# Molecular characterization of the *Oxalis tuberosa* Mol. collection maintained in the CIP's genebank

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## Abstract

The Andean ecosystem harbors more than 180 economically important crop plants in a variety of climates and habitats, along its extension. The oca crop (*Oxalis tuberosa* Mol.) is the most important tuber, after potato, in this ecosystem. The genetic diversity and the geographic distribution patterns of oca genebank maintained in the International Potato Center were investigated in the present study using AFLP markers. This collection holds 585 accessions from Argentina, Bolivia, Chile and Peru. Seven primer combinations were tested; obtaining 175 polymorphic markers for genetic diversity analysis. The UPGMA dendrogram showed three main clusters, two including Peruvian ocas and the other formed by ocas from southern Peru, Argentina, Bolivia and Chile. Accessions from southern Peru were found in the three groups, supporting that oca originated in this region. The molecular groups identified showed a relationship with the eco-region where they come from: groups 1 and 3 are found in the Central Andean wet Puna and group 2 in Central Andean Puna. The molecular variance analysis (AMOVA), indicated that Peru is the country with the greatest diversity found in the CIP collection.

**Keywords:** *Oxalis tuberosa*, oca, AFLP, Genetic diversity.

## Introduction

Among the ART´s have oca (Oxalis tuberosa Molina) considered the most important specie between all of the ART´s because its high range of adaptation and its high content of vitamins, micronutrients and starch, also for its grown ability under extremely atmospheric conditions.

The productivity of oca is low because its sow under dry conditions, and support dried and freezing like plague attacks and diseases. In the last years, Oca in Peru, had a harvest ground of 20000 ha, with a production of 116 tm., the centre of major yield is the department of Puno with approximately 8 tm/ha in 2003.

To maintain and protect this species of the genetic erosion we must collect from their origin place and developed genebanks and maintain the major quantity of interest genes of each species. This conservation makes in tidy way maintaining this accessions for undefined time trying to unchange their genetic constitution.

This work find to contribute to knowledge of the genetic diversity, using AFLP markers, determined lines of geographic distribution of the accessions of oca maintained in the genebank of the International potato Center.

## Plant material

585 accessions from the genebank maintained at the International Potato Center was studied: 448 from Peru (Piura, Cajamarca, Amazonas, Pasco, La Libertad, Ancash, Junín, Lima, Huancavelica, Ayacucho, Apurimac, Arequipa, Cuzco, Puno y Tacna), 75 from Bolivia (Cochabamba, La Paz, Beni, Oruro, Potosí y Tarija), 52 from Argentina (Salta y Jujuy) y 10 from Chile (Antofagasta y Tarapacá).

## Methods

### *AFLP procedure*

AFLP fingerprinting (technique) was carried out using a modified procedure of the one described by Vos et al, 1995, modified in CIP by (Zorrilla, 2006) The digestion of genomic DNA was carried out using enzyme

combination *Eco RI / Mse I*. A pre – amplification with primers complementary to the adapter sequences, having one additional nucleotide on their 3´ end, was done before the final amplification. In this one, primers were used with three additional nucleotides on their 3´ end. The amplification product was loaded on to a 6% (w/v) denaturing polyacrylamide gel and visualized with silver nitrate.

### *Data analysis*

***Data recording***. The size of the band was calculated by evaluation in a denaturing polyacrylamide gel, was put all the primer combination used in the investigation and also weight markers like plasmid sequencing pUC 18 and ladder 30 pair base. Each produced fragment for each primer combination was taken like an evaluation unit and sequentially numbered. The data was registered in a binary matrix with 1 if is present and 0 is if absent for each AFLP marker band.

***Dendrogram construction***. The data analysis was made using with Darwin program 5.0.144 (Perrier et al, 2003), the genetic dissimilarity was estimated by the Jaccard index. The genetic distance (1 - genetic similarity) between each accession was calculating using NTSys version 2.01 (Rohlf, 1997), based on the Sokal and Michener (1958) dissimilarity coefficient. Dendrograms were constructed employing the UPGMA (unweighted pair group with mean average) algorithm using the same program. The cophenetic matrices generated from the dendrograms or the similarity matrices used in clustering were compared by performing a Mantel test (Mantel 1967).

***AMOVA and population distance (ϕst)***. Based on the Euclidean distances, the analysis of molecular variance (AMOVA) procedure (Excoffier *et al.,* 1992) was applied to estimate variance components for AFLP genotypes.

Individual variation was partitioned within and between regions (countries).

***Nei's genetic diversity***. This method was made for genetic diversity analysis (heterozygosis) of a subdivided population, genetic diversity between and among populations.. (Nei, 1973)

***Principal Coordinates Analysis (PCoA)***. Was used for evaluated the distances between population, which is a very big space and very difficult to read, this method find minor dimension spaces where populations are closer for been analyzed.

