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Genetic diversity of guava (*Psidium guajava* L.) from Central Mexico revealed by morphological and RAPD markers

Análisis morfológico y molecular de la diversidad genética de guayaba (*Psidium guajava* L.) del Centro de México

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Abstract. Guava fruit produced in Calvillo, Aguascalientes (Mexico) is considered to be of the best quality in this country. Nevertheless, growers from this place empirically know that there is a noticeable variation among individual trees within the same orchard, and variation among individuals from different orchards. In order to have a clear evidence of this, morphology analysis of guava was performed taking data from seventy nine individuals out of thirty six orchards, while RAPD was performed on a subset of twenty six individuals. Similarity was found for morphology data ranging from 87-100%, while similarity from RAPD data ranged from 30-100%. Combined data of RAPD and morphology showed similarity greater than 80%. Clades from combined data sorted genotypes into clearly defined groups according to fruit shapes and banding pattern. These markers could be used as helper tools for breeding programs for guava genotypes of Mexico. Furthermore, these may help on the claims from growers when checking origin authenticity if packed guava from somewhere else is labeled as guava from Calvillo.

Keywords: Morphological analysis; RAPD; Variation; Guava.

Resumen. La fruta de guayaba cultivada en Calvillo Aguascalientes (México) es considerada como la de mayor calidad en el país. Sin embargo, los productores de este lugar empíricamente saben que hay una variación notable entre los árboles individuales dentro de la misma huerta, y entre individuos de diferentes huertas. Con el fin de tener una clara evidencia de esto, se realizó el análisis de la morfología de la guayaba tomando los datos de setenta y nueve individuos de treinta y seis huertas, mientras que un análisis genético tipo RAPD se realizó de un subconjunto de veintiséis individuos. Se encontró similitud para los datos de morfología que fueron desde 87 hasta 100%, mientras que la similitud de los datos de RAPD varió de 30-100%. Los datos combinados de RAPD y morfología mostraron una similitud mayor al 80%. Clades de datos combinados ordenó los genotipos en grupos claramente definidos de acuerdo a la forma de los frutos y patrón de bandas. Estos marcadores podrían ser utilizados como herramienta de ayuda para los programas de mejoramiento genético de genotipos de guayaba en México. Además, éstos podrían ser de gran ayuda a los reclamos de los productores para demostrar la autenticidad del origen del fruto cuando la guayaba de otros lugares se etiqueta como guayaba de Calvillo.

Palabras clave: Caracterización morfológica; RAPD; Variación; Guayaba.

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INTRODUCTION

Guava is a fleshy-fruited member of the Myrtaceae family that has attracted the attention due to its economic importance. Mexico ranks third after India and Pakistan on production of guava fruit (Quesada-Parga et al., 2005; Grattapaglia et al., 2012). In the Calvillo County, a region in Central Mexico, more than 400 guava orchards of variable area were included into the "Consejo de la Guayaba" records by local officers from the Ministry of Agriculture of Mexico in Aguascalientes; most of them (76%) in the range of 0-6 acres (SAGARPA, 2012). These orchards are the source of both fresh fruit and derived guava products like jellies, candies, liquor and cookies which are highly appreciated in Mexico and USA by Mexican immigrants. Guava genotypes native to Calvillo and surrounding areas from Zacatecas State with a total of about 12000 ha, are grown at altitudes ranging from 1500-1700 meters over the sea level (Perales-Cruz et al., 2005; Padilla-Ramírez et al., 2007), and their fruits are considered the best of Mexico. Nevertheless, fruit morphological variability is one of the major concerns for Mexican growers of the Central Region of Mexico since size, color of skin and flesh, fruit shape, flesh thickness and other attributes are key features to consider in guava quality both for exports or local consumption. Another concern of these growers is to find a genetic marker that could be used as identifier to discriminate local genotypes from others since some growers from other regions falsely label their guava as produced in Calvillo.

