Characterization of lulo (*Solanum quitoense* Lam.) genetic diversity in the department of Boyaca, Colombia

Caracterización de la diversidad genética de lulo (*Solanum quitoense* Lam.) en el departamento de Boyacá, Colombia

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Abstract

Lulo (*Solanum quitoense* Lam.) is an exotic fruit with major economic importance in the Andean region. It has gained importance in the agricultural sector due to organoleptic and cultivation characteristics. There are not studies on this plant genetic resource in the province of Neira, Boyaca department, Colombia. Given these concerns, the aim of this research was to characterize the genetic diversity with random amplified microsatellite markers (RAM). Twenty-one *S. quitoense* materials were collected in the Soaquira and Sacaneca, Boyaca-Colombia districts to calculate genetic diversity and similarity indices. In addition, the analysis with the Nei-Li coefficient formed three groups at 0.60 similarity level showing a loose distribution of the materials without a relationship with the geographical site or the presence or absence of thorns. The seven RAM markers generated 346 alleles, ranging between 43 and 48 for CT and TG, respectively. The average estimated heterozygosity values ranged from 0.33 for TG and 0.40 for CGA. The average value for all evaluated *S. quitoense* materials was 0.36, higher than the reported value in other studies on *S. quitoense* genetic diversity in Colombia. The average Fst value for the 20 evaluated materials was 0.14, showing a moderate genetic differentiation. Overall, the RAM markers identified genetic variability in the study area, the need for levels of subdivision and hierarchization greater than those considered in this research, which would have allowed a better use of the plant genetic diversity present in *S. quitoense* materials in the province of Neira-Boyaca, Colombia.

Keywords: Andean fruit, RAM microsatellites, *Solanum*, variability.

Resumen

El lulo (*Solanum quitoense* Lam.) es uno de los frutales exóticos de mayor importancia económica en la región andina, ha ganado importancia en el sector agrícola debido a sus características organolépticas y de cultivo. No existen estudios de este recurso fitogenético en la provincia de Neira, departamento de Boyacá, por lo cual el objetivo de la presente investigación fue caracterizar la diversidad genética con marcadores Microsátelites Amplificados al Azar (RAMs). Se colectaron 21 materiales de *S. quitoense* en los distritos de Soaquira y Sacaneca, departamento de Boyacá-Colombia, para calcular los índices de diversidad genética y similitud. El análisis mediante el coeficiente de Nei-Li permitió formar tres grupos a un nivel de similitud de 0.60, mostrando una distribución laxa de los materiales en donde no hubo correspondencia con sitio geográfico o la presencia o ausencia de espinas. Los siete cebadores RAMs generaron un total de 346 alelos que fluctuaron entre 43-48 para marcadores y marcador, respectivamente. Los valores de heterocigosidad promedio estimada variaron entre 0.33 para el cebador TG y 0.40 para CGA. El valor promedio para todos los materiales de *S. quitoense* evaluados fue de 0.36 más alto que lo reportado en otros estudios de diversidad genética en *S. quitoense* en Colombia. El valor de Fst promedio para los 21 materiales estudiados fue de 0.14, mostrando así una moderada diferenciación genética. Los marcadores RAMs permitieron identificar una alta variabilidad genética en la zona de estudio, la necesidad de niveles de subdivisión y jerarquizaciones mayores a los considerados en este estudio, lo cual permitiría un mejor aprovechamiento de la diversidad genética presente en los materiales de *S. quitoense* en la provincia de Neira.

Palabras clave: Frutal andino, microsátelites RAMs, *Solanum*, variabilidad.

Introduction

Lulo (*Solanum quitoense* Lam.) belongs to the *Solanum* genus and the Lasiocarpa section, which includes about 12 species (Bohs, 2004). In Latin America, economically profitable crops belong to the species *Solanum quitoense* and *Solanum sessiliflorum* (Heiser, 1985). In Colombia, *S. quitoense*, has gained importance in the agroindustrial sector for juices, yogurt, flavoring, soft drinks and processed foods because of its appearance, bittersweet flavor, and fragrance, ease of cultivation, continuous production, and high yields (Lobo *et al.,* 2007).

