



Revista Ceres

ISSN: 0034-737X

ceresonline@ufv.br

Universidade Federal de Viçosa
Brasil

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Revista Ceres, vol. 60, núm. 3, mayo-junio, 2013, pp. 428-431
Universidade Federal de Viçosa
Vicosa, Brasil

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Short Communication

Genetic diversity in sugar apple (*Annona squamosa* L.) by using RAPD markers

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ABSTRACT

Genetic diversity in a collection of 64 sugar apple accessions collected from different municipalities in northern Minas Gerais was assessed by RAPD analysis. Using 20 selected RAPD primers 167 fragments were generated, of which 48 were polymorphic (28.7%) producing an average of 2.4 polymorphic fragments per primer. Low percentage of polymorphism (< 29%) was observed by using the set of primers indicating low level of genetic variation among the 64 accessions evaluated. Genetic relationships were estimated using Jaccard's coefficient of similarity. Accessions from different municipalities clustered together indicating no correlation between molecular grouping and geographical origin. The dendrogram revealed five clusters. The first cluster grouped C19 and G29 accessions collected from the municipalities of Verdelândia and Monte Azul, respectively. The second cluster grouped G16 and B11 accessions collected from the municipalities of Monte Azul and Coração de Jesus, respectively. The remaining accessions were grouped in three clusters, with 8, 15 and 37 accessions, respectively. In summary, RAPD showed a low percentage of polymorphism in the germplasm collection.

Key words: accessions, molecular marker, variability, germplasm.

RESUMO

Diversidade genética em pinheira (*Annona squamosa* L.) por meio de marcadores RAPD

A diversidade genética da coleção de 64 acessos de pinheira, coletados em diferentes cidades, no norte do Estado de Minas Gerais, foi avaliada por meio do uso de marcadores RAPD. Foram selecionados 20 *primers* RAPD que geraram 167 fragmentos, dos quais 48 foram polimórficos (28,7%), produzindo uma média de 2,4 fragmentos polimórficos por *primer*. Baixa percentagem de polimorfismo foi obtida com o conjunto de *primers* (< 29%), indicando baixa variação genética entre os 64 acessos avaliados. As distâncias genéticas foram estimadas, utilizando-se o coeficiente de similaridade de Jaccard. Acessos de diferentes cidades foram agrupados em um mesmo grupo, indicando que não há correlação entre os agrupamentos moleculares e origem geográfica. O dendrograma revelou cinco grupos. O primeiro grupo reuniu os acessos C19 e G29, coletados nas cidades de Verdelândia e Monte Azul, respectivamente. O segundo grupo reuniu os acessos G16 e B11, coletados nas cidades de Monte Azul e Coração de Jesus, respectivamente. Os acessos remanescentes foram agrupados em três grupos, com oito, 15 e 37 acessos, respectivamente. O marcador RAPD apresentou baixo nível de polimorfismo entre os acessos avaliados.

Palavras-chave: acessos, marcador molecular, variabilidade, germoplasma.

Received: 16/04/2012; Accepted: 03/05/2013.

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INTRODUCTION

In Brazil, sugar apple (*Annona squamosa* L.) is an important fruit crop grown commercially in semiarid regions. There are few well-established breeding programs for sugar apple around the world. India, China and Taiwan have produced a few named cultivars that are propagated vegetatively (Nakasone & Paul, 1998). Annonaceae family presents a large intra and inter-specific variability offering an ample scope for studying genetic variation. Despite this great variability, germplasm banks that contain *Annona* spp., mainly *Annona squamosa*, are rare throughout the world, which is a limiting factor for selecting and crossing among elite cultivars (Pinto *et al.*, 2005). Brazil has the largest number of sugar apple accessions (92); therefore a systematic effort to evaluate these accessions will be fundamental to improve the species (IPGRI, 2000). In the past few years, morphological traits have been used as tools to characterize germplasm resources. Unfortunately, morphological characters tend to be influenced by environmental factors and problems with ambiguity are frequent. Molecular markers have provided a powerful tool for proper characterization of germplasm diversity (Williams *et al.*, 1990). PCR-based molecular techniques provide a variety of DNA markers for diversity analysis (Newbury & Ford-Lloyd, 1993), identification of cultivars (Matsumoto & Fukui, 1996), gene localization, and construction of linkage maps (Giovanni *et al.*, 2004). RAPD markers have been used for diversity analysis in cacao (Leal *et al.*, 2008), banana (Souza *et al.*, 2008) and, sugar apple (Bharad *et al.*, 2009, Ahmad *et al.*, 2010).