Molecular markers such as Restriction Fragment Length Polymorphisms (RFLP), Random Amplified Polymorphic DNAs (RAPD), Amplified Fragment Length Polymorphisms (AFLP), Simple Sequence Repeats (SSR) and Single Nucleotide Polymorphism (SNP), help to detect polymorphism at the DNA level, and may be used for genotyping and estimating genetic distances between populations, inbreeds or breeding genotypes. Furthermore, molecular markers used in breeding have been proved to be of good help, shortening the time required to develop new crop varieties (Provan et al., 1999; ISAAA, 2013). For instance, genetic homogeneity of regenerated guava plants from micropropagation and somatic embryogenesis was tested using SSR and Inter Simple Sequence Repeat (ISSR) markers with reliable results, finding monomorphic amplification patterns on the regenerated plants (Liu & Yang, 2012; Rai et al., 2012). On the opposite, SSR were used together with morphological descriptors to evaluate genetic diversity in wild Brazilian guavas (Nogueira et al., 2012). Further, SSR primers previously designed in guava were tested for identification and diversity studies on the genera *Psidium*, *Zyzygium* and *Eugenia, and* are useful for diversity studies in the Myrtaceae family (Valdés-Infante Herrero et al., 2012a).

Chen et al. (2007) considered RFLP and Denaturing Gradient Gel Electrophoresis (DGGE) as time-consuming and less efficient techniques on guava than specific amplifications of rDNA (18S and ITS), cpDNA (*trn*L intron and *trn*L-*trn*F IGS) or RAPD. Using the later with OPB17, OPG6, OPY15 and OPY18 primers, they obtained highly polymorphic RAPD patterns and concluded that RAPD is useful for Taiwanese guava cultivar discrimination. RAPDs also proved useful for discrimination of thirty-three Bangladeshi guava genotypes into two major groups and subgroups that reflect morphological characteristics and cultivar stages of domestication (Ahmed et al., 2011). Traits normally affected by environmental conditions are unreliable indicators *per se* of a plant genotype (Kujal et al., 2005). Therefore, the main goal of this work was to analyze the molecular markers and morphological variation among guava native genotypes from Aguascalientes, Mexico. The results obtained are intended to help breeding programs for this species in Mexico as well as to help to determine the identity of genotypes from Calvillo.

MATERIALS AND METHODS

Plant material and morphological and molecular analysis. Fully developed fruits and 5-10 green full developed leaves were collected from 79 random selected guava trees from 36 commercial orchards located in Calvillo, Aguascalientes. Together with the fruits collected from the selected trees, their leaves were photographed and their morphology was included as complementary data (Fig. 1, Table 1), described according to UPOV (1987) complemented with descriptions by Sánchez-Urdaneta & Peña-Valdivia (2011). Fruit descriptors were: shape at stalk end; width of neck in relation to that of fruit; diameter of calyx cavity in relation to that of fruit; ridged collar around calyx cavity; thickness of outer flesh in relation to core diameter, and thickness of outer flesh in relation to core diameter (Table 1). Additionally, flesh color, number of carpels, and fruit shape. Leaf descriptors were: shape; curvature in cross section; twisting; curvature of midrib; shape of base, and shape of tip (Table 1). From the sampled trees, 26 were selected for molecular analysis, based on apparent morphological differences.

Total genomic DNA extraction. Modifications to reported protocols (Doyle & Doyle, 1987; Padilla-Ramírez et al., 2002; Cheng et al., 2003) were used for obtaining DNA of good quality. Briefly: A sample of 0.5 g leaf tissue was ground with liquid nitrogen. A volume of 750 µL preheated Lysis buffer (100 mM TrisHCl pH 8, 50 mM EDTA pH 8, CTAB 2%, 2 M NaCl, 2% PVP-40 and 2% β-mercaptoethanol) was added to the sample and mixed thoroughly. The aqueous phase was subjected to two extractions with 24:1 chloroform: isoamylic alcohol, mixing during 15 minutes and centrifuged at 13000 rpm for 15 minutes. The supernatant was treated with 5 µL de RNase (10 mg/mL) and incubated at 37 °C during 30 minutes. DNA was precipitated with cold isopropanol,

