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SHORT COMMUNICATION

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.

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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.

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 $MgCl_2$ 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 8^e for windows).

Results

Results reflect the ability of ISSRs in revealing genetic variability within a limited sample of

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)

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. 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.

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,

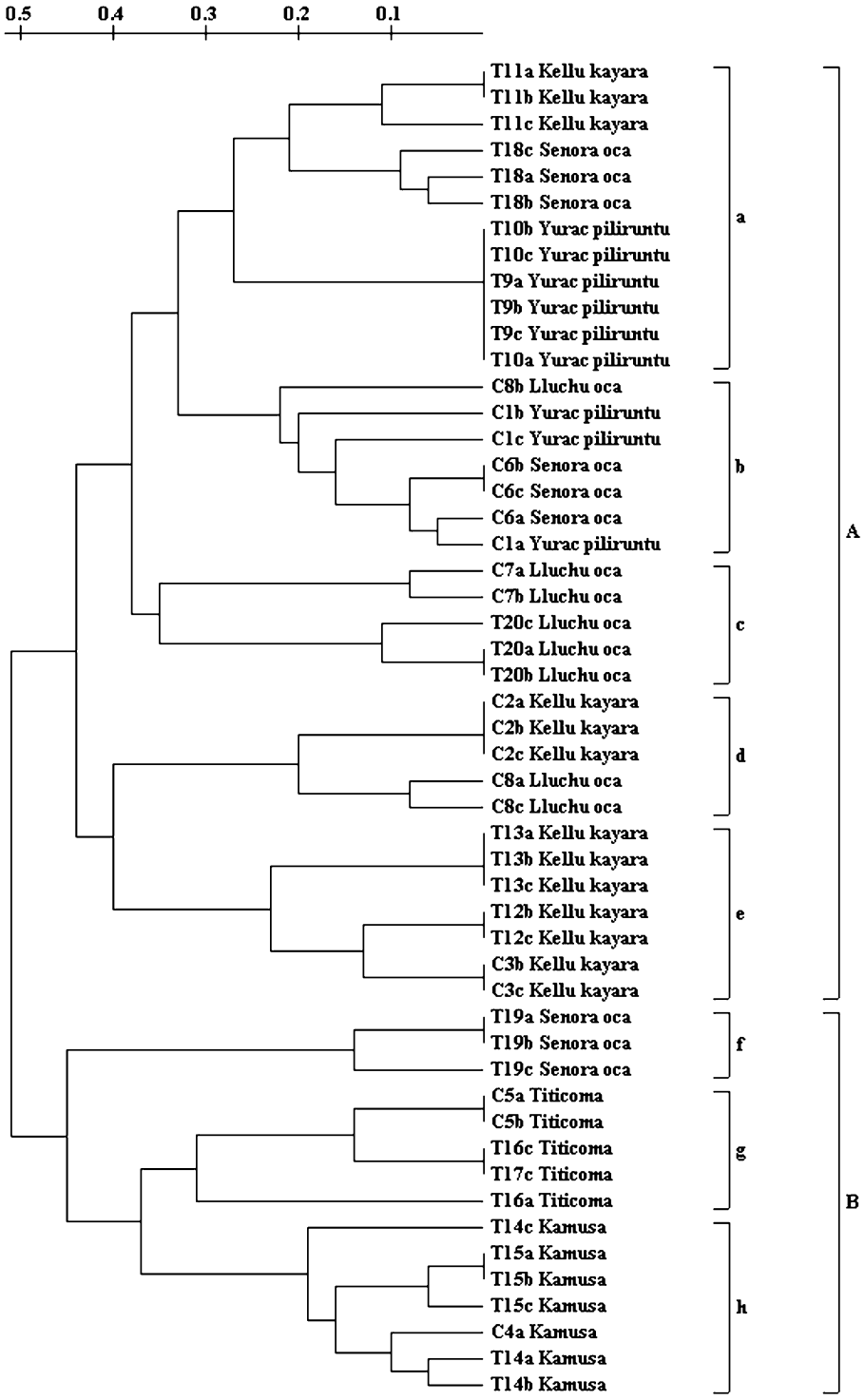

Fig. 1 Dendrogram based on ISSR polymorphism of the 51 oca's individuals representing 20 tuber lots conserved *in situ* and *ex situ*: application of unweighted paired group method algorithm (UPGMA) and distance of Dice

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

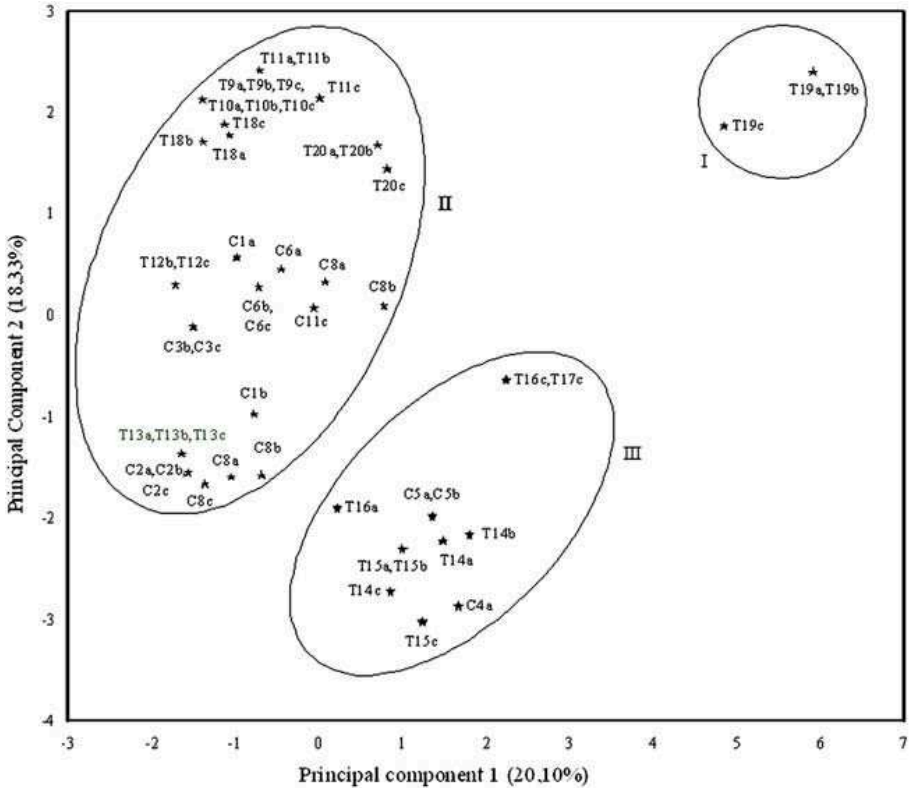

Fig. 2 Principal Component Analysis based on ISSR polymorphism of 51 oca's individuals representing 20 tuber lots conserved *in situ* and *ex situ*

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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