The objective of the present study was to assess the genetic diversity in 64 accessions of sugar apple germplasm by using RAPD markers.

MATERIALS AND METHODS

The plant material used in this study is listed in Table 1. The collection is located at experimental farm of State University of Montes Claros, Janaúba, Brazil. The germplasm collection included accessions that were collected from different municipalities located in the semiarid region.

Genomic DNA was isolated from young sugar apple leaves as described by Cheung *et al.* (1993). Twenty decamer primers (OPC 14, OPD 04, OPE 05, OPF 10, OPF 19, OPG 05, OPH 11, OPJ 20, OPL 03, OPM 08, OPN 11, OPO 10, OPQ 11, OPQ 19, OPV 14, OPV 15, OPW 02, OPX 14, OPY 20 and OPZ 18) were selected and used for amplification. RAPD reactions were performed as described by Williams *et al.* (1990). Only distinct and reproducible fragments produced by RAPD were scored. Estimated similarity coefficients were used to

construct a dendrogram using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The determination of the number of clusters was based on Mojena (1977), assuming $k=1.25$. The stability of the nodes in the dendrogram was verified using the bootstrap method with 1,000 bootstraps. The statistical analysis were carried out using the GENES software (Cruz, 2006).

RESULTS AND DISCUSSION

Using the 20 selected primers, a total of 167 fragments was generated, of which 48 were polymorphic (28.7%). The number of polymorphic fragments varied from 1 (OPE-05, OPO-10, OPW-02 and OPY-20) to 8 (OPF-19) with an average of 2.4 polymorphic fragments per primer.

The pair wise comparisons and dissimilarity matrix (data not shown) among the 64 accessions showed genetic dissimilarities ranging from a minimum of 0.0000 to a maximum of 0.17884. The most genetically diverse genotype was E4, collected from Montes Claros, which was 15.20% genetically related to all genotypes, while C2, collected from Verdelândia, was the most genetically similar (0.59%).

The similarity coefficients generated from RAPD data were used to construct a dendrogram. The dendrogram grouped the genotypes in five major clusters (I, II, III, IV and V) (Figure 1), based on a relative genetic distance of 40%, determined by Mojena (1977). The first cluster was formed by genotypes C19 and G29 (collected from Verdelândia and Monte Azul, respectively); the second cluster was formed by genotypes B11 and G16 (collected from Coração de Jesus and Monte Azul, respectively), and the clusters III, IV and V were formed by the remaining 60 accessions, with cluster V grouping most of accessions analyzed (57.8%). Accessions from different sites of collection were found to cluster together indicating no correlation between molecular groupings and their geographical origin. Dissimilarity values of 0% were observed between several accessions grouped in the III, IV and V clusters. In evaluating the values of dissimilarity of accessions by origin, it was observed that accessions from Verdelândia showed the smallest genetic distance.