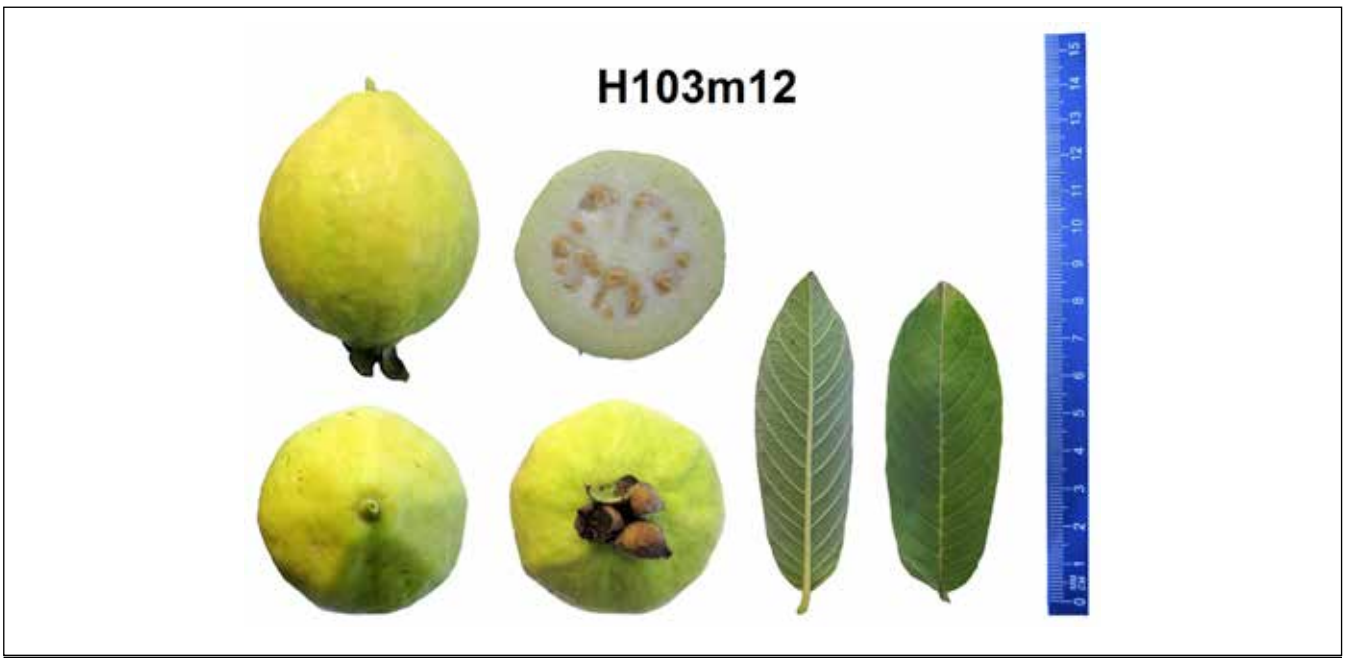

Fig. 1. Example of collected guava fruits showing a side, the top, the bottom and a traversal cut in order to show the carpels. Leaves show the abaxial and adaxial planes.
Fig. 1. Ejemplo de frutos de guayaba colectados donde se muestra un lado, la parte superior e inferior y un corte transversal con el fin de mostrar los carpelos. Las hojas muestran los ejes abaxial y adaxial.

Table 1. Descriptors for guava: Table of characteristics of fully developed leaf and fruit (UPOV, 1987).
Tabla 1. Descriptores de guayaba: Tabla de característica de hoja totalmente desarrollada y fruto (UPOV, 1987).

