	VI523	1	0	2	3	1	5	1	2	3	1	2	3	0	0	3	0	0	2
Pusqo	VI532	3	0	2	3	2	7	1	2	2	1	2	2	0	0	2	0	0	4
Puka pusqo	VI328	3	0	2	3	2	7	1	2	2	1	2	2	0	0	2	0	0	4
Qello pusqo	VI324	1	0	2	3	1	5	1	2	3	1	2	3	0	0	3	0	0	2
Yana pusqo	VI419	1	1	2	3	3	7	2	3	2	1	2	12	0	0	3	8	3	2
Yuraq pusqo	AM575	1	0	2	3	1	5	1	2	3	1	2	3	0	0	3	0	0	2

Twelve accessions represent the variety called *Pusqo*, while the other 4 are called *Puka pusqo*, *Qello pusqo*, *Yana pusqo* and *Yuraq pusqo*. In *Quechua* language, "*puka", "qello",* "*yana" and* "*yuraq"* respectively means red, yellow, black and white. These observations confirm the importance of tuber color in the subvarietal characterisation.

The subvarieties *Pusqo*, *Yuraq pusqo* and *Qello pusqo* are identical for the 18 observed characters. These accessions, although displaying identical phenotype, were identified by the farmers with different vernacular names. The subvariety *Puka pusqo* is characterized by greyish red stems (C1=3), and yellowish (C12=2) and cylindrical (C18=4) tubers. One should mention that the accession VI532 called *Pusqo* is morphologically similar to the accession VI328 called *Puka pusqo*. This probably reflects an error in the denomination of the accession VI532. The subvariety *Yana pusqo* is characterized by a dark greyish tuber (C12=12) and by the presence of a dark secondary tuber flesh color (C16=8).

Similar observation can be made for the varieties *Panti*, *Misitu* and *Ushpacha* (data not shown) and reflect the perception of a variety, defined by the farmers on the basis of three morphological characters: tuber color, tuber shape and stem color.

4. Discussion

Endemic in the high Andes, oca is poorly known and used outside its area of origin. Cultivated by the Andean rural populations, this species is of particular importance to ensure the livelihood of the poorest Andean farmers and represents also a part of their traditions.

The field study we conducted enlightened different factors of the production system that could influence the varietal diversity of the oca in the microcentre of Cusco. First, the tuber flow linked to the participation to biodiversity fairs and exchange of tubers between farmers allow the farmers to keep a diversified lot of oca. In addition, the participation of the farmers into ecotourism activities may reduce the importance of agriculture in these areas, leading to a lack of varieties. In this context, it is of particular importance to associate different methods for germplasm conservation (*in situ* / *ex situ*).

Morphological characterization showing high values of Gower's distance confirms the high morphological diversity found within the oca species (IPGRI/CIP, 2001; Emshwiller, 2006; Cadima Fuentes, 2006). Three features of the Andean farming system can be considered as key elements in the creation and the maintenance of diversity in the case of vegetatively reproduced crop species, particularly in the case of the oca in the microcentre of Cusco. (i) Firstly, this diversity can be linked to the ecological and geographical distribution of varieties across the Andean region, explaining their adaptation to different climatic and soil conditions (Castillo, 1995). Vegetative reproduction of these species in a wide range of microenvironments, has conducted to the development of morphologically distinct clones. Andean farmers have undeniably contributed to the generation of this diversity (Quiros *et al.*, 1992; Iriondo *et al.*, 2008). Indeed, with the obligation to respond to very diverse edaphic and climatic conditions encountered in the Andes, farmers have selected and preserved as most varieties as possible, with a vertical control of ecological zones ensuring, as a result, food security to their families (Ortega, 1997; Terrazas & Valdivia, 1998). (ii) Secondly, the possible presence of viable botanical seeds from oca could also be involved in the creation and maintenance of new varieties. In addition to the conservation of existing varieties through vegetative propagation, new forms from sexual reproduction could be selected and maintained by the Andean farmers, intentionally or not. This has already been noted in other species such as cassava (Elias *et al.*, 2001) and potato (Brush *et al.*, 1981; Quiros *et al.*, 1992); and could be investigated for the oca. (iii) Thirdly, the flow of tubers represents a form of germplasm migration and a significant mechanism in the emergence of diversity in the Andean agricultural systems. In addition to the tubers selected from their own fields, farmers obtain new tubers from other farmers or local markets (Espinoza, 2001). The biodiversity fairs promote exchanges of varieties between participants (farmers of the same or different communities) and facilitate the widespread dispersal of a significant genetic diversity (Espinoza, 2001).