The percentage of polymorphism obtained in this study (28.7%) was very low compared to other studies in *Annona* species. Ronning *et al.* (1995) used 15 RAPD primers to evaluate the genetic variability of atemoya, sugar apple and cherimoya and observed highly distinct and polymorphic patterns for the genotypes studied. Bharad *et al.* (2009) analyzed 11 sugar apple genotypes using five RAPD primers, and found 73% polymorphism, with all genotypes clustered in three groups. A recent study using

Table 1. Accession identification (AI) and collection site of 64 sugar apple germplasm located in Janaúba, Minas Gerais, Brazil, used for RAPD analysis

AI	Collection site	AI	Collection site	AI	Collection site
A 3	Pai Pedro	C 14	Verdelândia	E 3	Montes Claros
A 5	Pai Pedro	C 15	Verdelândia	E 4	Montes Claros
A 15	Pai Pedro	C 16	Verdelândia	E 8	Montes Claros
A 32	Pai Pedro	C 17	Verdelândia	E 18	Montes Claros
A 33	Pai Pedro	C 18	Verdelândia	E 21	Montes Claros
B 6	Coração de Jesus	C 19	Verdelândia	F 7	Francisco Sá
B 11	Coração de Jesus	C 21	Verdelândia	F 13	Francisco Sá
B 15	Coração de Jesus	C 22	Verdelândia	F 15	Francisco Sá
B 24	Coração de Jesus	C 23	Verdelândia	F 20	Francisco Sá
B 35	Coração de Jesus	C 24	Verdelândia	F 25	Francisco Sá
C 1	Verdelândia	C 25	Verdelândia	G 11	Monte Azul
C 2	Verdelândia	C 26	Verdelândia	G 16	Monte Azul
C 3	Verdelândia	C 27	Verdelândia	G 25	Monte Azul
C 4	Verdelândia	C 28	Verdelândia	G 26	Monte Azul
C 5	Verdelândia	C 29	Verdelândia	G 29	Monte Azul
C 6	Verdelândia	C 30	Verdelândia	H 3	Janaúba
C 7	Verdelândia	C 31	Verdelândia	H 11	Janaúba
C 8	Verdelândia	D 2	Jaíba	H 25	Janaúba
C 9	Verdelândia	D 5	Jaíba	H 26	Janaúba
C 10	Verdelândia	D 17	Jaíba	H 29	Janaúba
C 11	Verdelândia	D 25	Jaíba	-	-
C 13	Verdelândia	D 26	Jaíba	-	-

