Genetic Variability of the Colombian Collection of Lulo (*Solanum quitoense* Lam.) and Related Species of Section *Lasiocarpa*

Variabilidad Genética de la Colección Colombiana de Lulo (*Solanum quitoense* Lam.) y Especies Relacionadas de la Sección *Lasiocarpa*

Paola Andrea Fory Sánchez[1]; Inés Sánchez Mosquera[2]; Adriana Bohórquez Cháux[3]; Hernando Ramírez[4]; Clara Inés Medina Cano[5] y Mario Lobo Arias[6]

Abstract. *An AFLP genetic variability study of the Colombian collection of lulo* ***Solanum quitoense*** *and related species of section Lasiocarpa was carried out. The aim was to elucidate the genetic polymorphism of such collection and interspecific affinities. Two primer combinations, E-ACG/M-CAT and E-ACG-M-CTC, were chosen out of 30 evaluated combinations based on their high expression levels of polymorphism. The UPGMA dendrogram, obtained through similarity analysis, exhibited systemic power with discrimination at species level and between the groups of Andean and Amazonian taxa from section* ***Lasiocarpa****, with a clear separation between* ***Lasiocarpa*** *species and outgroup taxa. Clustering patterns regarding the geographic origin of the accessions, as well as between materials of the* ***S. quitoense*** *botanical varieties* ***septentrionale*** *and* ***quitoense*** *were not evident. Wider genetic variability was observed in the wild section* ***Lasiocarpa*** *species than in the cultivated ones* ***S. quitoense*** *and* ***S. sessiliflorum****. The interespecific hybrids between* ***S. hirtum*** *and* ***S. quitoense*** *and backcrosses of these with* ***S. quitoense*** *exhibited greater variability in comparison to* ***S. quitoense****. The above, indicated the feasibility of the use of* ***S. hirtum*** *to broaden the genetic base of the cultivated species, with potentiality of* ***S. psudolulo*** *and* ***S. vestissimum*** *for the same purpose. The results obtained with the multiple analysis of correspondence were in agreement with those of the similarity index analysis, and the neighbor joining analysis with high "bootstrap" values, which indicates that the support of each cluster for the studied species is well represented.*

Key words: AFLP, clustering patterns, dendrograms, genetic base broadening, molecular characterization.

Resumen. *Se realizó un estudio de la variabilidad genética de la colección colombiana de lulo* ***Solanum quitoense*** *Lam y especies relacionadas de la sección* ***Lasiocarpa****, por medio de marcadores AFLP, con el fin de conocer el polimorfismo y las afinidades entre materiales y taxa. Para tal fin se emplearon marcadores moleculares AFLP, y se hicieron análisis de conglomerados, con los cuales se construyeron dendrogramas a través del algoritmo UPGMA. Los marcadores empleados mostraron valor sistemático ya que permitieron la separación de grupos por especies y entre taxa de la Amazonía y de los Andes, con aislamiento de* ***Solanum*** *de otras secciones, empleados como grupo de comparación. En el estudio no fue evidente el agrupamiento de los materiales por zonas de origen e igualmente no hubo un patrón de asociación de los materiales por variedad botánica:* ***septentrionale*** *y* ***quitoense*** *del taxón* ***S. quitoense.*** *También se observó menor variabilidad genética en las especies cultivadas* ***S. quitoense*** *(andina) y* ***S. sessiliflorum*** *(amazónica), con relación a las silvestres. Los híbridos interespecíficos entre el taxón silvestre* ***S. hirtum*** *y el cultivado* ***S. quitoense*** *y los retrocruzamientos de éstos hacia* ***S. quitoense****, incluidos en el estudio, señalaron el potencial de ampliación de la base genética del taxón cultivado por esta vía, con posibilidades en este sentido a partir de los taxa* ***S. pseudolulo*** *y* ***S. vestissimum*** *(andinos). El análisis de correspondencia múltiple y el dendrograma obtenido por el algoritmo "vecino más próximo", exhibieron alta congruencia con el obtenido por UPGMA, con altos valores de remuestreo ("bootstrap").*

Palabras clave: AFLP, ampliación de la base genética dendrogramas, patrones de agrupamiento.

Lulo, (*Solanum quitoense* Lam), Solanaceae, is an Andean fruit, of the *Lasiocarpa* section, which includes, according to different researchers, between 11 and 13 species of shrubs or small trees (Whalen *et al*., 1983, Heiser, 1996, Bohs, 2004). This set of species is distributed in the northeast part of South America, with presence of taxa in the Guianas and the northern Brazil (Bohs, 2004). Some of the species of the section produce edible fruits and two of these, *Solanum quitoense* and *Solanum sessiliflorum*,

[1] Research Assistant. International Agricultural Tropical Center - CIAT. A.A. 6713, Cali, Colombia. <p.fory@cgiar.org>
[2] Researcher. International Agricultural Tropical Center - CIAT. A.A. 6713, Cali, Colombia. <i.sanchez@cgiar.org>
[3] Researcher. International Agricultural Tropical Center - CIAT. A.A. 6713, Cali, Colombia. <a.bohorquez@cgiar.org>
[4] Associated Professor. Universidad Nacional de Colombia, Sede Palmira. Facultad de Ciencias Agropecuarias. A.A. 237, Palmira, Colombia. <hramirez@palmira.unal.edu.co>
[5] Researcher. Corporación Colombiana de Investigación Agropecuaria – CORPOICA, C.I. "La Selva". A.A. 100, Rionegro, Colombia. <cmedina@corpoica.org.co>
[6] Researcher. Corporación Colombiana de Investigación Agropecuaria - CORPOICA , C.I. "La Selva". A.A. 100, Rionegro, Colombia and Associated Professor. Universidad Nacional de Colombia, Sede Medellín. Facultad de Ciencias Agropecuarias. A.A. 1779, Medellín, Colombia. <mlobo@corpoica.org.co>

Recibido: Julio 19 de 2010; Aceptado: Febrero 21 de 2011.

cocona or cubiú, are economically important crops in Latin America (Heiser, 1969, 1985). The primary center of diversity of the fruit includes Colombia, Ecuador and Peru, with presence of wild related species in Venezuela, Brazil, Central America and one taxon in Asia (Whalen *et al*., 1983, Heiser and Anderson, 1999, Lobo and Medina, 2000). Lulo was introduced and it is cultivated in Venezuela, Panama, Guatemala and Costa Rica (Heiser and Anderson, 1999; Lobo and Medina, 2000; Bohs, 2004), with distribution of the cultivated species between 1200 and 2300 masl (Heiser and Anderson, 1999; Lobo and Medina, 2000).