Another key element in farmers' management processes of Andean tubers diversity is the system used by the farmers to name their varieties, linked to their morphological characters. Andean farmers employ a range of morphological characters (mainly based on tuber shape and color) to identify their varieties using their own varietal name (Cadima *et al*., 2003). As also observed for other crops like potato (Brush *et al.*, 1981; Jackson *et al.*, 1980; Zimmerer, 1991), cassava (Salick *et al*., 1997; Elias *et al*., 2000-2001) or maize (Louette & Smale, 2000), Andean farmers establish a unit (set of tuber plants) that they select, exchange, and maintain. In most studies the genetic diversity managed in traditional farming systems is estimated by the number of different varietal names, but these do not always coincide (Wood & Lenne, 1997). The related hypothesis that number of varietal names corresponds to the actual diversity should necessarily be tested because the expertise of farmers to name and manage their varieties may have important consequences in terms of Andean tubers diversity management and conservation. Previous studies based on molecular characterisation of oca showed that varieties are heterogeneous and present intravarietal diversity (Malice *et al*., 2007; Pissard *et al*., 2008; Emshwiller, 2006). We also observed an intravarietal, even if only morphological descriptors were used to characterize oca varieties. This unexpected result enlightens the underestimation of genetic diversity found in farmers' fields. Anyway, when characters used by farmers to describe their varieties are considered, description of the varieties is consistent with their varietal names. So, based on the farmer's concept, a variety of oca corresponds to a distinct phenotypic entity. These names reflect the phenotypic characteristics and are quite reliable indications of genetic diversity. On the contrary, about one third of the studied varieties presented variability in the phenotypic characters used by farmers to identify them. It seems that, even for key traits, farmers do not maintain strict purity in their varieties.

In summary, high morphological diversity between the oca accessions studied on-farm in the diversity microcentre of Cusco has been shown. In addition, the existence of morphological intravarietal diversity has been highlighted. System used to assign vernacular name to the oca varieties is linked to the morphological characters used by the farmers to identify their varieties. Our results provide additional criteria for variety identification and also raise important questions about the most effective way to conserve diversity.

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CHAPTER VII: Discussions and Conclusions - Genetic diversity and structure of Andean tuber crop species: a tentative unifying model

Discussions and Conclusions

Genetic diversity and structure of Andean tuber crop species: a tentative unifying model

Genetic diversity and structure of Andean tuber crop species (oca, ulluco and mashua) have been insufficiently investigated. However, the risk of genetic erosion for these species has recently revealed the importance to develop accurate *in situ* and *ex situ* conservation programs (see chapter II).

In addition, Andean tubers constitute a representative model to study genetic diversity and structure of neglected vegetatively propagated crop species. Indeed, these crops are cultivated in traditional agroecosystems, which are of particular interest. In fact, the genetic diversity in such systems is generally high; it is not unusual to encounter several varieties of a species in the same field and farming practices are very often based on local natural resources. Furthermore, in the absence of significant breeding programs and deep genetic studies, the evolution processes of Andean tubers were mainly guided by selection pressures imposed by farmers and farming environment.

Genetic diversity and structure of Andean tubers, an overview

Population structure is defined as the distribution of genetic variation among the various taxonomic units that can be, in our case study, tuber samples, varieties or morphotypes (Jarvis *et al*., 2000). The genetic structure of a species may also refer to the partitioning of its variation over space (region, localities, altitudinal gradient) and through time. Farmers' decisions in response to cultural, socio-economic or environmental factors also contribute to the structuration of the genetic diversity of a species in traditional farming system. **Genetic**

diversity is the genetic variation present in a population or species. Genetic diversity can be evaluated either at physiological, biochemical, morphological or molecular levels (Jarvis *et al.*, 2000). In this work, we focused on the two latter to study genetic diversity and population structure of Andean tuber crops.

We showed that Andean agriculture preserves high diversity, at the inter-specific level but also at the intra-specific one, in terms of number of local varieties. Andean tubers are not only presenting a high diversity, but this diversity is reflected by numerous parameters such adapted traditional farming practices, ethnobotanical data, typical dishes… To understand genetic diversity and structure of these cultivated species, a mandatory step is to appreciate the interrelationship between men (farmers and their families), their crops and the environment. To reach this goal, we analysed morphological, molecular, geographical, environmental and cultural information.

In this thesis, we focused on the diversity and structure of oca, ulluco and mashua varieties in farmer's fields (*in situ* – see chapters III, V and VI) and in genebanks (*ex situ* – see chapters III and IV), in Peru and Bolivia.

