Genetic relationships between nine *Annona muricata* L. accessions using RAPD markers

Jennifer Brown, Hernán Laurentín*, Martha Dávila

Departamento de Ciencias Biológicas, Decanato de Agronomía, Universidad Centroccidental Lisandro Alvarado, Apartado 400, Barquisimeto, Venezuela
hlaurentin@ucla.edu.ve
mdavila@ucla.edu.ve

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Abstract — Introduction. *Annona muricata* L. is a fruit tree species of tropical origin whose fruit, the soursop, although having great potential, is not commercially exploited enough. So there is little information on this species and, in particular, on its germplasm characterization. Consequently, the purpose of our study was to estimate the genetic variability between nine soursop accessions using the RAPD marker technique. **Materials and methods**. By means of RAPD analysis, using the Jaccard's coefficient, a similarity matrix was generated between nine accessions, seven of them collected in Venezuela and two in Brazil. With these, a phenogram was obtained using UPGMA clustering analysis. The ordering of the accessions was also achieved by means of a principal component analysis. **Results**. Seventeen RAPD fragments were obtained, of which 14 were polymorphic. Average similarity was 0.5333, and ranged from 0.2627 to 1.000. The phenogram identified two groups, equal to principal coordinates analysis. The Venezuelan accessions showed more variability when compared with the Brazilian ones (Jaccard's coefficient of 0.5038 and 0.5442, respectively). **Discussion**. Compared with other studies on various fruit trees, that carried out here on *A. muricata* underlined a great genetic variability. The situation is thus favorable to undertake in Venezuela a breeding program in this still under-exploited fruit species.

Venezuela / *Annona muricata* / genetic variation / classification

Proximité génétique de neuf accessions d'*Annona muricata* L. étudiées à l'aide de marqueurs RAPD.

Résumé — Introduction. *Annona muricata* L. est une espèce végétale d'origine tropicale dont le fruit, le corossol, bien que présentant un grand potentiel, n'est pas suffisamment valorisé. Il y donc peu d'informations sur cette espèce et, en particulier, sur l'évaluation de ses ressources génétiques. Notre étude a cherché à évaluer la variabilité génétique existant parmi neuf accessions de corossol présentes au Venezuela en utilisant la technique des marqueurs RAPD (amplification de fragments de DNA). **Matériel et méthodes**. L'analyse par RAPD et l'utilisation du coefficient de Jaccard ont permis de produire une matrice de similitude au sein des neuf accessions analysées dont sept provenaient du Venezuela et deux du Brésil. Un phénogramme a été obtenu en utilisant une analyse typologique basée sur la méthode de groupement de paires non pondérées avec moyennes arithmétiques (UPGMA). Le classement des accessions a été également réalisé par une analyse en composantes principales. **Résultats**. Dix-sept fragments de DNA amplifié ont été obtenus dont 14 ont été polymorphes. La similitude moyenne a été de 0,5333 et, dans l'ensemble des mesures, elle a varié de 0,2627 à 1,000. Le phénogramme a permis d'identifier deux groupes parmi les neuf accessions, les mêmes que ceux révélés par l'analyse en composantes principales. Les accessions vénézuéliennes ont montré plus de variabilité parmi elles que lorsque comparées avec les accessions brésiliennes (coefficient de Jaccard de 0,5038 et 0,5442, respectivement). **Discussion**. Par rapport à d'autres études sur différents arbres fruitiers, celle effectuée ici sur *A. muricata* a mis en évidence une grande variabilité génétique. La situation est donc favorable pour entreprendre au Venezuela un programme d'amélioration de cette espèce encore sous-exploitée.

Venezuela / *Annona muricata* / variation génétique / classification

* *Correspondence and reprints*

Fruits, 2003, vol. 58, p. 255–259

DOI: 10.1051/fruits:2003013

***RESUMEN ESPAÑOL**, p. 259*

1. Introduction

The Annonaceae family (Magnolians) includes about 2500 species in 140 genera [1]. The Annona genus is one of four of importance with regard to fruit [2]. It contains more than 118 species of which *Annona muricata* L., *A. squamosa* L., *A. reticulata* L., *A. diversifolia* Saff and *A. cherimola* Mil L. [3] have been reported as the most important ones. *A. muricata* originated in the lowlands of tropical America, both in Central America and the Peruvian valleys. It is considered to be the best species suited to processing, because of the excellent flavor of its pulp and high recovery of large fruits.