The plant does not have any archaeological reports (Heiser, 1985). It was found and described by the Spanish conquerors in Ecuador and Colombia (Patiño, 1962), countries that are the main producers of the species. Lobo (2004), considers Colombia as the nuclear center of the taxon, which is based on genetic and linguistic arguments. In this country ancestral attributes are found, with a noticeable pattern of domestication that extends from the south of Colombia to Ecuador, in which most of the planted materials, unlike the Colombian species, lack of thorns, which is a derived character (Whalen *et al*., 1981, Lobo, 2000). Besides the above, in Colombia there is the greater wealth of species of the *Lasiocarpa* section, with eight taxa of this biological set. As far as the linguistic concept, in Ecuador the fruit is named with the Spanish word "naranjilla", whereas in Colombia it is denominated "lulo" word of quechua origin (Patiño, 1962), with the use of other names at local level as: "Machak-ve", in the Kamsá dialect (Schultes, 1949) and "Monai" in the Tunebo language (Lobo, 2004).

Lulo is not a completely domesticated species (Lobo, 1991, 2000). The previous affirmation is based on the fact that it exhibits a series of characteristics corresponding to individuals of the wild-weed complex, such as: allogamy, narrow ecological adaptation in the spontaneous populations, thorns in stems and leaves, anthocyianins in different organs, fruits covered by trichomes, seed dormancy, elevated number of seeds by berry (Lobo, 2000), non-plastic andromonoecy (Miller and Diggle, 2003), fast browning juice, and leaves with ideoblasts containing calcium oxalate crystals (Medina, 2003). This last characteristic is a natural mechanism of defense against herbivory in spontaneous populations. The above agrees with the affirmed by Gepts (2002), who indicated that the fruit trees have been considered crops with a partial syndrome of domesticación, and display some but not most or all of the domestication traits (Gepts, 2002).

The starting point of any crop is the planting material, in which there is the genetic information for all the development and production processes of the plant. Those include its architecture, the productive capacity in specific environments, the insect and disease resistance, the tolerance to abiotic factors, and the quality aspects required by different consumer types (Lobo, 2002). The above deserves greater relevance in the case of fruit species, taking into account the investments to be made until they start production, by their relatively long vegetative periods (Lobo, 2006).

In Colombia there are planted around 4500 hectares of lulo, with an annual production of 35000 to 38910 tons and an average yield of 8.7 ton/ha (Ministry of Agriculture and Rural Development, 1997; cited by Lobo and Medina, 2000). Besides the above, the production of about 1000 hectares is imported from Ecuador (Lobo, 2000, 2004). The fruit is used for fresh consumption and also it has an important and increasing demand by the juice industry.

Lulo is planted in the Colombia, as it is the case of most of the Andean fruits, by sexual seed of farmer local varieties (Lobo, 2006). These, generally are heterogenous and heterocygous, because they are produced by open pollination, especially in areas of concentration of crops, in which several farmer materials are planted at the same locality. With this species, the only improved material offered is the clone "La Selva", which started with an interspecific hybridization between *Solanum hirtum* and *Solanum quitoense* sent by professor Heiser of the University of Indiana; the hybrid was backcrossed twice towards *S. quitoense*, by Lobo and Navarro; with selection and cloning of three plants in the F2, of the second backcross, and release of the improved cultivar (Bernal *et al*., 1998).

For the development of a sustainable lulo breeding program, a collection, was developed and incorporated to the National System of Plant Germplasm Banks of Colombia. Such collection is constituted mainly by local farmer varieties, and wild and feral populations of related species from the *Lasiocarpa* section, either collected in Colombia or obtained from other national and international institutions that hold genetic resources (Lobo *et al*., 2000). The potential of this collection

depends on the knowledge of the genetic variability of the metapopulation in conservation. In this connection, it has been indicated that one of the main reasons for the reduced use of the materials of the germplasm banks is the lack of information of the genetic variability of such collections (Ordás, Malvar and de Ron, 1994).

Based on the above, the Global Plan of Action for the Conservation and Sustainable Utililization of Plant Genetic Resources for Food and Agriculture, included, as one of the priority activities, the increase of germplasm characterization and evaluation processes (FAO, 1996). In such document, it was indicated that most genebank accessions have not been well characterized and evaluated, a situation that leads to the under use of collections, resulting in high conservation costs in relation to the derived benefits (FAO, 1996). Additionally, the importance of the integration of molecular and phenotypic information has been stressed, in the so called "industrial biological research era", (Sobral, 2002). The above allows the integration of several information levels and the identification of relationships between genotype and phenotype, which has been the driving question underlying genetics since Darwin and Mendel (Sobral, 2002).

A series of processes of characterization and evaluation of diverse nature has been made, with the Colombian collection of lulo and related species, including the molecular study reported in the present paper, carried out with AFLP markers. AFLP exhibits a series of advantages such as: the great number of fragments originated and revealed in one gel, its high power of detection of genetic variability, and the specificity of amplification (Ferreira and Grattapaglia, 1998). Other research work carried out include: genetic variation by Conserved Ortholog, COS II, markers (Enciso-Rodríguez *et al*., 2010), evaluation and morphologic characterization (Sahaza and Henao, 2001; Lobo *et al*., 2007), physiological evaluation (Medina, 2003; Medina *et al*; 2004a, 2004b; Medina *et al*., 2005), morphologic and chemical characterization and evaluation (Bermeo, 2005), chemical evaluation (Lobo and Medina, 2000) and allozymes variability analysis (data not published). With the results of the present AFLP molecular work, it is possible to obtain putative relationships between the molecular markers and phenotypic attributes, for further genetic verification of such relations.

MATERIALS AND METHODS

Plant materials. In the study, were included accessions from lulo and 6 related section *Lasiocarpa* species, and populations of other *Solanum* taxa as outgroup. Additionally, interespecific hybrids between S. hirtum and *S. quitoense* and a group, called collectively as backcrosses, which includes: backcrosses of the above hybrids to *S. hirtum*, the 3 clones that conform cv. Lulo "La Selva", and one F2 clone derived from cv "La Selva", were also used, for a total of 159 evaluated populations (Table 1). From the field collection, established at "La Selva" Research Center, Rionegro, Antioquia, young leaves were harvested and frozen in liquid nitrogen for DNA extraction and tissue was collected from a composite sample of 5 plants per accession.

DNA extraction. The molecular work was carried out at the CIAT's (International Agricultural Tropical Center) Biotechnology Laboratory, Palmira, Colombia. Total DNA was extracted according to the method developed by Dellaporta *et al*. (1983). The DNA quality was verified by electrophoresis in 0.8% agarose gels.