## Result and discussion

### *AFLP combinations for oca*

A total of 292 EcoRI/MseI primer combinations were tested for polymorphic bands, intensity of bands and repeatability. The best 7 combinations were used for the molecular characterization of the 585 accessions of *O. tuberosa* collection. The 82% of the AFLP markers detected were polymorphic, 175 from a total of 213. (*Table1*).

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**Table1. AFLP combinations used and percentage of polymorphism**

| Combination | Fragment Sizes | Total Markers | Polymorphic markers | Percentage of polymorphism |
|---|---|---|---|---|
| E35ACA/M59CTA | 500 -169 | 31 | 28 | 90 |
| E35ACA/M60CTC | 515 -118 | 27 | 21 | 77 |
| E39AGA/M40AGC | 515 -155 | 30 | 20 | 66 |
| E40AGC/M35ACA | 295 -153 | 29 | 29 | 100 |
| E42AGT/M54CCT | 515 -130 | 28 | 23 | 82 |
| E42AGT/M60CTC | 300 -122 | 28 | 24 | 85 |
| E45ATG/M51CCA | 405 -140 | 40 | 30 | 75 |
| Total | **515-118** | **213** | **175** | **82** |

The oca shows less polymorphism in comparison to potato; Kim *et al.* (1998) obtained 466 markers with 7 combinations in *Solanum tuberosum* meanwhile we have obtained 213 markers in oca with the same number of

combinations. We believe that it is a consequence of the size of the genome that is smaller for oca. Also, the high number of monomorphic bands would be a consequence of its polyploidy (x=8); thus the absence of a marker can be detected only when it is not present in any of the eight homologous chromosomes.

### *Clustering analysis*

The dendrogram was drawn using the Jaccard's distance and the UPGMA algorithm. Three groups were clearly observed (*Figure 1*). Group I included accessions from departments of the Northern Peru (Cajamarca, Piura, Amazonas y La Libertad), Central Peru (Lima, Ancash, Pasco, Junín, y Huancavelica) and Southern Peru (Ayacucho, Apurimac, Cuzco, Arequipa, Tacna y Puno) and one accession from Bolivia. Group II included mostly accessions from Puno, together with Argentina, Bolivia and Chile. Group III included accessions from the departments of North, Center and South Peru. The Principal Coordinates Analysis indicates shows that the Groups I and III, that include Peruvian accessions, are closely related (*Figure 2*). The South of Peru seems to be the place where it was originally domesticated as it was previously reported (Arbizu y Tapia 1992, Emshwiller 2002, Pissard *et al.* 2006) because accessions from this area are distributed in the three molecular groups.