In Venezuela, *A. muricata* or soursop has great potential for international marketing as a delicacy [4]. However, there are factors which restrict its big-scale production: scarce existing basic information about its management [5], lack of commercial varieties, nonexistence of breeding programs [6], skin fragility, irregular shape and fruit softness, which limit machine processing, for example. Germplasm collection is basic for setting up a breeding program [6]; in this sense, it is necessary to establish genetic relationships between accessions from one species, as these can be useful for organizing germplasm, identifying cultivars and helping in the selection of parents for hybridization [7].

To achieve this objective, it is possible to use the RAPD (random amplified polymorphic DNA) technique which is based on the amplification of genomic DNA fragments using 10 base pair oligonucleotides as random primers [8, 9].

The objective of the present study was to establish genetic relationships between nine soursop accessions and to assess the existing genetic variation and the potential among the accessions to start a breeding program.

2. Materials and methods

Nine accessions of *A. muricata* (*table I*), seven from the Germplasm Bank of the *Centro Nacional de Investigaciones Agropecuarias*" (National Center of Farming Research), Maracay, Venezuela, and two accessions from Bahía, Brazil, were used in this research. The DNA was isolated from young, thoroughly developed leaves, according to Saghai-Maroof *et al.*'s techniques [10]. The amplification protocol was carried out in a Perkin Elmer Gene Amp PCR System 2400 thermal cycler, utilizing four primers from the OPH series of Operon Technologies (*table II*) during 40 cycles. The amplification products were separated through electrophoresis in 1.5% agarose gel with TBE buffer, stained with ethidium

Table I.
Accessions of *Annona muricata* studied to establish genetic relationships using RAPD markers (Venezuela).

Accession number	Name	Source
1	Palmarito 2	CENIAP, Venezuela
2	Palmarito 3	CENIAP, Venezuela
3	Palmarito 4	CENIAP, Venezuela
4	Palmarito 5	CENIAP, Venezuela
5	Amado	CENIAP, Venezuela
6	Playón 6	CENIAP, Venezuela
7	Playón 7	CENIAP, Venezuela
8	Brasil 1	Bahía, Brasil
9	Brasil 2	Bahía, Brasil

Table II.
Nucleotide sequences of four primers from the OPH series used for the amplification protocol involved in the study of genetic relationships between nine *Annona muricata* L. accessions using RAPD markers (Venezuela).

Primer	Nucleotide sequence
OPH-01	5' GGTCGGAGAA 3'
OPH-02	5' TCGGACGTGA 3'
OPH-05	5' AGTCGTCCCC 3'
OPH-07	5' CTGCATCGTG 3'

bromide and observed under ultraviolet light. As a molecular size marker, a 100 bp ladder of undigested bacteriophage DNA was included. The amplification products were recorded in the binary system (1: band presence, 0: band absence). From the band presence or absence matrix, another similarity matrix was generated through calculation of the Jaccard's coefficient. Using the UPGMA (Unweighted Pair Group Method with Arithmetical averages) clustering analysis, a phenogram was obtained, which allowed the observation of the genetic relationships between the assessed accessions.

In addition, a principal component analysis was done to observe which bands were more discriminant among the assessed accessions and to obtain a new ordering of them. This analysis was done with a centrated matrix from the Jaccard's similarity matrix previously obtained. All this analysis was done using the NTSYS v.2.02j program [11].

3. Results

The use of RAPD was successful in distinguishing soursop (*Annona muricata* L.) accessions. Using four primers on the nine soursop accessions, 17 bands were obtained, with a size ranging from (250 to 2500) bp, of which 14 (82.35%) turned out to be polymorphic and three (17.65%) monomorphic. Polymorphic bands were obtained in three out of the four primers used, which also generated single bands for some accessions; thus, OPH2 permitted the production of two bands of 250 bp and 2000 bp for the Amado and Playón 6 accessions, respectively; OPH1 generated a 350 bp band for Palmarito 4, and OPH7 a 1700 bp band for Palmarito 2.

When Jaccard's coefficient was used, mean similarity among the nine accessions was 0.5333%, with minimum and maximum values being (0.2627 and 1.0000)%, respectively. The highest similarity was 100% between the two accessions collected in Brazil, whereas the most different ones were Amado and Palmarito 2. When graphing the similarity relationships using the UPGMA algorithm, a cophenetic correlation coefficient of 0.9703 was obtained. The resulting phenogram shows two different groups, one formed by Amado and Palmarito 4, and another by the rest (*figure 1*).