AFLP analysis. The work was done employing the methodology proposed by Vos *et al*. (1983). AFLP assays were performed in duplicate and only those patterns obtained clearly twice were scored. For the study the kit AFLP Analysis System (Invitrogen™) was used, with modifications suggested for plant DNA, following catalog instructions. Isolated DNA was digested by using the restriction enzymes EcoR I and Mse I. Amplified regions were electrophoresed in TBE 0.5X buffer in 6% polyacrilamide gel, and visualized by silver nitrate staining. The amplification process was carried out in a M.J. Research Inc. Programmable Control Thermocycler (PTC-100).

Data analysis. Dendograms were obtained from matrices based on AFLP patterns, using the clustering method of UPGMA (unweighted pair group method using arithmetic averages), described by Sneath and Sokal (1973). For the above, matrices for AFLP were obtained from the presence of absence of each band, scored as 1 and 0. Similarities between individuals were estimated by the Dice index (1945), also known as Nei-Li coefficient (Nei and Li, 1979), using NTSYS-PC, version 2.1 (Rohlf, 2000).

Additionally, an Analysis of Multiple Correspondence, (MCA), was done with the procedure COSRRESP of the statistical program SAS (SAS Institute, 1989),

to find structures of the population in study, and an analysis of neighbor-joining was made, through the program PAUP 4.0 to determine the confidence levels of the tree nodes. Also, a bootstrap analysis, with 1.000 replications was applied through the program 4.0 for the same purpose.

Table 1. Species and number of accessions by taxa included in the molecular characterization study, from the Colombian lulo and related species collection*

Species	Number of accessions
Andean species	
S. quitoense	74
S. pseudolulo	31
S. hirtum	7
S. vestissimum	7
S. pectinatum	5
Amazonian species	
S. sessiliflorum	11
S. stramonifolium	1
Interspecific crosses	
S. hirtum X *S. quitoense*	4
Backcrosses	
(*S. hirtum* X *S. quitoense*) X *S. quitoense***	10
Outgroup species	
S. atropurpureum	1
S. jilo	1
S. capsicoides	2
S. mammosum	2
S. marginatum	1
S. spp	2
Total	**159**

* Colombian Plant Genetic Resources System

** Including clones from cv "La Selva"

RESULTS AND DISCUSSION

DNA extraction. Good quality and adequate concentration of DNA was obtained with the Dellaporta *et al*. (1983) extraction method. The average yield of total DNA was between 20 and 150 ng by mg of tissue, which was sufficient for the molecular analysis with the AFLP technique. Additionally, the test of digestion, with one of the restriction enzymes (EcoR I), used in the technique, corroborated the good quality of the extracted DNA.

AFLP molecular markers. Out of the 30 evaluated oligonucleotid pairwise, the most polymorphic were the combinations E-ACG/M-CAT and E-ACG/M-CTC, with a total of 206 and 170 polymorphic bands, respectively. The reading rank was made between 50-400 pb (Figure 1). The preliminary tests showed that the technique of the AFLP is highly polymorphic and reproducible, conferring a high degree of credibility. Similar results have been reported by several researchers using this technique with other species of the Solanaceae family (Christian *et al*., 1996; Saliba-Colombani *et al*., 2000; Acquadro *et al*., 2003 y Toquica *et al*., 2003).

Similarity analysis. The similarity analysis, estimated by the Dice coefficient (1945), calculated with the data of both combinations of primers, showed a greater number of genome sampled sites than those obtained independently by each one of the combinations. The UPGMA-AFLP dendogram, is shown in Figure 2. As may be appreciated, the AFLP results exhibited systematic power, with conformation of clusters by species of each one of the 7 section *Lasiocarpa* taxa included in the study. These results agree with

the ones obtained by means of cladìstic analysis of morphologic characteristics (Heiser 1972; Whalen *et al*., 1981); isoenzymatic analysis (Whalen and Caruso, 1983; Bruneau *et al*. 1995), and karyotipic studies (Bernardello *et al*., 1994). Whalen *et al*. (1981) affirmed that internal barriers for hybridization, between *Lasiocarpa* species are generally well developed and include stylar incompatibility, hybrid seed abortion, and partial hybrid sterility. Molecular studies based on chloroplast ndhF sequence data using a broad range of sampling from *Solanum* indicate that section *Lasiocarpa* may be a relatively basal lineage within the *Leptostemonum* clade and that it may be sister to *Solanum* section *Acanthophora* (Olmstead and Palmer, 1997; Bohs, 2005). The systematic power of the AFLP-UPGMA dendrogram is supported also by the clear separation of the *Solanum* outgroup species, being located at both ends of the obtained tree, with similarity levels with the section *Lasiocarpa* taxa from 12 to 28%.

As shown in Figure 2, greater intraspecific polymorphism was obtained with the wild related species of section *Lasiocarpa* than with the cultivated ones. In such connection the highest intraspecific variability was exhibited by *S. hirtum*, *S. pseudolulo* and *S. vestissimum* accessions, with similarity values that oscillated between 76 to 98%, 77 to