However, this species is still in domestication process and have some important traits as follows: allogamy, narrow ecological adaptation, presence of thorns on stems and leaves, anthocyanins in different organs, trichomes, latency and high number of seeds per berry, andromonoecy, rapid oxidation of the juice and presence of ideoblasts in the leaves, as seen in individuals in the weed-wild complex (Lobo, 2006).

In Colombia, three systematic collection processes have been conducted for *S. quitoense*, and related taxa, covering the departments of Antioquia, Boyaca, Caldas, Cauca, Huila, Magdalena, Nariño, Norte de Santander, Putumayo, Quindio, Santander, Tolima and Valle del Cauca (Lobo *et al.*, 2007). The potential use of a collection depends on the materials knowledge. In this country, studies conducted for characterization and evaluation of *S. quitoense* collections and related species, have allowed exhibiting the existence of polymorphism (Fory *et al.*, 2010; Lobo *et al.*, 2007; Riascos *et al.*, 2012). Similarly, molecular studies have revealed significant polymorphism (Fory *et al.*, 2010), and a study conducted with AFLP markers (Fory *et al.*, 2010), found greater genetic variability in interspecific hybrids, *S. hirtum* × *S. quitoense,* as compared to the parentals, which points out the potential for increasing the genetic basis of *S. quitoense* for breeding programs.

The advance of fruit genetic breeding programs by conventional procedures is difficult and expensive, mainly due to the very long growing seasons, inter- and intraspecific incompatibility, presence of important attributes in wild species and high degree of heterozygosity (Lobo, 2006). The use of biotechnological tools in these programs would improve the selection process, facilitating control and management of the genetic basis of populations, saving resources and ensuring genetic purity of new varieties (Moose & Mumm, 2008). Therefore, it is necessary to implement strategies for the evaluation and characterization of *S. quitoense* materials with farmers in different regions of the country.

Among the known molecular markers, RAM (Random Amplified Microsatellites) are very useful for measuring genetic diversity in plants and animals to detect differences among families, species and within species. In addition, Muñoz *et al.* (2008), showed that the variation basis of individuals, have allowed the selection of specific regions within the DNA molecule for determined studies, the number of detectable polymorphisms is theoretically unlimited and allows for the analysis of information that is both expressed and non-expressed. This method is feasible to implement in small laboratories in terms of equipment and facilities cost; it requires no prior sequencing knowledge or the use of radioactive isotopes; therefore, can be used in characterization studies of genetic diversity (Morillo *et al.*, 2014).

In order to establish a strategy and plant management for phytogenetic resources for *S. quitoense* in the department of Boyaca- Colombia especially in the Province of Neira, it is necessary to start studies on morphological, agronomic, physiological and molecular characterizations to know the genetic diversity and generate basic necessary information to obtain sustainable solutions for the problems of low levels of technology in production, common in *S. quitoense* cultivation, such as lack of homogeneity in organoleptic characteristics in materials used in beverages and jams, genetic transformation, which allows for a shorter growth cycle for production in less time and tolerance to pests and diseases throughout plant breeding program (Lobo *et al.,* 2007). Within this context, this research aimed to establish a molecular characterization of lulo (*Solanum quitoense* Lam.) materials in the Province of Neira, Boyaca-Colombia using random amplified microsatellite markers (RAM).

Materials and methods

Biological material

Young leaves were collected from a total of 21 *S. quitoense* materials in the province of Neira, municipality of Pachavita in the Soaquira district, department of Boyaca, Colombia; located at an altitude of 1985 m. a. s. l. with an average temperature of 16°C and in the Sacaneca district, at an altitude of 2148 m. a. s. l., with an average temperature of 17°C (Table1).