Organ	Morphological descriptors	Categories					
Fully developed leaf	Shape	round	ovate	obovate	trullate	obtrullate	oblong
	Curvature in cross section	weak	medium	strong			
	Twisting	present	absent				
	Curvature of midrib	present	absent				
	Shape of base	obtuse	rounded	cordate			
	Shape of tip	attenuate	apiculate	acute	obtuse	rounded	
Fruit	Shape at stalk end	broadly rounded	rounded	truncate	pointed	necked	
	Width of neck in relation to that of fruit	narrow	medium	broad			
	Diameter of calyx cavity in relation to that of fruit	small	medium	large			
	Ridged collar around calyx cavity	inconspicuous	conspicuous				
	Thickness of outer flesh in relation to core diameter	thin	medium	thick			

the pellet was washed with 70 µL sterile water and stored at -20 °C. DNA concentration was measured using a spectrophotometer (Eppendorf BioPhotometer plus, Fisher Scientific). Complementary samples were checked to detect DNA degradation on Sodium Borate Buffer (Brody & Kern, 2004) 1% agarose gels at 180 V during 15 minutes.

Polymerase chain reaction. Thirty decamer primers were tested (series OPA1-20 and OPB1-10, Operon Technologies) for their usefulness to generate polymorphic banding pattern among guava genotypes. PCR reactions were done using the kit *Ready to go RAPD analysis Beads*® (GE Life Sciences). The reactions were performed similar as described by the supplier,

except for the total volume per reaction which was set to one half (8.5 mL of dissolved beads, 3 mL 20-250 ng/mL DNA, 1 mL 30 mM of the selected primer). On the other hand, using a custom-made master mix, the reactions were set to 25 mL final volume, as follows: 2.5 mL 10X PCR Buffer, 1.5 mL 25 mM $MgCl_2$, 0.5 mL 10 mM dNTPs, 1 mL 30 mM of the selected primer, 0.3 mL 5 U/mL Taq DNA polymerase, 5 mL 20-250 ng/mL DNA, 14.2 mL ddH_2O. PCR runs were done using a Techne TC-512 thermal cycler, starting with 5 min denaturation (95 °C) step and 45 amplification cycles (1 min 94 °C, 1 min 35 °C, 2 min 72 °C); this program was adapted from Feria-Romero (2008). Amplification products were checked using TBE 1.5% agarose gels at 100 V during 1.5 hours. Molecular weight was determined using a 1 Kb ladder (New England BioLabs Inc.) as a reference.

Cladistics analysis. Banding pattern data from RAPD (binary data) and morphological data (class-grouped as described in Table 1) were analyzed separately using the software "Primer 5" (version 5.2.8 for Windows) calculating similarity by Bray-Curtis with square root transformation to construct a similarity table which, in turn, was used to build the dendrograms based on group average (Clarke & Gorley, 2005). Spearman Rank correlation of the variables considered for the similarity matrix was performed ten times maximum and reporting the lowest calculated value, taking five variables at a time per run for all of the dendrograms.

RESULTS AND DISCUSSION

Cladistics analysis based on RAPDs. Distinctive polymorphic bands were found after the preliminary test of the twenty primers from the OPA series (1-20) and ten from the OPB series (1-10). Some of these primers produced five to seven polymorphic bands in the range of 0.5 to 3 kb, depending on the guava genotype (Fig. 2, Table 2). In most cases the

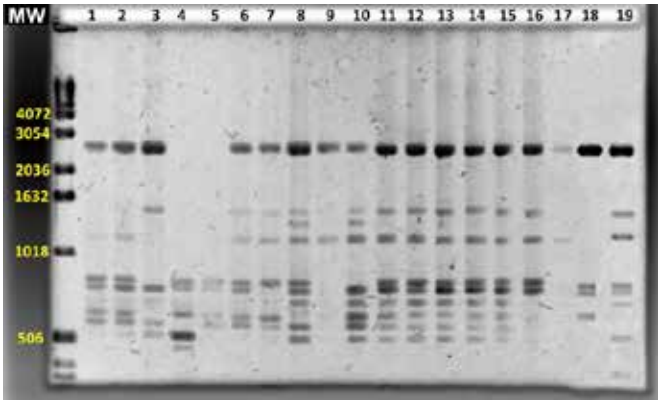

Fig. 2. RAPD banding pattern obtained with primer OPB1 and 19 DNA guava samples from Central Mexico showing polymorphism. MW= Molecular weight marker.
Fig. 2. Patrón de bandeo de RAPD obtenido con primer OPB1 y 19 muestras de ADN de guayaba del Centro de México mostrando polimorfismo. MW= Marcador de peso molecular.