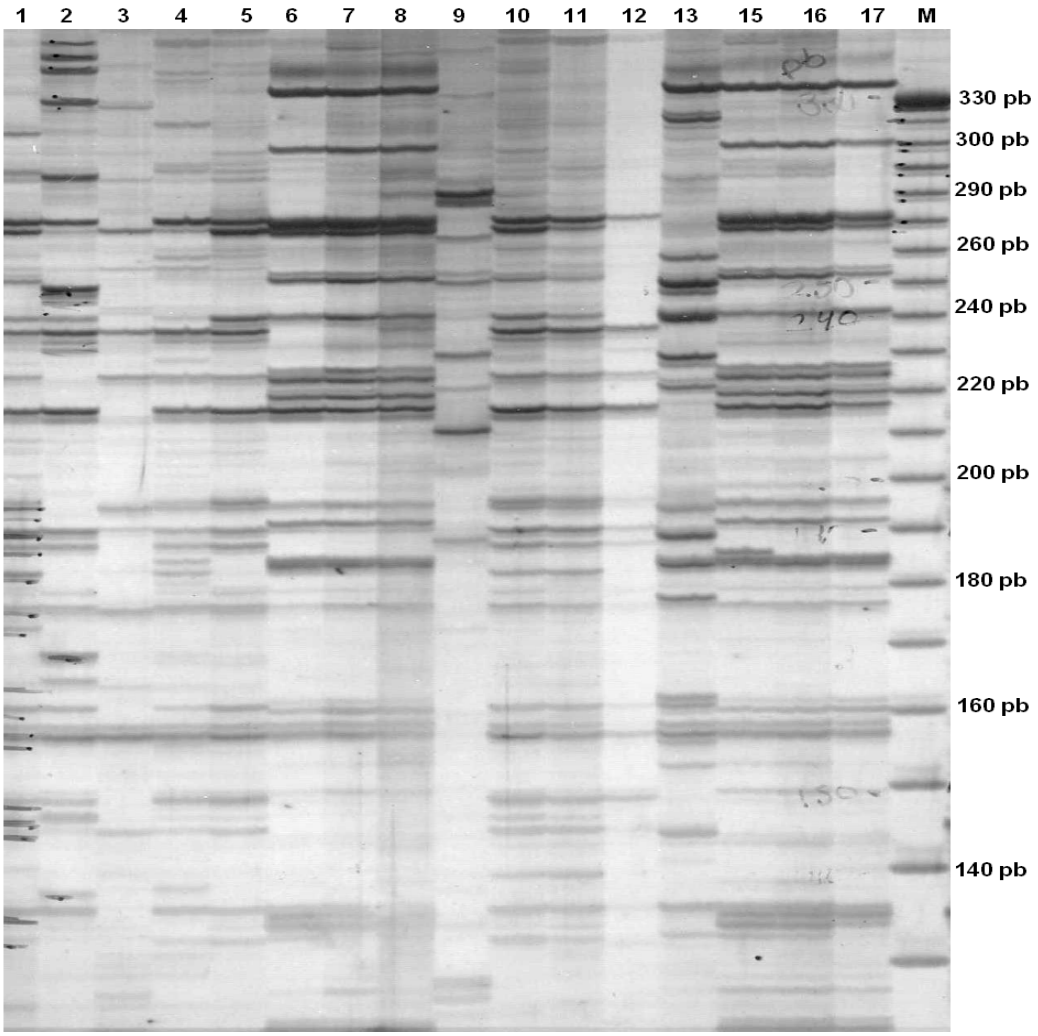

Figure 1. AFLP pattern of bands, obtained with some of the studied Colombian lulo and related species accessions, employing the primer combination E-ACG/CAT.

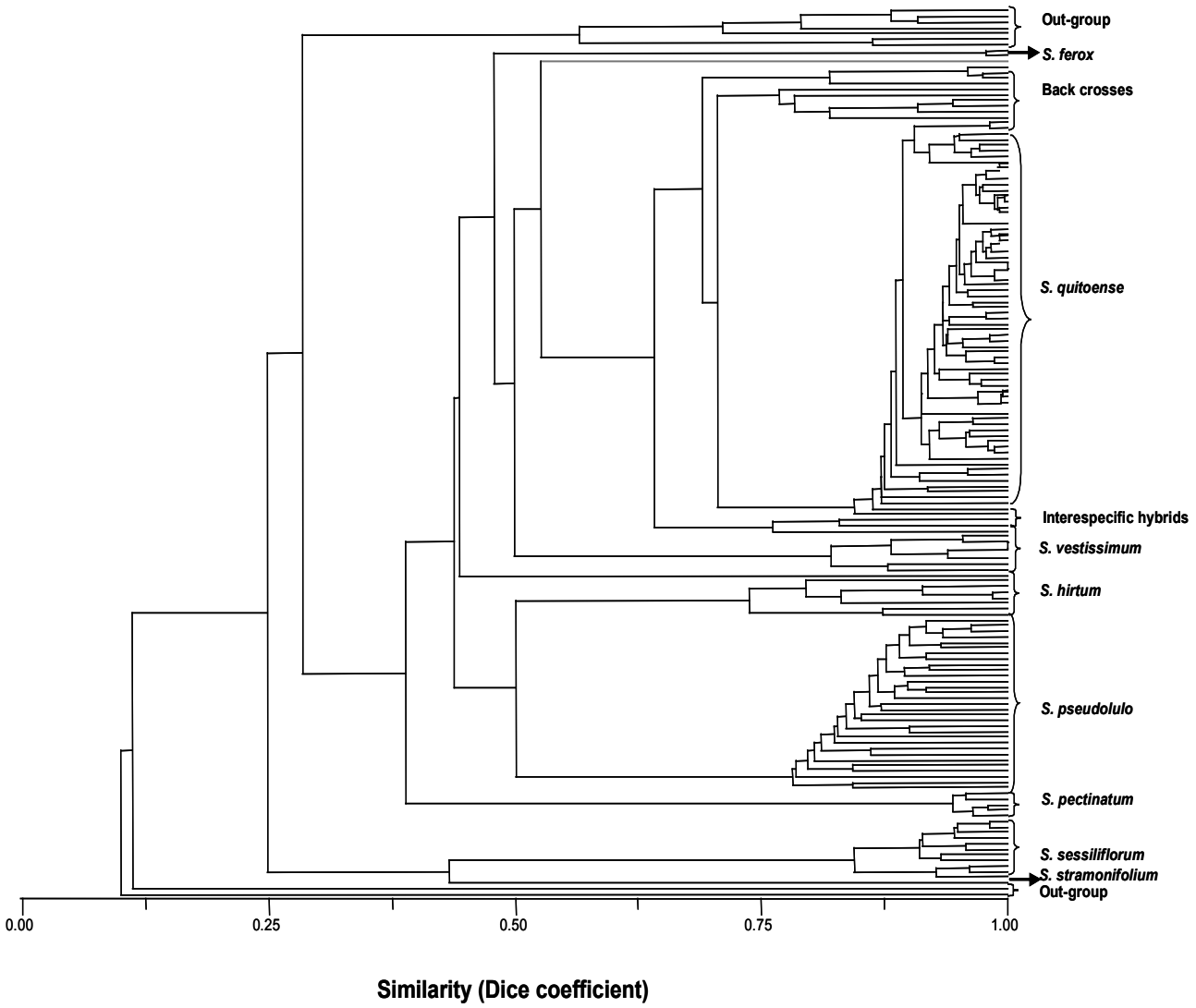

Figure 2. AFLP molecular dendrogram of Colombian lulo and related species collection.

96%, and 83 to 100% respectively. In contrast, the cultivated species *S. quitoense* displayed similarity levels between accessions between 85 and 100%, being completely similar just two populations. *S. sessiliflorum*, planted at the Amazon region exhibited similarity values among their demes between 0.85 and 0.98%. The *Lasiocarpa* species *S. ferox* and *S. pectinatum*, exhibited the lowest variability, which can be due to the reduced accession number included in the study. The other Amazonian taxon *S. stramonifolium*, was represented just for one material, exhibiting a clustering pattern with the other species from this region, *S. sessiliflorum*. It is to say that there was no a clear relationship at intraspecific level between clustering patterns and site of origin of the populations, based on the comparisons of the clusters and the passport data (not included). Also, it was not appraised an evident clustering pattern, in *S. quitoense*, based on the presence or absence of thorns, criteria that has been used to classify the botanical varieties septentrionale and quitoense respectively (Whalen *et al*., 1981). The narrow genetic variability, in cultivated materials has been related with the "founder effect", which designates the establishment of a new population by a few individuals who carry a small fraction of the genetic variability of the parental population (Mayr, 1942), whose effect is low genetic variability in the new population (Ladzinsky 1985). Another cause of the above is the small population sizes in local

varieties, which causes the loss of alleles by genetic drift (diverse authors, mentioned by Spillane and Gepts, 2001).