Concerning *in situ* studies, we considered a variety as a group of plants identified by farmers under a single name and considered by farmers as a part of the same unit (see chapters III, V and VI). Similar definition of the variety has been used by diverse authors (e.g. maize, Louette *et al.*, 1997 – sorghum, Alvarez *et al.*, 2005 – cassava, Elias *et al.*, 2001a).

The ISSR markers have revealed considerable genetic variation both within and between oca and mashua varieties (see chapters III and VI, and chapter VI, respectively). Variation was slightly higher for the mashua.

Using both molecular and morphological marker systems, many heterogeneous varieties were found for the oca, ulluco and mashua species collected from different locations and conservation strategies (*in situ* or *ex situ*). Anyway, these varieties show a wide range of intra-varietal variation, as some of them were found monomorphic and others presented a high intra-varietal variability (see chapters III, V and VI).

Diversity maintained *in situ* and *ex situ* has been compared in the case of oca varieties (see chapter III), and appeared quite lower in *ex situ* compared to *in situ* collections.

In *ex situ* conservation, samples are conserved in the field genebanks as accessions regrouped into morphotypes. These latter were defined as a set of accessions with identical morphological characters. ISSR markers revealed a genetic variability within the morphotypes identified in ulluco (see chapter IV).

Furthermore, we enlightened the fact that genetic structure of ulluco is influenced by the geographical distances (generally, the closer the accessions are geographically, the more genetically similar they are; see chapter IV). These findings reveal the importance of geography on the diversity pattern.

A tentative unifying model

Following these results, we conceived a model encompassing the evolutionary processes that shape genetic diversity and structure of Andean tubers. We also tried to enlighten the importance of intrinsic characteristics of the species (**reproductive characteristics**) as well as specificities of the **Andean agricultural system** (summarized in figure 11 as **sociocultural**, **economic and environmental factors**). In this context, we think that **farmers influence** the genetic variability of their crops through a range of actions. Indeed, crops are not only the result of natural factors, such as **mutation** and **natural selection**, but also and particularly, of **human selection and management**.

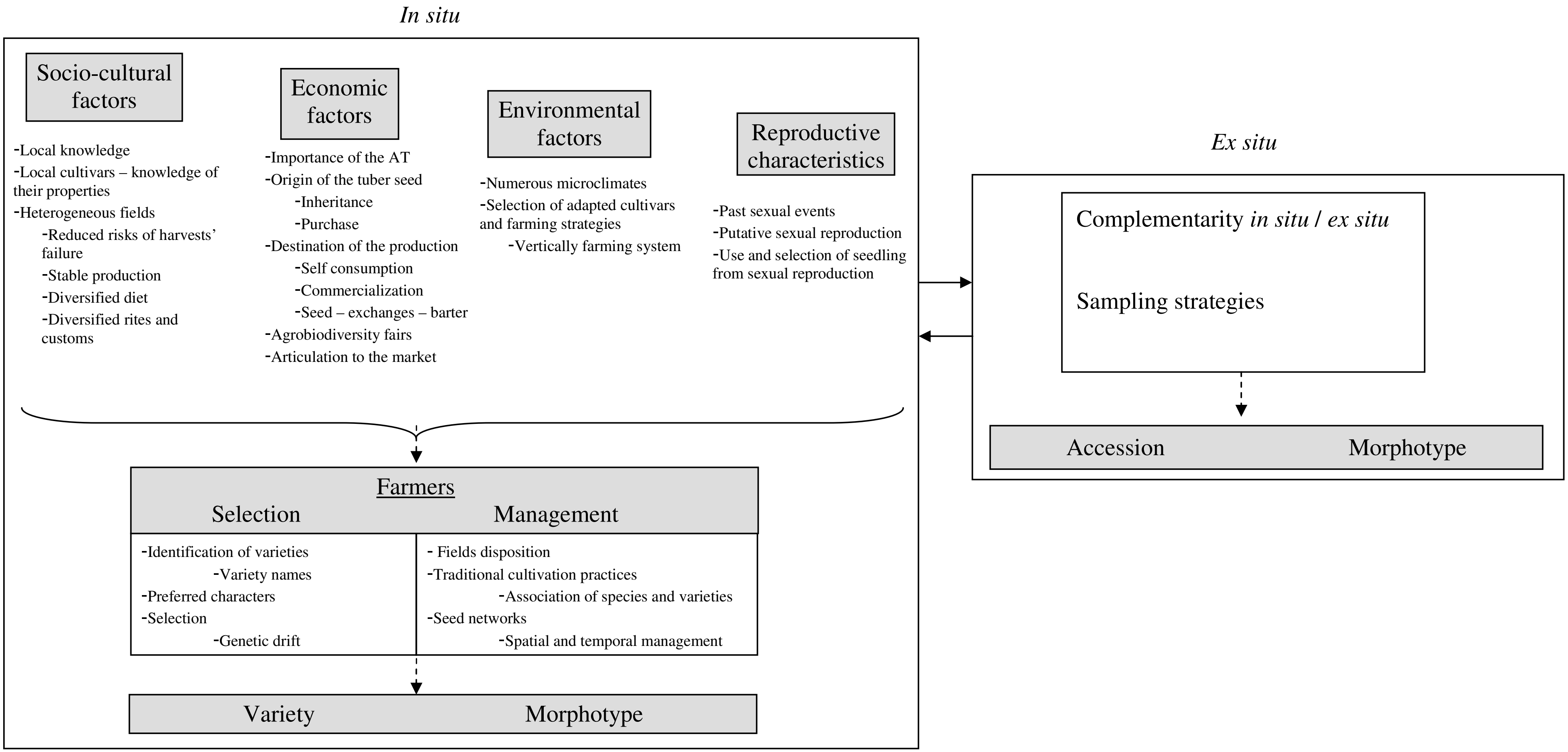
These observations share several similarities with other vegetatively reproduced species in traditional agrosystems, like potato (Brush *et al*., 1981), cassava (Elias *et al*., 2001a,b) or yam (Scarcelli *et al*., 2006); and even sexually reproduced crops like maize (Pressoir & Berthaud, 2004).