Molecular characterization was carried out at the Universidad Pedagógica y Tecnológica de Colombia. Boyacá-Tunja, Colombia. DNA extraction was according to Dellaporta *et al.* (1983).

Farm N° individuals	District	Altitude (m.a.s.l.)	Description	Latitude	Longitude
La playa (6)	Soaquira	2234	Wild or mixed Lulo	5°08´26″	73°23´52″
Carrizal (5)	Soaquira	2404	Smooth or thorny Lulo	5°06´27″	72°26´63″
San Eduardo (4)	Soaquira	2320	Smooth or thorny Lulo	4°03´24″	73°34´54″
La estrella (4)	Soaquira	2186	Smooth or thorny Lulo	2°05″17″	74°24´33″
San Antonio (2)	Sacaneca	2148	Smooth or thorny Lulo	5°45´54″	73°14´53″

Molecular characterization

Genomic DNA of displayed samples was in 0.8% agarose gels, stained with z-vision in a MaxiCell Primo EC-340 electrophoresis gel system chamber. A Hoefer Dyna Quant 200 fluorometer, was used to determine the concentration of each genotype and HPLC water, was used for the dilution (10 ng μL^{-1}), at a total volume of 100 μL and stored at -20°C. Seven markers, synthesized by Technologies Inc. (Table 2), were used for RAM analysis.

Table 2. Markers used in the RAM microsatellite technique

Markers	Sequence (5´ to 3´)
CCA	DDB(CCA)$_5$
CGA	DHB(CGA)$_5$
ACA	BDB(ACA)$_5$
AG	HBH(AG)$_7$A
CT	DYD(CT)$_7$C
TG	HVH(TG)$_7$T
CA	DBDA(CA)$_7$

For RAM amplification, a reaction cocktail was prepared in a sterile microcentrifuge tube (1.5 mL) at a final volume of 25 µL. The reaction mixture was prepared with 1X buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1U DNA Taq polymerase, 2 uM of marker primer and 10 ng of genomic DNA.

The amplification was carried out in a PTC 100 Programmable Thermal Controller thermocycler (MJ. Research, Inc.). Initial denaturation was at 95 ° C for 5 min; denaturation at 95 ° C for 30 sec, annealing at a temperature of 50 ° C (primer AG CA), 55 ° C (primer CCA-TG-CT) and 58 ° C (primer CGA) for 45 sec, one final extension of 72 ° C for 2 min, 37 cycles from denaturation to extension, and a extension at 72 ° C for 7 min. Products were separated by electrophoresis in 1.2% high resolution agarose gel at 90 volts for 3 h, displayed in an ultraviolet light transilluminator.

Statistic analysis

A binary matrix was created, absence (zero) and presence (one). The genetic similarity among individuals was calculated using the Nei-Li similarity coefficient. The grouping analysis was carried out with UPGMA method and a dendrogram was created with the NTSYS statistical package (Numerical Taxonomy System for personal Computer, version 2.02 PC ™). The unbiased heterozygosity and polymorphic loci percentage were estimated to evaluate the genetic diversity using the TFPGA (Tools for Population Genetic Analyses) statistical package, version 1.3. ®.

Results and discussion

The analysis with the Nei-Li coefficient at a 0.60 level of similarity differentiated in *S. quitoense* materials into three groups (Figure 1). Group I, had three *S. quitoense* materials from the Soaquira district-Boyaca, Colombia in the following farms: La Playa, San Eduardo and Carrizal, which were characterized as wild *S. quitoense* material, conserved by farmers in that area, exhibiting traits of incipient domestication.
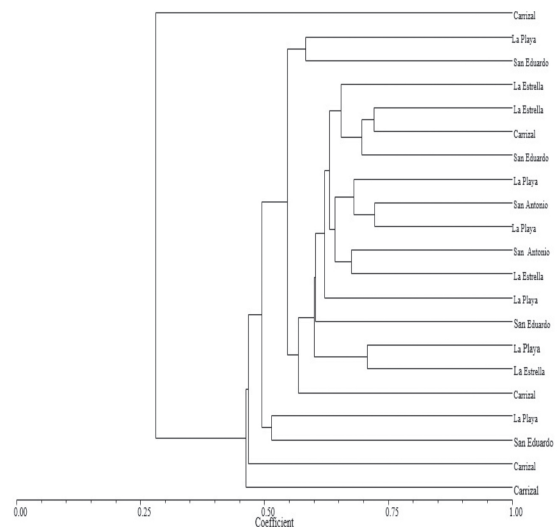
Figure 1. Dendrogram of 21 lulo materials (*S. quitoense* Lam.) based on the coefficient of Nei and Li.