The non evident clustering pattern, of *S. quitoense,* based on origin places of the accessions, is attributed to the fact that lulo cultivators, move from one place to other in Colombia, with their seeds (Lobo, 2000), which cause genetic flow. The previous fact could explain the relatively low intraspecific variability of the studied lulo accessions, in conjunction with the fact that AFLP are dominant markers, which could underestimate the genetic charge of the studied demes, because *S. quitoense* is a cross pollinated species.

The lack of a pattern of clustering of lulo accessions, in relation to the presence and absence of thorns, could be attributed, to the genetic migration between these two types of materials. Both are frequently cultivated in Colombia at the same geographical local zones, under different irradiation conditions (Lobo, 2006). This derives from different requirements, in this sense, by the prickly and the unarmed materials (Medina, 2004; Medina *et al*., 2006). Usually, the populations with thorns grow better in agroforestry systems, and the ones without thorns under full sunshine conditions (Lobo, 2006).

Another outstanding result derived from the AFLP-UPGMA dendrogram was a clear separation, in the section *Lasiocarpa*, between de Andean species (*S. quitoense, S. hirtum, S. pseudolulo, S. vestissimum* and *S. pectinatum*) and the Amazonian ones (*S. stramonifolium* and *S. sessiliflorum*). Similar results were obtained by Bruneau *et al*. (1995); the authors found, in a phylogenetic context, two groups based on restriction DNA patterns, obtained from chloroplasts, which were compared with morphologic and isoenzymatic studies made by different authors (Whalen and Caruso, 1983). Bruneau *et al*. (1995), found a first group conformed by the Andean species: *S. quitoense, S. hirtum, S. pseudolulo, S. vestissimum* and *S. pectinatum,* and a second group of three amazonian taxa: *S. stramonifolium, S. sessiliflorum* and *S. albidum* (not included in section *Lasiocarpa*).

In relation to the obtained results, Whalen and Caruso (1983), indicated that most of the populations of *Solanum* section *Lasiocarpa* proved to be highly monomorphic at allozyme loci and quoted the affirmation of Soule (1976), who stated that "because natural populations of most species are ordinarily small an sparse, we assume that their genetic poverty is size related". In the previous sense, and since the AFLP markers are dominant, it is important to make genetic characterizations using codominant markers to reveal the heterocigocity of the populations of the Colombian collection. Bethaud and its collaborators (2001) indicated that the genetic migration between local populations has been generally underestimated. This indicates the importance of uncover the recessive alleles present in the studied materials, aspect which is more relevant in the case of allogamous species, as is lulo. In connection with the above, there is in progress a study of characterization using different isozyme loci.

Taking in consideration the apparent low level of intraspecific variability and the diverse levels of similarity between the taxa clusters, a broadening of the genetic base of lulo, could be obtained through interspecific hybridization. The species with greater potential for achievement of the above is *S. hirtum* by their sexual compatibility with *S. quitoense* with production of fertile hybrids (Whalen *et al*., 1981; Heiser, 1985; Lobo, 2006). In the context just mentioned, it can be observed in the dendrogram (Figure 2), at both sides of the *S. quitoense* cluster, groups of materials derived from interespecific hybridization between *S. quitoens*e and *S. hirtum*. At the left side there is a conglomerate of hybrids from both parents, and at the right side, another integrated by interespecific hybrids between these species backcrossed to the cultivated ancestor. In both cases an increase in variability could be appreciated. It has been indicated (Jørgensen and Mauricio, 2005) that interbreeding among species can lead to the creation of novel genotypes and morphologies that lead to adaptation. In connection with the above, Lobo and his group has started a process of broadening of the genetic base of lulo, throughout interespecific hybridization between five populations of *S. hirtum* and ten of *S. quitoense* with backcrossing to *S. quitoense* accesions (Lobo, 2000, 2002, 2004a. 2004b, 2006). Also, the first Colombian improved lulo, cultivar "La Selva", was obtained, by Lobo and other researchers, employing the same procedure (Bernal *et al*., 1998). Another important fact of *Solanum hirtum*, as a germplam source, is the fact that this species is the most widespread and variable taxa of section Lasiocarpa, extending from Mexico through Central America, Colombia and Venezuela to Trinidad (Whalen *et al*., 1981).

Another potential taxa for gene transfer to broadening the genetic base of lulo, are: *S. pseudolulo* and *S. vestissimum.* The first taxon is endemic from Colombia and it is found from 200 to 2000 masl, in different environments (Whalen *et al*., 1981). Crosses between *S. pseudolulo* with *S. hirtum* are difficult to obtain, by using the first taxon as female parent (Whalen *et al*., 1981), with later embryo rescue. Lobo (2006), obtained a fertile interespecific hybrid without embryo rescue, in one combination of genotypes from both taxa (Lobo, 2006). By these means it could be evaluated the possibility of using the hybrid as a bridge to transfer genetic variability between *S. pseudolulo* and *S. quitoense.* Also *S. pseudolulo* produces interspecific fertile hybrids as female parent crossed to *S. vestissimum*, and the last taxon could be crossed with *S. quitoense* as pollen receptor, employing embryo rescue (Whalen *et al*., 1981).

Multiple correspondence analysis, MCA. This procedure is a descriptive technique of multivaried analysis that allows visualizing in a graphical way the existing relations between groups of individuals. The MCA procedure, unlike the similarity analysis that gives equal weight to all the bands, confers greater weight to the unique ones. The obtained results, included in Figure 3, explained most of the variation in three dimensions.

As it can be observed, there is an evident distribution of the seven species of the section *Lasiocarpa*, as discreet groups, in the three-dimensional space. Thus, in the first dimension, it can be appreciated separation between *S. sesiliflorum* and *S. straminifolium* from the outgroup species; the second dimension establishes a marked difference of all the Andean species of section *Lasiocarpa* and the third dimension shows the separation between the different species included as outgroup taxa and the cluster conformed by *S. quitoense*, the hybrids between *S. hirtum* and *S. quitoense*, the clones from lulo cv "La Selva" and backcrosses of the hybrids to *S. quitoense*. The results of the MCA analysis agreed closely with those obtained with the similarity one, included in the