- ***Reproductive characteristics of the species***

Today, sexual reproduction is uncommon and setting seed is limited in oca and ulluco. In contrast, mashua flowers profusely and sets many viable seeds with high germination rates (see Chapter II). However, in farming conditions, propagation of these three species is exclusively realized through tubers, and botanical seed propagation by farmers has never been scientifically described in the literature.

Despite a long history of asexual propagation, it is probable that oca and ulluco maintained variation attributable to **past sexual processes**. Diversity now encountered is thus very likely relictual. In order to conserve and maintain existing genetic diversity, the Andean farmer has simply carried out clonal tuber propagation of existing cultivars, a process reinforced by the following factors: true-to-type breeding, the necessity to reduce the crop duration and prevalence of vegetatively propagated crops in Andean agriculture (Grau *et al.*, 2003).

Clonality of varieties is expected under strict vegetative propagation. The high proportion of polyclonal varieties observed in this work (see chapters III, V and VI) suggests a possible **use of seedlings from putative sexual reproduction** in Andean tuber species. For domesticated plants vegetatively propagated by man, the impact of sexual reproduction and recombination on genetic diversity is largely unknown. However, examples of management by farmers of individuals resulting from sexual reproduction have been reported, e.g. potato (*Solanum* spp., Johns & Keen, 1986; Quiros *et al.*, 1992), cassava (Boster, 1985; Salick *et al.*, 1997; Emperaire *et al.*, 1998; Elias *et al.*, 2001a,b).



AT = Andean Tubers

Figure 11: Genetic diversity and structure of Andean tuber crop species, a tentative unifying model.

In addition, the coexistence of different varieties of Andean tubers species in the same field or in neighbouring fields is common and favourable to genetic exchange between individuals through sexual reproduction. This situation may therefore lead to the production of new genotypes and phenotypes on which human and natural selection can operate. Concomitantly with the conservation of existing varieties through the traditional planting procedures, new forms originating from seedlings may be conserved, unconsciously or not, by Andean farmers.

Jackson *et al.* (1980) give an interesting example of the impact of **seedling from sexual reproduction** on genetic diversity and structure of the potato species, as well as mixture of species and varieties of Andean tubers (oca, ulluco and masshua) in farmers' fields due to the presence of **volunteers from the previous years**. According to these authors, potatoes' tubers from seedlings are usually small and, due to this small size, are preferentially used for planting the following year rather than being eaten. A similar supposition could be made for oca, ulluco or mashua.

In conclusion, the extent of incorporation of seed-derived tubers can not be completely excluded in Andean tubers species but should be confirmed by field studies. The way farming practices encourage or eliminate these sexually produced plants found in fields, and thereby influence genetic diversity and structure of their crops, needs to be investigated.

- ***Socio-cultural factors***

Traditional agroecosystems are characterized by the interaction between the environment and the farmers. A significant element of these systems is their level of plant diversity in time and space, and the traditional planting procedures used to maintain crop genetic diversity (Jackson *et al*., 1980; Brush *et al*., 1981).