This group contained material from the Carrizal farm, which had the lowest similarity value (0.25) with respect to rest of *S. quitoense* materials. This have allowed establishing specific morphological traits of *S. quitoense* materials and geographical separation, because these materials were collected at higher altitudes.

Group II, had *S. quitoense* materials collected in two districts, Soaquira and Sacaneca- Boyaca,

Colombia demonstrating that gene flow may exist among these materials, throughout seed exchanges by farmers in this area. These *S. quitoense* materials were smooth (thornless) or thorny, thick and mixed, characterized by high coefficients of variation in the qualitative and quantitative characteristics. Traits, which are favorable for marketing, helping to solve the problem of reduced fruit size and weight in this country (Riascos *et al.*, 2012).

Group III, with a level of similarity of 0.50, contained three *S. quitoense* materials, which were collected at the Carrizal farm, which had the highest genetic distance (0.45) from the other evaluated materials, which corroborates the fact that the materials of this farm, are highly variable and wild, maintained by this farmer and found at a higher altitude than the others.

In general, the groupings had a loose distribution of *S. quitoense* materials, with no relationship with the districts, but showed an exchange or gene flow among them. Furthermore, there were no associations for the presence or absence of thorns, as has been reported in other studies on *S. quitoense* genetic diversity (Riascos *et al.*, 2012). The obtained results in this research, are different from those obtained by Fory *et al.* (2010), who reported low variability in cultivated species of *S. quitoense,* using AFLP markers. This difference is associated with the type of marker as follows: AFLP and RAM markers, are dominant and COS II markers are co-dominant, and are designed based on conserved exon in order to amplify intron sequences, which increases the possibility to find polymorphism.

The seven RAM markers used in this study generated 346 alleles, which ranged from 43 for the CT marker to 48 for TG marker, with molecular weights between 300 and 1000 bp (Table 3). The number of bands was suitable for estimating parameters of genetic diversity when compared with the obtained results in other studies on *S. quitoense* and other Solanaceae species (Saliba-Colombani *et al.*, 2000; Toquica *et al.*, 2003; Fory *et al.*, 2010). TG marker, performed the greatest contribution to the observed variation with a 0.22 Fst, which means it can be useful for other studies on the genetic diversity in the *Solanum* genus.

The average estimated heterozygosity values ranged from 0.33 for TG marker to 0.40 for CGA marker. The average value for all evaluated *S. quitoense* materials, was 0.36. In addition, the fact that *S. quitoense* is an allogamic species, but can also experience self-pollination and be an andromonoecious species must be taken into account (Enciso-Rodríguez *et al.* 2010). Ge *et al.* (2013), when analyzing the genetic diversity and structure of eggplant populations (*Solanum melongena* L.) in China using simple sequence repeat markers, found an expected heterozygosity value of 0.32, lower than the value reported in this study.

Other genetic diversity studies with codominant microsatellite markers on different species (*S. elaeagnifolium, S. rostratum* and *S. tuberosum* spp. *tuberosum*) of the Solanaceae family, have shown higher heterozygosity values, demonstrating the high variability present in these species and the potential of these markers to determine the genetic structure and diversity in natural populations (Vallejo-Marín *et al.*, 2011; Zhu *et al.*, 2013).