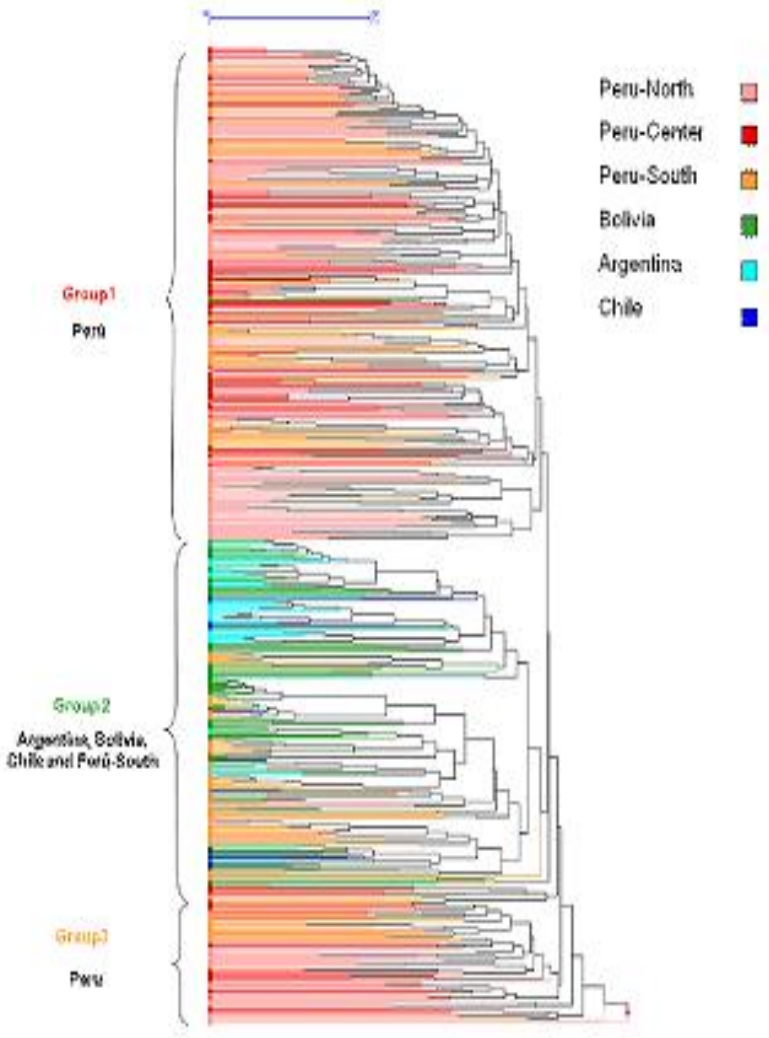


**Figure 1.**

**Clustering analysis of 585 oca accessions using Jaccard's distance and UPGMA algorithm. The dendrogram was drawn with the software Darwin 4.0.**

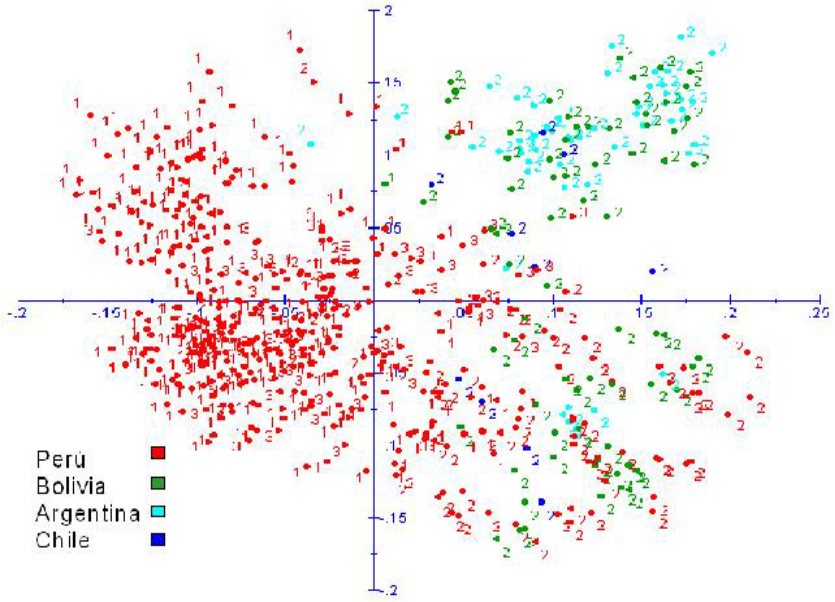


**Figure 2. Representation of the two main axes obtained from the Principal Coordinates Analysis of 585 oca accessions. Axes X (6.3%) and axes Y (4.17%) represent the 10.47% of total variability using the software Darwin 4.0**

### *Geographical patterns of genetic diversity.*

The Jaccard's disimilarity and the Nei's index were calculated to estimate genetic diversity in every country and the whole collection. The results indicate that Peru is the country with the highest genetic diversity of oca, with the highest values of Jaccard's dissimilarity (0.410) and Nei's index (0.31); and Argentina is the least diverse country with 0.309 and 0.19, respectively (*Table 2).* Also, the Analysis of Molecular Variance (AMOVA) reveals that the genetic variation is 6.77% between countries, which is significant. Although, the main source of genetic variation is individuals within countries (*Table 3).*

**Table 2. Jaccard's dissimilarity and Nei's index**

| País o Región | Jaccard's dissimilarity | Nei's index |
|---|---|---|
| Argentina (52 accessions) | 0.309 | 0.19 |
| Bolivia (75 accessions) | 0.351 | 0.22 |
| Chile (10 accessions) | 0.359 | 0.20 |
| Perú (448 accessions) | 0.410* | 0.31 |

*This average was calculated as a (1-similarity)

**Table 3. Analysis of Molecular Variance (AMOVA) by country**

| Source of variation | *df.* | Percentage of variation |
|---|---|---|
| Between countries | 3 | 6.77 |
| Indivi. within countries (Perú, Bolivia, Argentina y Chile) | 582 | 93.23 |
| **Total** | **585** | **100.00** |

In the study of Pissard *et al.* (2006) in a set of the oca accessions maintained at CIP genebank using another type of marker called ISSR, it was also found that Peruvian ocas are the most diverse compared to the ocas of Bolivia, Chile and Argentina.

## Conclusions

In conclusion, the molecular clustering of the 585 accessions from the CIP genebank shows three groups, two of them formed by accessions from Peru and one formed by accessions from Argentina, Bolivia and Chile. Peru is the most diverse country and there is a geographic pattern of genetic diversity due to genetic variation among different countries is significant.

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