The principal component analysis accounted in its three first axes for 53.70% of the variation, with bands 4 (500 bp, primer OPH7) and the single band for the genus Palmarito 4 (350 bp, primer OPH1) being the highest supplier for axis 1; the single band for genus Playón 6 (200 bp) and band 11 (2100 bp), both from primer OPH2, to the second axis, and the single band for genus Palmarito 2 (primer OPH7)

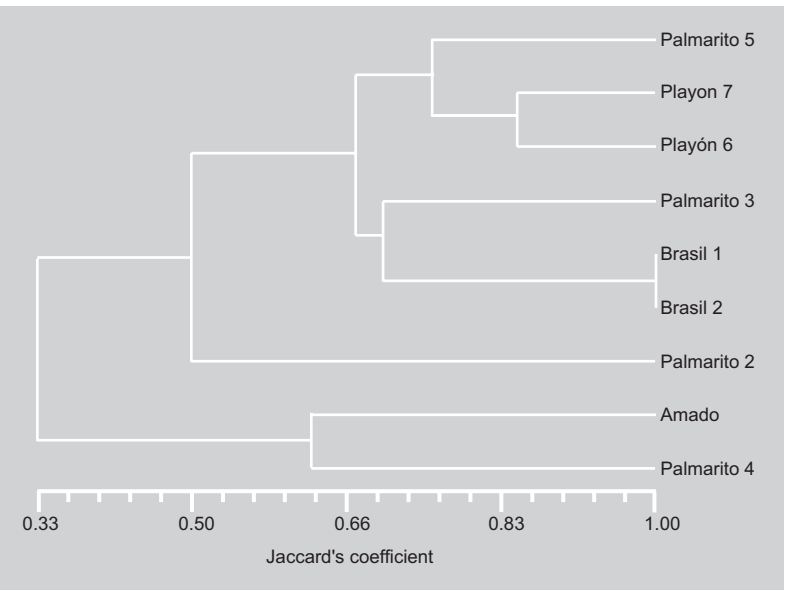

Figure 1.
Phenogram of nine accessions of *Annona muricata* L. based on Jaccard's coefficient similarity values (Venezuela).

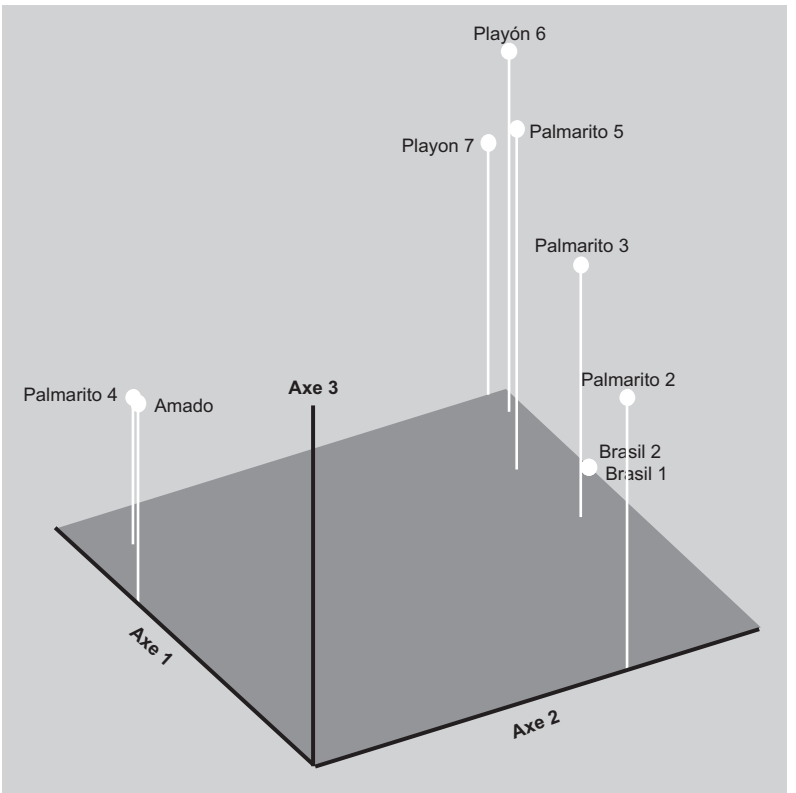

Figure 2.
Three-dimensional graph from the principal component analysis obtained to discriminate nine accessions of *Annona muricata* L. in Venezuela.

to the third axis, showing an accession order (*figure 2*) very similar to the order obtained with the phenogram. The first axis separates Amado and Palmarito 4 from the rest as these accessions are the only ones that have band 4, which is the major supplier to that axis. In addition, because Palmarito 4 has a single band which is a high supplier of the same axis, it is separated from Amado. The second axis separates Playón 6, Playón 7 and Palmarito 5 from the rest as these show band 11, one of the highest contributions to this axis. The third axis separates Palmarito 2 from the rest because it has a single band with great contribution to that axis. In addition, Brazil 1 and Brazil 2 have no band 14, which is second in contribution to axis 3.