Table 2. Number of bands obtained per primer, and total polymorphic bands obtained with the 30 primers.
Tabla 2. Número de bandas obtenidas por primer, y el número total de bandas polimórficas contando los 30 primers.

No.	Primer	Sequence	Bands	No.	Primer	Sequence	Bands
1	OPA-01	CAGGCCCTTC	2	16	OPA-16	AGCCAGCGAA	3
2	OPA-02	TGCCGAGCTG	5	17	OPA-17	GACCGCTTGT	5
3	OPA-03	AGTCAGCCAC	1	18	OPA-18	AGGTGACCGT	7
4	OPA-04	AATCGGGCTG	0	19	OPA-19	CAAACGTCGG	2
5	OPA-05	AGGGGTCTTG	6	20	OPA-20	GTTGCGATCC	2
6	OPA-06	GGTCCCTGAC	3	21	OPB-01	GTTTCGCTCC	6
7	OPA-07	GAAACGGGTG	6	22	OPB-02	TGATCCCTGG	1
8	OPA-08	GTGACGTAGG	0	23	OPB-03	CATCCCCCTG	1
9	OPA-09	GGGTAACGCC	3	24	OPB-04	GGACTGGAGT	0
10	OPA-10	GTGATCGCAG	7	25	OPB-05	TGCGCCCTTC	4
11	OPA-11	CAATCGCCGT	2	26	OPB-06	TGCTCTGCCC	5
12	OPA-12	TCGGCGATAG	2	27	OPB-07	GGTGACGCAG	1
13	OPA-13	CAGCACCCAC	5	28	OPB-08	GTCCACACGG	5
14	OPA-14	TCTGTGCTGG	3	29	OPB-09	TGGGGGACTC	6
15	OPA-15	TTCCGAACCC	0	30	OPB-10	CTGCTGGGAC	4