Local knowledge about farming practices is especially rich in the case of native crops because of the farmers' experience accumulated over centuries. In this context, **heterogeneous fields**, with associations of different varieties and species, are particularly fitted in marginal and fluctuating environments (diverse altitudes and microclimates). This strategy is thought to support diversity of diet (flavours, textures, shapes and colours mixture), income source, yield stability, risk reduction, limited insect and disease incidence.

In addition to the cultural practices, Andean farmers have a wide local knowledge of their local cultivars' properties. Farmers recognize **advantages and disadvantages of their local cultivars** for production, food, culinary uses and medicinal properties. Farmers applying this knowledge are able to obtain the best productivity, adaptability and resistance from their cultivars (Terrazas & Valdivia, 1998).

Thus, farmers produce, consume and trade Andean tubers according to their production system characteristics and consumption preferences. Currently, the reduction of genetic diversity pattern is also associated with the **loss of traditional knowledge**, especially about traditional uses of the plant products or the tubers (Altierri & Merrick, 1987; Quiros *et al*., 1992; Bianco & Sachs, 1998; Terrazas & Valdivia, 1998; Tapia, 2001).

- ***Economic factors***

Andean tubers (oca, ulluco and mashua) are of **particular importance** in traditional rural communities. They are cultivated for their edible tubers in small areas under traditional agricultural systems and marginal conditions, in all Andean countries (mainly in Peru, Ecuador and Bolivia) and at high altitudes (2800 to 4100 meters). These tubers constitute, together with the potato species (*Solanum* sp.), Andean farmers' staple crops and the main source of income (Hernández Bermejo & León, 1992).

As shown in chapter VI in the case of oca, **destination** that families give to the production of Andean tubers is multiple (self-consumption, commercialisation, planting material, barter with other products like maize…) (Bianco & Sachs, 1998; Villarroel, 2001). Ulluco is principally cropped to be commercialized (Gonzales *et al.*, 2003) and mashua is destined to self-consumption and livestock feed (Grau *et al.*, 2003).

Also, **biodiversity fairs** are of particular importance for the dynamics of Andean tuber species and the exchange of varieties between participating farmers (from a particular community or from various ones). These fairs allow the dispersion of a great genetic richness, but especially of a limited number of varieties that are preferred on the market (Espinoza, 2001). As a consequence, varieties that do not have **market demand** are cultivated on a limited scale by Andean farmers and are threatened of disappearance in farmers' fields. Terrazas & Valdivia (1998) observed in Candelaria (Bolivia) that the number of varieties tended to decrease, and attribute their observation to market orientation. In fact, three or four cultivars are commercialized and are the most common crops among the microcentre of Candelaria. A similar observation was made by Tapia (2001), where mashua is disappearing since it is confined to a few elderly farm families for self-consumption, without current economic projections in the market.

In conclusion, the influence of the market threatens agrobiodiversity and eliminates the less utilized Andean crops, reducing genetic diversity maintained *in situ*.

- ***Environmental factors***

The Andean region presents remarkable geographical contrasts, creating **countless microclimates** (Castillo, 1995). These numerous local environmental conditions have acted as selecting forces during centuries; Andean farmers exploit natural plant adaptations and the result is the creation of a wide range of cultivars presenting differential characteristics (tolerance to soil type, moisture, temperature, reproductive mechanism, …).

Microclimatic conditions vary mainly with the altitude (Terrazas & Valdivia, 1998). To cope with these numerous microclimates, farmers establish their fields at different elevations, according to a **vertically farming system**. This has been largely developed in the case of Candelaria (see chapter II) but has also been reported at many other locations (Brush, 1991; Castillo, 1995; Villarroel, 2001). Moreover, farmers develop and maintain **adapted cultivars and farming strategies** in accordance to these microclimates.

In conclusion in a diversified ecological context (covering numerous microclimates), Andean farmers have built a complicated farming system, in accordance to their subsistence strategy and to environmental conditions.

How farmers model genetic diversity

Crops are not only influenced by natural factors, such as mutations and natural selection, but also by **human management and selection**. In fact, farmers' decisions have a great impact on local crop diversity (Jarvis *et al.*, 2000).

Fields may be large or small, close to each other or widely separated. Depending on the reproductive biology of the crop (oca and ulluco *vs.* mashua in our case – see chapter II) this spatial organization influence genetic diversity and structure of the crops (see discussion chapter VI).