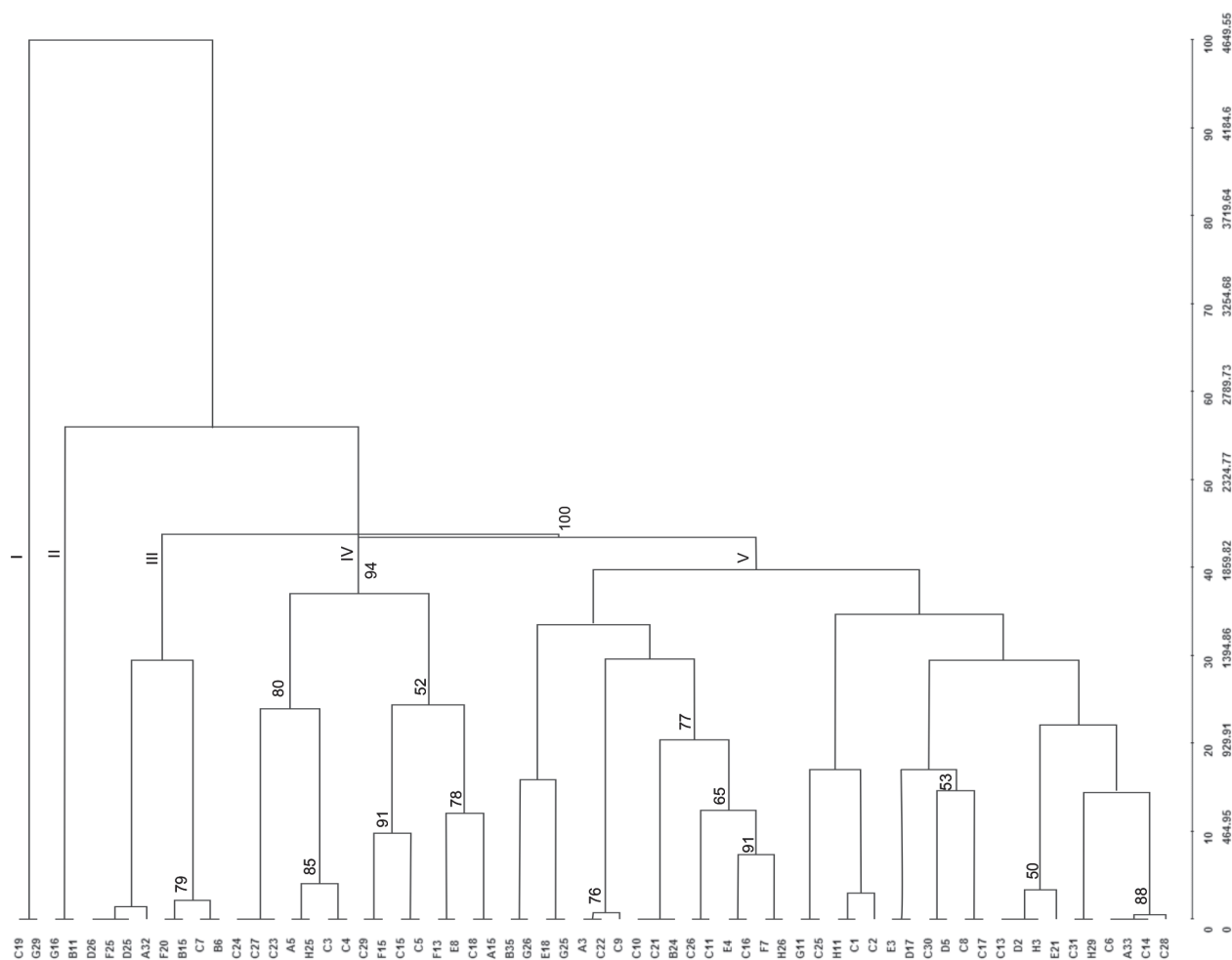


Figure 1. Dendrogram depicting 64 sugar apple accessions from a germplasm collection in North Minas Gerais, Brazil. Similarities were estimated based on the UPGMA method. The values refer to bootstrap values greater than 50%.

20 RAPD and 30 ISSR primers to assess the genetic diversity in four *Annona* species found levels of polymorphisms of 52% and 58% and divided them into two and three major clusters, respectively (Ahmad *et al.*, 2010).

Although sugar apple is open-pollinated species and the plants evaluated in this study were propagated by seedlings and collected from different locations, low values of genetic dissimilarity were observed. The greatest genetic distance observed in this study (17.88%) was between accessions collected from Montes Claros and Verdelândia, distanced 250 km from each other. However, values of dissimilarity of 17.00% were obtained from accessions collected in Montes Claros city from the same orchard. Telles *et al.* (2003) evaluated the genetic diversity of *Annona crassiflora* Mart and found a smaller genetic distance among individuals of the same location due to high rate of ancestry in common. These authors suggest that a greater genetic variability is necessary and can be achieved by collection of few individuals from a number of different locations rather than a large number of individuals from the same location. In the present study 45.3% of the accessions were collected from the city of Verdelândia.

Montes Claros is the convergent center of agricultural activities in the region of northern Minas Gerais. Most farmers purchase agricultural inputs, seedlings and seeds in the Central Market located in the city of Montes Claros. Although the collection of accessions are originated from eight different cities, representing an area of about of about 16.720 Km², the sugar apple orchards might have been established from fruits purchased in the Central Market. This fact could be increasing the probability of common ancestry and may explain the high similarity observed between the accessions evaluated.

Furthermore the low genetic diversity in the present study may be associated with the number and type of primers tested. Therefore, future studies should test additional primers and in larger numbers aiming at additional loci for the potential differentiation of accessions and validation of genetic variability, if any exists.

CONCLUSION

Amplification of genomic DNA of 64 sugar apple accessions from Northern Minas Gerais, Brazil, by using analysis of 20 RAPD showed a low percentage of polymorphism.

ACKNOWLEDGEMENTS

The authors thank FAPEMIG and CNPq for supporting this work.

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