The coefficient of genetic differentiation (Fst = 0.14 ± 0.01), suggests an intermediate level of genetic differentiation, which may be due to the state of domestication of the evaluated materials and may also indicate that there is a moderate relationship between the geographical location and genetic group (Morillo *et al.*, 2014). The molecular variance analysis AMOVA, showed the genetic variation observed in the evaluated *S. quitoense* materials, was mainly within groups, with 89 % (Table 4). This high variation could indicate the presence of higher levels of subdivision and hierarchy. The remaining 11% was due to the component of genetic variance among groups, which was significant (P≤0.001). Such genetic variation among groups, might be used for conservation and breeding of this species. Similar results have been reported in other studies of genetic diversity in the genus *Solanum* using microsatellite markers (De Galarreta *et al.,* 2007; Mercati *et al.,* 2015; Zhou *et al.,* 2015).

Table 4. Analysis of molecular variance (AMOVA) for the formed groups

Source of variation	Gl.	SC	CM	%
Among groups	2	210.97	105.48	11
Within groups	18	1145.02	63.61	89
Total	20	1356.00	----	100

Table 3. Parameters of genetic diversity in the evaluated *S. quitoense* materials

Marker	N° Loci	Estimated He	Polymorphic loci % (95%)	Fst	SD
ACA	44	0.35	88.6	0.11	0.02
AG	46	0.38	100.0	0.19	0.04
CA	47	0.36	95.7	0.08	0.02
CCA	45	0.39	95.6	0.06	0.02
CT	43	0.34	90.7	0.12	0.03
TG	48	0.33	89.6	0.22	0.03
CGA	47	0.40	100	0.17	0.04
TOTAL	**346**	**0.36**	**94.4**	**0.14**	**0.01**

Results of this study are consistent with Lobo (2006), who found greater variation at the subgroup or subpopulation level, which is correlated to the geographical distribution of *S. quitoense*

in this country, because Colombia is a center of origin and has diverse agro-ecological conditions, which are appropriate for this species. Geographical barriers, adaptation to different ecological niches, the reproduction mode of the species, and type of molecular markers used, could influence results (Lobo *et al.*, 2007).

In general, high genetic variability was observed in *S. quitoense* materials, both cultivated and wild, contrary to what was reported by Fory *et al.* (2010), who found a reduced genetic variability in cultivated species of *S. quitoense* and species related to the Lasiocarpa section of the Colombian collection, which was mainly attributed to the founder effect. Owing to the low degree of domestication, is common that exist a high heterozygosity in Andean fruits, due to a mechanism of adaptation and survival to changing environmental conditions. However, *S. quitoense* is a primitive plant with a low degree of domestication, and stability and adaptability, which are restricted to very specific niches with direct natural variation and evolutionary processes (Lobo, 2006). Knowledge on the producers and communities has enabled the development and maintenance of local and primitive varieties (or so-called folk varieties), which have been the basis for the development of most crops, and are continuously subjected to selective pressures imposed by the spatiotemporal dynamics of growth and human being selection processes (Lobo, 2006).

The use of molecular markers in the evaluation and characterization of wild germplasm, provides great opportunity for a more precise determination of the relationships and genetic structure and evolution of the populations. In addition, it is an important tool for gene transfer assisted by markers to identify variants at the genome level. The introgression of interest traits throughout interspecific hybridization and conservation of wild materials is an alternative, which can help to identify elite materials that are adapted to local conditions and meet the needs of farmers, producers and consumers.

Conclusion

Lulo (*Solanum quitoense* Lam.) materials were grouped into three groups, which did not present any associations strictly related to the geographic area or thorn presence or absence. This research study revealed the existence of genetic variability in the evaluated *S. quitoense* materials in the Province of Neira-Boyaca, Colombia, which are readily available to be used in breeding programs aimed to find elite materials in this area.

Acknowledgements

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