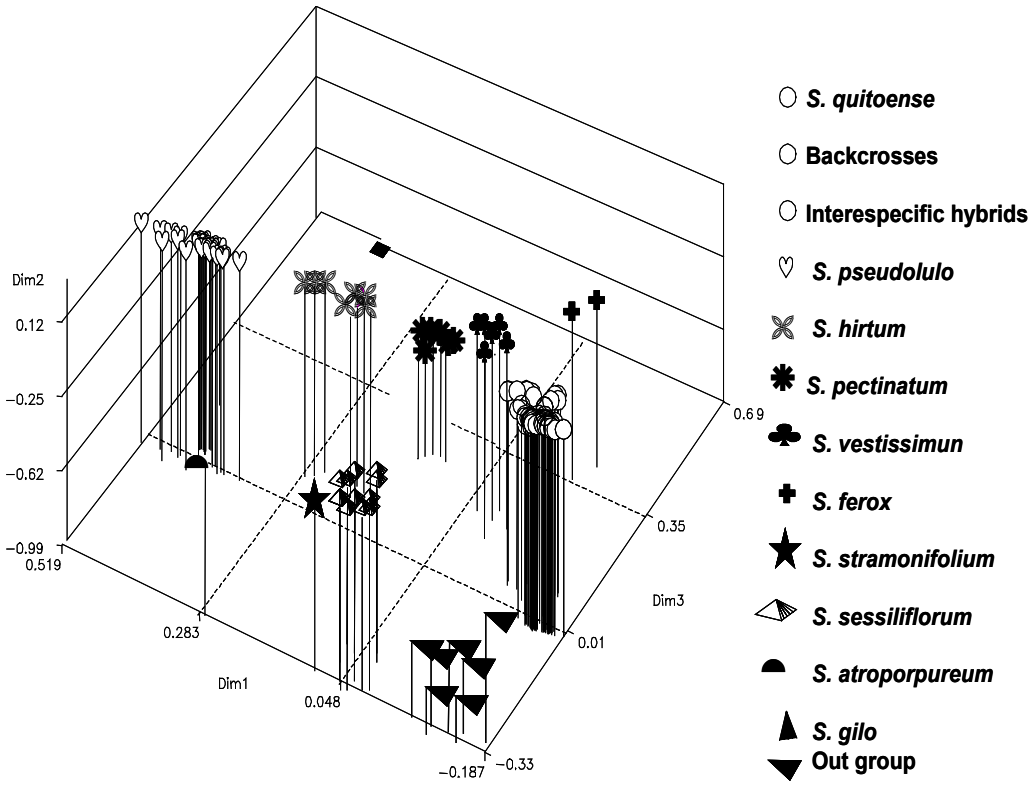

Figure 3. Tridimensional graph obtained for the Colombian lulo and related species collection with the Multiple Correspondence Analysis, MCA.

AFLP-UPGMA dendrogram, constructed with the Dice similarity coefficient.

Neighbor-joining análisis. From the 159 accessions analyzed with the AFLP, those non informative, that could cause bias to the result, were excluded from the analysis. *S. jilo* was the used species for rooting the tree, as outgroup. Additionally, the nodes of the respective tree were evaluated through "bootstrap" with 1,000 replications for distance to obtain confidence probability. The dendrogram, from the Neighbor-joining analysis, obtained from the Dice similarity coefficient (1945), is not included by its high coincidence with the one obtained by the UPGMA algorithm, discussed previously. Bootstrap values of 100% were obtained for clusters of the 7 section *Lasiocarpa* studied species, which indicates an adequate representation of the clustering pattern of the tree branches for each one of these taxa.

Taking into account the results obtained with both algorithms, the structure represented in the UPGMA dendrogram Figure 2, is congruent with the ones obtained in morphologic and isoenzymatic characterization processes done by Whalen *et al*. (1981) and Whalen and Caruso (1983), respectively and do not agree with the results of Bruneau *et al*. (1995). The discrepancies with this study may reside in the type of analyzed DNA. Whereas Bruneau *et al*. (1995) used cpADN, a structure highly conserved, which is used to obtain more precise data about the evolutionary history of the species, in this investigation nuclear DNA was used, which is a structure relatively more dynamic, which is subjected to recombination, selection and gene flow.

Considering bootstrap results, the group formed by all the Andean species of the section *Lasiocarpa*, displays three sub-groups. The first conformed by *S. hirtum* and *S. pseudolulo*, supported by a branch with 87% bootstrap value; which indicates that these two species are closely related to each other. Similar results were found in different phylogenetic studies made by Whalen *et al*. (1981) and Whalen and Caruso (1983). The second sub-group is conformed by *S. quitoense* and *S. vestissimum* with a bootstrap value of 66%. Finally, a third sub-group conformed by *S. pectinatum* accessions, species that was not included in the morphologic analysis performed by Whalen *et al*. (1981). The last taxa, *S. pectinatum*, was suggested to be closely related to *S. quitoense,* based on an isoenzyme diversity analysis done by Whalen and Caruso (1983), hypothesis that was supported by previous studies with flavonoids. These, relationships were not found in a combined analysis (isozymes and cpDNA), made by Bruneau *et al*. (1995). In the present study, another important clustering pattern is the one conformed by the Amazonian section *Lasiocarpa* species *S. sessiliflorum* and *S. stramonifolium*, with a bootstrap value of 99%.

The bootstrap values, obtained in the present research, give support and confidence to clustering patterns of one Amazonian and another Andean group of section *Lasiocarpa* species. The presence of Amazonian and Andean species has been reported in others Solanaceae genus, between which are included *Capsicum*, and *Cyphomandra*, genus proposed to be transferred to *Solanum* (Bohs, 1995), section *Pachyphylla* (Bohs and Olmstead, 1999). In the section *Lasiocarpa*, sterile hybrids have been obtained between *S. quitoense* and *S. sesiliflorum*, Andean and Amazonian cultivated species respectively (Heiser, 1993; Heiser and Anderson, 1999).

CONCLUSIONS

AFLP molecular markers exhibited systematic power, with clustering of section *Lasiocarpa* accesions at intraspecific level and discrimination of the Lasiocarpa taxa from other *Solanum* species included as outgroup.

The molecular markers discriminated in the section *Lasiocarpa* between the clusters of Andean species: *S. quitoense, S. hirtum, S. pseudolulo, S. vestissimun* and *S. pectinatum* and those of the Amazonian species: *S. sessiliflorum* and *S. stramonifolium*.

Greater genetic variability was found in the wild species section *Lasiocarpa* in comparison with the cultivated taxa, *S. quitoense* and *S. sessiliflorum*. This can be attributable to the founder effect in the last ones, and reduced population sizes of the planted farmer materials, which may cause lost of alleles by genetic drift.

In *S. quitoense* it was not evident a pattern of clustering of the materials in relation to the origin sites, which was attributed to movement of the farmers from a zone to another one, taking with them their local seeds.

Also, in *S. quitoense* there was not a clustering pattern, discriminating populations with spines and

unarmed ones, criteria that has been employed for the classification of the botanical varieties septentrionale (with thorns) and *quitoense* (without thorns). The previous result could derive from the fact that both types of materials are planted in the same regions, which causes genetic flow between them.