Another important factor is the **association of different Andean tuber species and varieties in the same field** to mediate potential environmental stresses. Indeed, varieties show different tuber yields and adaptations to climatic conditions or to pests. Moreover, farmers regularly apply an appropriate system of **spatial and temporal tuber seed management** of Andean tubers' germplasm (see chapter II). Farmers move their varieties vertically among fields situated at different altitude levels. Crop diversity and vertically farming systems allow to reduce the risk of varieties and production loss, assuring the survival and harvest of at least a few plants in case of damage (Biancho & Sachs, 1998; Terrazas & Valdivia, 1998; Flores *et al.*, 2003).

The great diversity of these fields is handled by farmers, who identify and select existing varieties using numerous **variety names**. Recognizing these names is important because the "farmer-named variety" is the unit that farmers manage and select over time. All farming processes (management of biodiversity and selection practices) are based on the notion of **variety**. Variety names are defined on the basis of a few morphological characteristics of the tuber and the other plant organs (see chapter VI), but may also be related to various other features like the original source of the material, agronomic performance, use of the material (such as rapid cooking time, taste, use for straw or other parts of the plant), role in a religious ceremony, …. Therefore, it is not surprising that we observed a great number of variety names (see chapters III, V and VI) but also a large inconsistency in the variety names (see chapter VI).

As a consequence, a variety defined by a farmer, even if supposed clonal in the case of vegetatively reproduced crop species like Andean tubers, may be complex and consists of diverse genetic components. In fact, we found that varieties are not uniform units, neither from the morphological (see chapter VI) nor from the molecular characterization (see chapters III and V).

Some of the agromorphological traits are **preferred**, or valued, by the farmer; the farmer chooses to plant a particular variety because some of its distinguishing characteristics are desirable. The traits farmers value in their varieties may relate to agronomy (e.g. yield, drought resistance), use (e.g. cooking or fodder quality), aesthetics (e.g. colour, shape) and market demand. Farmers **select** among the plants in the crop population to maintain these desirable characteristics and to increase the prevalence of other valued traits in the population over time.

In conclusion farmers modify the amount and structuration of genetic variability of their Andean tuber crops, directly by selecting preferred varieties, and indirectly by managing the the cropping system (including planting, protecting, weeding and harvesting the plants as well as processing the tubers or the harvested products).

Morphological markers vs. molecular markers

Our results show a good correlation between morphological and molecular (ISSR) markers (see chapter IV). Moreover, the consistency of the results found using these two marker systems corroborates the reliability of the methods used throughout this thesis work. Morphological descriptors and molecular (ISSR) technique may be considered as complementary and, when possible, have to be combined for an increased resolving power of genetic diversity analysis.

Molecular markers seem to be better appropriate for deeper evaluation of genetic diversity and structure of the species. In fact, the ISSR technique, even if dominant markers, proved to be a very useful tool in this investigation (good reproducibility, low cost and good ability to detect polymorphism).

The use of morphological data is of great value in crop diversity studies but presents some limitations; the most widely recognized being the influence of environmental conditions. Their application leads to the definition of morphotypes in the case of *ex situ* conservation in genebanks, and of varieties in the case of *in situ* farmers' fields conservation. **Morphological descriptors** (published for oca and ulluco (IPGGRI/CIP, 2001, 2003) and in preparation for publication for mashua) are suitable for the usual management of field genebanks and the definition of morphotypes (see chapter IV). But, for their application in farmers' fields, they should be adapted in accordance with the view of the farmers (see chapter VI). Indeed, large variability remain within varieties in the fields and several morphological characters appear most important than others for the farmers to define a variety, e.g. tuber variables (see chapter VI). Tuber characteristics are those that present the lowest level of variability within varieties, as they do for other crops like cassava (Elias *et al.*, 2001a). Other variables, not represented in the published descriptors, are undoubtedly used by farmers to recognize their varieties and should be informative to study the factors influencing varieties conservation in the field (e.g. agronomic data, market demand, culinary and cultural uses; Cadima *et al.*, 2004).

Implications for in situ/ex situ complementarity and sampling procedures

There are two fundamental approaches for the conservation of genetic diversity: the *ex situ* and *in situ* conservation. While the objective of *ex situ* conservation is mainly to preserve germplasm without any modifications, the genetic diversity maintained *in situ* in farmers' fields is fashioned by a complex range of factors over time (see the case study in Chapter II).

Both *in* situ and *ex* situ conservations present its specific advantages and disadvantages (see chapter II) and these two methods are presented to be used in a complementary manner (Brush, 1991; Piergiovanni & Laghetti, 1999; Jarvis *et al.*, 2000). The management of genebanks, complementary to the *in situ* conservation, raise an essential question: whether the germplasm maintained in the genebank still represents the *in situ* populations from which it was collected, sometimes a long time ago. Thus, it is of prime importance for conservation as well as for practical applications to compare levels of genetic diversity and structuration resulting from these two conservation strategies.