4. Discussion

The similarity among the studied soursop accessions, considering it was calculated based on the presence or absence of RAPDs, is an inversely proportional measure to the existing genetic variability. The wide range of the Jaccard's coefficient values thus shows the genetic variability among the studied accessions.

If we group them according to the country of origin, it is clear that there is a higher genetic variability when comparing the Venezuelan accessions (mean Jaccard's coefficient 0.5038) with the Brazilian samples studied (mean Jaccard's coefficient 0.5442). This is reaffirmed by *figures 1* and *2* where it is observed that the two Brazilian accessions are grouped with the Venezuelan accessions Palmarito 2, Palmarito 3, Palmarito 5, Playón 6 and Playón 7 and is clearly different from the cluster formed by the Venezuelan accessions Palmarito 4 and Amado. These findings clearly demonstrate the existing but hardly considered variability in the soursop germplasm collected in Venezuela.

Other studies on molecular variability in *A. muricata* L. have not been reported. Observing the existing similarity among the nine soursop accessions and comparing it with the similarity determined within other fruit species using, as in this case, RAPD and the Jaccard's coefficient, it can be stated that there is a wide genetic variability in this work. In the study of 35 accessions of the gender *Citrus*, including ten species and five interspecific hybrids, a minimum coefficient of 0.77 was obtained, which means that there is a great similarity among the accessions included in the study [12]. These results contrast with the wide genetic variability found in this work between the accessions studied within the species *A. muricata*, where all the included accessions belong to the same species. In a coconut study of 17 populations [13], a similarity range between 0.763 and 0.928 was obtained. In avocado, a study of 16 accessions [14] showed a mean similarity of 60%, with ranges between (45 and 85)%. In both studies, a greater similarity was observed, which means there is less genetic variability than that obtained for *A. muricata* in this work.

All this evidence points to the great genetic variability existing between the studied accessions, a very favorable condition for obtaining a genetically variable

basic population from which to start a breeding program in this underexploited fruit species in Venezuela.

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Proximidad genética de nueve accesiones de *Annona muricata* L. estudiadas mediante marcadores RAPD.

Resumen — Introducción. *Annona muricata* L. es una especie vegetal de origen tropical cuyo fruto, la guanábana, aunque tiene un gran potencial, no está bastante valorizado. Existe, pues, poca información sobre esta especie y, en particular, sobre la evaluación de sus recursos genéticos. Nuestro estudio intentó evaluar la variabilidad genética existente en nueve accesiones de guanábana presentes en Venezuela empleando la técnica de marcadores RAPD (amplificación de fragmentos de DNA). **Material y métodos**. El análisis mediante RAPD y el empleo del coeficiente de Jaccard permitieron producir una matriz de similitud en las nueve accesiones analizadas de las cuales siete procedían de Venezuela y dos de Brasil. Se obtuvo un fenograma mediante un análisis tipológico basado en el método de agrupamiento de pares no ponderados con medias aritméticas (UPGMA). La clasificación de las accesiones también se realizó mediante análisis de componentes principales. **Resultados**. Se obtuvieron diecisiete fragmentos de DNA amplificados de los que 14 fueron polimórficos. La similitud media fue de 0,5333 y, en el conjunto de medidas, varió de 0,2627 a 1,000. El fenograma permitió identificar dos grupos dentro de las nueve accesiones, los mismos que reveló el análisis de componentes principales. Las accesiones venezolanas mostraron mayor variabilidad entre ellas que comparadas con las brasileñas (coeficiente de Jaccard de 0,5038 y 0,5442, respectivamente). **Discusión**. Con relación a otros estudios sobre diferentes árboles frutales, éste trabajo que aquí presentamos sobre *A. muricata* puso de manifiesto una gran variabilidad genética. Estamos, pues, ante una situación favorable para acometer en Venezuela un programa de mejora de esta especie aún infraexplotada.

Venezuela / *Annona muricata* / variación genética / clasificación