Broadening of the genetic base of *S. quitoense* could be achieved by interespecific hybridization with *S. hirtum*, which is facilitated by crossing compatibility between both taxa.

The above is supported by the increase in polymorphism obtained with interspecific hybrids between both species and backcrosses to the cultivated taxon, evaluated in this research.

Another alternative for broadening the "lulo" genetic base is from the Andean related species *S. pseudolulo* and *S. vestissimum*, which requires the employment of genetic bridges in the first case, and embryo rescue in the second one.

It is recommended to employ codominant molecular markers to uncover the recessive alleles of the studied populations of section *Lasiocarpa* species, taking into account the heterocigocity of this species, by their allogamy.

Ample confidence of the clustering patterns of the current research was obtained through the employ of MCM analysis and the bootstrap procedure.

ACKNOWLEDGEMENTS

The authors acknowledge COLCIENCIAS, Colombia for financial support, the collaboration of all the personnel of CIAT Biotechnology Laboratory directed by Doctor Joe Tohme, and Maria Cristina Duque and Doctor Jaison Rauscher for their help in the statistical analysis.

BIBLIOGRAPHY

Acquadro, A., E. Portis and S. Lanteri. 2002. Development of microsatellite markers in pepper (*Capsicum annum* L.), using Microsatellite-Amplified Fragment Polymorphism (M-AFLP). *Capsicum* and Eggplant Newsletter 21: 25-28.

Bernal, J., M. Lobo y M. Londoño. 1998. Documento de presentación del material "Lulo La Selva". Corpoica. Rionegro, junio de 1998. 77 p.

Bernardello, L.M., C.M. Heiser and M. Piazzano. 1994. Kariotypic studies in *Solanum* section of *Lasiocarpa* (Solanaceae). American Journal of Botany 81(1): 95-103.

Berthaud, J., J.C. Clement, L. Empraire, D. Louette, M. Pinton, J. Sanou and G. Seccond. 2001. The role of local-level geneflow in enhancing and maintaining genetic diversity. p. 81-103. In: Cooper, H.D. Spillane, C. Hodgkin. (eds.). Broadening the genetic base of crop production. CABI Publishing Willingford, Oxford, UK.

Bohs, L. 1995. Transfer of *Cyphomandra* (Solanaceae) and its species to *Solanum*. Taxon 44(4): 583-587.

Bohs, L. and R.G. Olmstead. 1999. *Solanum* phylogeny inferred from chloroplast DNA sequence data. p. 97-110. In: Nee, M. D.E. Syman, R.M. Lester, and J.P. Jessop. (edits.). Solanaceae IV: Advances in biology and utilization. Royal Botanical Gardens, Kew, U:K.

Bohs, L. 2004. A Chloroplast DNA phylogeny of *Solanum* section *Lasiocarpa*. Systematic Botany 29(1): 177-187.

Bohs, L. 2005. Major clades in *Solanum* based on ndhF sequence data. p. 27-49. In: R.C. Keating, V.C. Hollowell and T.B. Croat. (eds.). A festschrift for William G. D'Arcy: the legacy of a taxonomist. Monographs in Systematic Botany from the Missouri Botanical Garden, Vol. 104. St. Louis, MO.

Bruneau, A., E.E. Dickson and S. Knapp. 1995. Congruence of chloroplast the DNA restriction site characters with morphological and isozyme data in *Solanum* sect *Lasiocarpa*. Canadian Journal of Botany 73: 1151-1167

Cleveland, D.A., D. Soleri and S.E. Smith. 2000. A Biological Framework for Understanding Farmer's Plant Breeding. Economic Botany 54: 377-394.

Christian, W., V. Bachem Rutger, S. De Bruijn, D. Vreugdenhil, M. Zabeau and R. Visser. 1996. Visualization of differential gene expression using a novel method of RNA fingerprinting based on AFLP: Analysis of gene expression during potato tuber development. The Plant Journal 9(5): 745-753.

Dice, L.R. 1945. Measures of the amount of ecologic association between species. Ecology 26(3): 297-302.

Dellaporta, S., J. Wood and J.B. Hicks. 1983. A plant DNA minipreparation: version II. Plant Molecular Biology Reporter 1: 19-21.

Enciso-Rodríguez, F., R. Martínez, M. Lobo and L.S. Barrero. 2010. Genetic variation in the Solanaceae fruit bearing species lulo and tree tomato revealed by Conserved Ortholog (COSII) markers. Genetics and Molecular Biology 33(2): 271-278.

Food and Agricultural Organization, FAO. 1996. Global plan of action for the conservation and sustainable utilization of plant genetic resources for food and agriculture. FAO, Rome. 63 p.

Ferreira, M.E. y D. Grattapaglia. 1998. Introducción al uso de marcadores moleculares en el análisis genético. Embrapa, Brasilia, DF, Brasil. 221 p.

Gepts, P. 2002. A Comparison between crop domestication, classical plant breeding and genetic engineering. Crop Science 42(6): 1780-1790.

Heiser, C. 1969. Nightshades: the paradoxical plants. Freeman and Co., San Francisco, CA, U.S.A. 200 p.

Heiser, C. 1972. The relationships of the naranjilla (*Solanum quitoense*) and their relatives. Biotropica 4(2): 77-84.

Heiser, C. 1985. Ethnobotany of the naranjilla (*Solanum quitoense*) and its relatives. Economic Botany 39(1): 4-11.

Heiser, C. 1987. Origins of *Solanum lasiocarpum* and *S. repandum.* American Journal of Botany 74(7): 1045-1048.

Heiser, C. 1993. The naranjilla (*Solanum quitoense*), the cocona (*Solanum sessiliflorum*) and their hybrid. p. 29-34. In: Gustafson *et al*. (eds.). Gene conservation and exploitation. Plenum Press, New York, USA. 234 p.

Heiser, C. and G. Anderson. 1999. "New" Solanums. p. 379-384. In: Janick, J. (edit.). Perspectives on new crops and new uses. ASHS Press, Alexandria, VA. 462 p.

Jørgensen, S. and R. Mauricio. 2005. Hybridization as a source of evolutionary novelty: leaf shape in a Hawaian composite. Genetica 123(1-2): 171-179.

Ladsinsky, G. 1985. Founder effect in crop-plant evolution. Economic Botany 39(2):191-199.

Lobo, M. 1991. Perspectivas de la siembra del lulo o naranjilla (*Solanum quitoense* Lam.). Boletín Técnico. Universidad Nacional de Colombia, Facultad de Ciencias Agropecuarias, Palmira 2(2): 125-132.