The complementarity of the techniques (*in situ* vs. *ex situ*) and their influence on the germplasm have been studied at the variety level, in the case of oca (see chapter III). This study (performed at the molecular level) pointed out the influence of the conservation strategy on the genetic diversity of oca varieties. Moreover, intravarietal diversity was higher *in situ* than *ex situ*.

At this time, due to the relatively short *ex situ* conservation history of oca in Toralapa (where germplasm is preserved *ex situ*), the deviation we observed between *in situ* and *ex situ* conservation systems was explained by different way of tubers' management in the two systems In Toralapa, the integrity of all accessions of oca conserved in the field genebank is verified each year comparing the morphological descriptors. Moreover, during the regeneration cycles, the field collection may have been subjected to genetic drift. In fact, accessions and morphotypes maintained *ex situ* are variable entities (see chapters IV and V); and genetic composition of the accessions evolve due to the procedure of regenerations themselves (number of tubers collected and planted at each regeneration cycle).

In contrast, in Candelaria (where the germplasm is maintained *in situ)*, farmers use positive selection to improve quality of their tubers, by identifying the best plants on the basis of a range of interesting properties; they use tubers of these selected plants for the next generation. Year after year, the genetic structure of the oca population could be modified by the selection for agromorphological characteristics. Moreover, as varieties present genetic variability (see chapters III, V and VI), the observed deviation between *in situ* and *ex situ* conservation could also be explained by a sampling bias. Indeed, when varieties are compared only on the basis of their variety names, the danger is to compare accessions with the same name but which are of different varieties (see chapters III, IV and VI).

Our results suggest that in the future, **sampling** in the fields needs special attention, mainly on the importance to sample all the diversity found in these fields (intra- and inter-varietal diversity). Levels of genetic diversity between samples maintained *ex situ* and varieties found *in situ* have to be assessed and compared at the moment of the sampling, and subsequently have to be periodically controlled in order to highlight a possible evolution of one these two complementary samples.

At small scale, our results confirmed the high genetic diversity **at the range of the microcentre** (see chapters III, V and IV). Collecting in fields in the same microcentre seem to be of particular interest in the case of oca and ulluco, since the diversity of these species appears to be divided into separated genepools (see chapter VI). For mashua, due to its reproduction mode (see chapter II and discussion chapter VI), collecting in neighbouring fields may be less important since it would probably lead to the sampling of similar genotypes, i.e. belonging to the same genepool (see chapter VI). Gene flow homogenizes population structure and counteracts the effects of drift and diversifying selection.

In addition, in the range of the microcentre, genetic structure and diversity should not be influenced by altitude, because of the vertically farming system developed by farmers, in which fields are disposed at different elevations with movements of varieties among altitude levels through time (see chapter II).

The amount of diversity found at a small scale (in farmer's field, community) is expected to be high, but we also highlighted an "isolation by distance" phenomenon in the case of ulluco. **At a larger scale**, samples could be then taken in various regions, and even countries, in order to enlarge the genetic variability found (see chapter IV).

Conclusions and perspectives

Scientists try to understand how species evolve and which mechanisms are in cause (genetic, biological, habitat, climatic modifications …). They also seek to highlight how biodiversity is structured and maintained, as well as what are past and present causes of genetic erosion.

During our investigation, we analyzed the genetic diversity and structure of the three neglected Andean tuber species (oca, ulluco and mashua), using both morphological and molecular (ISSR) markers. Our objective was here to present and compare the contributions of both *in situ* and *ex situ* conservation, in order to explore the complementarity of these two methods of germplasm preservation in the particular case of three Andean tuber species (oca, ulluco and mashua).

Our results have been discussed according to the reproductive biology of the species and the Andean agricultural system, as summarized in figure 11. This unifying model of the genetic diversity and structure of traditional neglected vegetatively propagated crop species allows a better understanding of the complex processes governing the creation and the maintenance of genetic diversity in Andean tuber crop species. These findings are essential to improve collection management and genetic resources conservation of neglected Andean tuber crop species threatened by genetic erosion. In fact, genetic erosion is commonly observed *in situ*, due to the most profitable choice made by farmers to crop only some varieties, and not to invest in conservation of the varieties that are less favoured by the market. One of the causes of farmers' decisions to 'disinvest' in biodiversity lie in the lack of incentives offered by current markets and other institutions. The solution could be to develop mechanisms that provide a different set of incentives. One mechanism could be the valorisation of the favoured varieties for alternative uses, nutritional value, agronomic characteristics within potential markets. New uses for these tuber crops include starches, baby food or sweetener for diabetics, showing their high potential for short term development. Moreover, their usefulness in highland areas where food production is limited by frost and the high cost of fertiliser and seed should also be assessed.

Our research has undoubtedly provided new scientific elements to the subject. The originality of this work lies in the overall investigation of diversity in three species of Andean tubers. Analyses were conducted at different scales, from the field to the region. Different aspects are discussed simultaneously (*in situ* and *ex situ* conservations - diversity within and between varieties - socio-economic, agronomic, cultural data – inconsistency of vernacular names - geographical distribution of diversity - molecular and morphological variation). We tested the contribution of agricultural practices in the creation and preservation of the diversity of vegetatively reproduced crop species, at inter- and intra-specific levels. We compared the complementarity of *in* situ and *ex situ* conservation strategies in the case of the oca species, which showed the necessity to develop strategies for the management of diversity in the fields. All these aspects where resumed in a theoretical model of evolutionary processes that shape the genetic diversity of vegetatively propagated crops.

Anyway, additional investigations about genetic resources evaluation and conservation of Andean tubers, and in general of minor crops, need to be performed.

First, as molecular markers have shown their usefulness to improve the comprehension of the *ex situ* collections and the *in situ* conservation, their use should be promoted. The choice of the marker usually depends on the purpose for which the marker system has to be used. Co-dominant markers could be developed for the oca, ulluco and mashua species. Co-dominant markers are in general reliable and highly reproducible, and have a wide range of applications in population genetic studies. However, the development of co-dominant markers can be a time consuming, laborious and expensive process. Dominant markers, like the ISSR markers used in the present study, are comparatively less laborious, faster and cheaper to develop and use. These characteristics make them attractive for species where no other co-dominant markers or DNA sequence data are available.

Moreover, several aspects like the impact of sexual reproduction, the clarification of naming system of the varieties, a better comprehension of traditional knowledge, the analysis of germplasm evolution with time under *in situ* and *ex situ* conservation, the constitution of core collection, … deserve deeper studies to determine their influence on genetic diversity and structure of Andean tuber species.

All societies depend, directly or indirectly, on biodiversity and biological resources. In particular in the Andes, diversity of tubers species and varieties is fundamental for the existence of communities living in marginal areas and for food security of million of peasants' families. As a common strategy to minimize the risks due to harvest failure, farmers often grow many different crops and varieties, so that in extreme weather some fields are likely to produce harvestable crops. Now that problems of genetic erosion emerges more severely, new questions arise including what would be the consequences of declining diversity in the functioning of ecological and farming systems.

Indigenous peoples universally use biodiversity as a buffer against variation and disaster. This is especially important among indigenous societies living on margins of fragile and changing ecological, economic, and political systems. Biodiversity itself is threatened by climatic change and genetic erosion. Nonetheless, as climate change threatens biodiversity, the major protection that indigenous people have against variation and change is simultaneously decreasing. Their primary tool for adaptation is at risk.

In this context, it appears crucial to see biodiversity not only as a scientific concern, but as a concern for all the society: how to find a balance between economic constraints, climate change, durable development and nature protection?

However, even if scientists claim the urgent need to protect nature, the real solutions are not in their hands but strongly related with political decisions. Biodiversity has a low priority in government decisions, which is at least partially due to the difficult evaluation of what biodiversity provides to the society, either in the form of natural resources or ecotourism, of molecules for pharmaceutical and industrial uses, or genes. The identification of genes and their functions is more easily achieved and new mechanisms and technologies allow us to understand the molecular base, at the genome level. Some neglected crops or wild species could gain economic value by the simple fact that they contain genes that could potentially be used to change the genetic background of cultivated plants.

The value of biodiversity is not simply to describe and quantify. Moreover, many parts of the ecosystem are not traded and therefore do not have a market price. In Andean agrosystems, traditional knowledge and relationships between human culture and conservation of biodiversity is a cultural heritage that has been passed down from generation to generation, and of which we now know the intrinsic value. It is important to recognize that local knowledge supports the survival of cultural and biological diversity.

This reflection is important for the Andean countries that are among the regions harbouring the greatest wealth in natural resources and biodiversity. However in these countries where malnutrition and unemployment are important problems, a low priority is usually assigned to environment and natural resources (*versus* economic development). The poorest farmers in the world are ironically those who are richest in biodiversity.

The future will necessarily depend on how society and scientists will organize themselves to influence policy.

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