Lobo, M. 2000. Papel de la variabilidad genética en el desarrollo de los frutales andinos como alternativa productiva. p. 27-36. En: Memorias III Seminario de Frutales de Clima Frío Moderado. C.D.T.F. Manizales, Noviembre 15-17.

Lobo, M. 2004a. Posibilidades y perspectivas del desarrollo de programas de mejoramiento en frutales andinos. Visión conceptual. p. 463-471. En: Memorias V Seminario Nacional e Internacional de Frutales. C.D.T.F. Universidad Nacional de Colombia, Sede Manizales, CDC, Universidad de Caldas, Corpoica. 11 al 13 de agosto de 2004. Manizales, Colombia.

Lobo, M. 2004b. Recursos genéticos de especies frutales. p. 1-13. En: Memorias VIII Congreso Venezolano de Fruticultura. Maracaibo, Venezuela, 6 al 9 de Junio.

Lobo, M. 2006. Recursos genéticos y mejoramiento de frutales andinos. Una vision conceptual. Revista Corpoica 7(2):40-54.

Lobo, M. e C.I. Medina. 2000. Lulo (*Solanum quitoense* Lam). En: Caracterizaçaõ de frutas nativas da América Latina. Série Frutas Nativas. Ediçaõ comemorativa do 30º aniversario da Sociedade Brasileira de Fruticultura.

Lobo, M., C.I. Medina, O. Delgado y A. Bermeo. 2007. Variabilidad morfológica de la colección colombiana de lulo (*Solanum quitoense* Lam.) y espécies relacionadas de la sección *Lasiocarpa*. Revista Facultad Nacional de Agronomía Medellín 60 (2): 3939-3964.

Medina, C.I. 2003. Estudio de algunos aspectos fisiológicos del lulo (*Solanum quitoense* Lam.) en el bosque húmedo montano bajo del oriente antioqueño. Tesis M.Sc. Universidad Nacional de Colombia, Facultad de Ciencias Agropecuarias, Departamento de Ciencias Agronómicas, Medellín, Colombia. 249 p.

Medina, C.I., M. Lobo, E. Martinez y N. Riaño. 2004. Estudios fisiológicos en lulo en el bosque húmedo montano bajo del oriente antioqueño. I. Crecimiento vegetativo. p. 455. En: Memorias V Seminario Nacional e

Internacional de Frutales. C.D.T.F. Universidad Nacional de Colombia, Sede Manizales, CDC, Universidad de Caldas, Corpoica. Agosto 11-13, Manizales.

Medina, C.I., E. Martínez, M. Lobo y N. Riaño. 2005. Distribución de la fitomasa en distintas etapas del crecimiento del lulo (*Solanum quitoense* Lam,) a plena exposición solar en el oriente antioqueño. p. 47. En: Memorias XXXV Congreso COMALFI. Fisiología de la Producción de Plantaciones Tropicales para un Mercado Global. Agosto 17-19. Medellín, Colombia.

Medina, C.I., E. Martinez, M. Lobo, J.C. López y N. Riaño. 2006. Comportamiento bioquímico y del intercambio gaseoso del lulo (*Solanum quitonse* Lam.) a plena exposición solar en el bosque húmedo montano bajo. Revista Facultad Nacional de Agronomía Medellín 59(1): 3123-3146.

Miller, J.S., and P. Diggle. 2003. Diversification of andromonoecy in *Solanum* section *Lasiocarpa*: the roles of phenotypic plasticity and architecture. American Journal of Botany 90(5): 707-715.

Mayr, E. 1942. Systematics and the origin of species. Columbia University Press, New York, USA.

Nei, M. and W. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA. 76: 5269-5273.

Olmsted, R.G. and J.D. Palmer. 1997. Implications for the phenology, classification and biogeography of *Solanum* from cpDNA restricition site variation. Systematic Botany 22:19-29.

Patiño, V.M. 1962. Edible fruits of Solanum In South America Historic and Geographic References. Botanical Museum Leaflets Harvard University. 19(10):215-234.

Patiño, V.M. 2002. Historia y Dispersión de los Frutales Nativos del Geotrópico. Cali, Colombia. Publicación CIAT No. 326. p. 225-230.

Rolph, F. 2000. NTSYS-PC. Numerical Taxonomy in multivariate Análisis System. Version 2.02. User's guide. Exeter Software. Setauket, New York, USA.

Sahaza, D.P. y M.L. Henao. 2001. Evaluación y caracterización morfológica del lulo (*Solanum quitoense* Lam). Trabajo de Investigación Ingeniero Agrónomo. Universidad Nacional de Colombia, Facultad de Ciencias Agropecuarias, Medellín. 57 p.

Saliba-Colombani, V., M. Causse, L. Gervan and J. Philouze. 2000. Efficiency of RFLP, RAPD, and AFLP markers for the construction of an intraspecific map of the tomato genome. Genome 43(1): 29-40.

Schultes, R.E. 1949. Plantae Colombianae XII, De plantis principaliter Amazoniae Colombianae investigations. Botanical Museum Leaflets, Harvard University 14(2): 21-47.

Sobral, B.W.S. 2002. The role of bioinformatics in germplasm conservation and use. p. 171-178. In: Engels, J.M.M., V. Ramantha-Rao, A.H.D. Brown and M.T. Jackson (eds.). Managing plant genetic diversity. CABI Publishing, Oxford. 487 p.

Soule, M. 1976. Allozyme variation: Its determinants in space and time. p. 60-77. In: Ayala, F.J. (ed.). Molecular evolution. Sinauer, Sunderland, Massachusetts. 277 p.

Spillane, C. and P. Gepts. 2001. Evolutionary and genetic perspectives on the dynamics of crop gene pools. p. 25-70. In: Cooper, H.D, Spillane, C. and Hodkin. (eds.). Broadening the genetic base of crop production. CABI, Walling, Oxford, UK. 474 p.

Toquica, S.P., F. Rodriguez, E. Martínez, M.C. Duque and J. Tohme. 2003. Molecular characterization by AFLPs of *Capsicum* germplasm fron the Amazon Departament in Colombia, Characterization by AFLPs of *Capsicum*. Genetic Resources and Crop Evolution 50: 639-647.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Van de Lee, M. Horness, A. Frijters, J. Pot, J. Peleman, M. Kuiper and M. Zabeau. 1995. AFLP: a new téchnique for DNA fingerprinting. Nucleic Acids Research. 23(21): 4407-4414.

Whalen M, D. D.E. Costich, C.B. Heiser. 1981. Taxonomy of *Solanum* Section *Lasiocarpa*. Gentes Herbarum 12: 41-129.

Whalen, M. and E. Caruso. 1983. Philogeny in *Solanum* sect. *Lasiocarpa* (Solanaceae): congruence of morphological and molecular data. Systematic Botany 8: 369-380.