Total polymorphic bands = 97

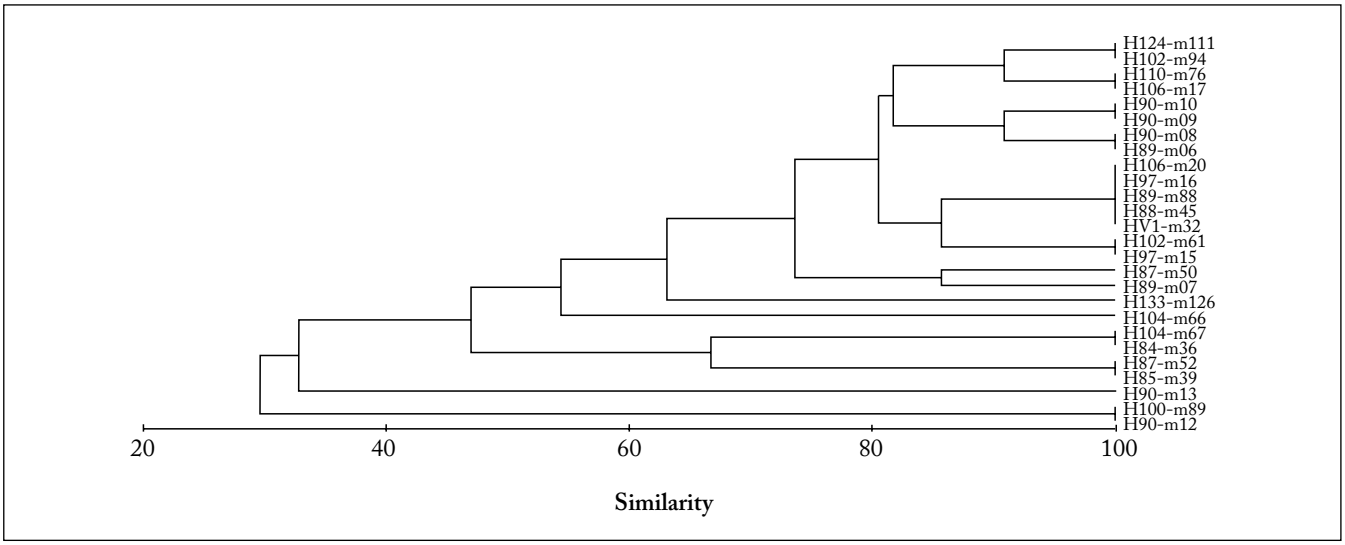

Fig. 3. Cladistics analysis based on RAPD performed on DNA samples of guava from Calvillo, Aguascalientes (Central Mexico).
Fig. 3. Análisis cladístico basado en RAPD realizado con muestras de ADN de guayaba de Calvillo, Aguascalientes (Centro de México).

number of amplicons was low, depending on the combination of genotype and selected primer. Based on that, dendrograms were constructed combining 12 polymorphic bands (Fig. 3).

In general terms, similarity calculated from RAPD of twenty six genotypes ranged from 30 to 100% (rank correlation r= 0.927), and the grouping did not correspond to that found for morphological characters. Other works point the possibility of relating groupings from the guava RAPD dendrograms to fruit morphology or culturing conditions. Chen et al. (2007) were able to identify on the dendrogram constructed from RAPD, mainly two major groups of guava: the commercial cultivars and the wild genotypes. The latter group included two subgroups which roughly clustered white- and red-flesh guavas, respectively. No specific banding was related to these groups. Similarly, Ahmed et al. (2011) using OPA02 and OPA03 for fingerprinting (33.2% polymorphic banding) found genetic distances between 33 guava genotypes calculated between 0.5253 and 0.6631. These authors claim that groupings in the dendrograms relate closely with morphology and that most cultivated genotypes were located in one cluster. Bajpai et al. (2008) using RAPD and Directed Amplification of Minisatellite DNA (DAMD) markers on 22 guava accessions obtained two different dendrograms with genetic distances from 5 to 43%. According to them, genotypes from Indo-Gangetic plains clustered together.

Cladistics analysis of morphological data. All of the morphological measurements made on fruit and leaves were done according to UPOV (1987) guidelines complemented with descriptions by Sánchez-Urdaneta & Peña-Valdivia (2011). Six of these descriptors were evaluated on full mature fruit (shape at stalk end, width of neck in relation to that of fruit, diameter of calyx cavity in relation to that of fruit, ridged collar around calyx cavity, thickness of outer flesh in relation to core diameter, carpel number and pulp color), and the other three on well-developed leaves (shape of base, shape of tip and shape of the whole leaf). To make a close comparison to RAPD results, morphological data were analyzed for almost the same group of genotypes used for RAPDs. Nevertheless, morphological data showed a similarity range from 87 to 98% (Fig. 4), which strongly differs from the similarity found for RAPDs. Clades were mostly defined by fruit characters, with a rank correlation r= 0.91. These results have some agreement to those of Kujal et al. (2005), who mentioned that RAPD data had broader divergence than data from morphological characters. The reason may be that during the evolutionary process, neutral markers such as DNA polymorphisms which do not contribute to the fitness of the individuals are not subjected to the same selective pressure as markers from coding genes.

A more comprehensive morphological analysis was performed with 79 accessions (which include the same twenty three genotypes mentioned in Figure 3) having collectable and fully developed fruits. According to the results, similarity ranged from 87-100%, with a rank correlation r= 0.909 (very close to the previous values). At 87% similarity, two main groups were noticeable (Fig. 5): Group A corresponds to guava genotypes with rounded fruits. This group was divided into three subgroups with 92-93% similarity; two of them (A1 and A2) had in common fruits with four carpels. A1 included genotypes having fruits with medium thickness of flesh and very round end of the fruit (the one that is attached to the pedicel). A2 showed most fruits with round end, pulp thickness ranged between medium and thick and most of the genotypes had oblong leaf shape. In the A3 group, genotypes were clustered because most of the fruits showed five carpels,

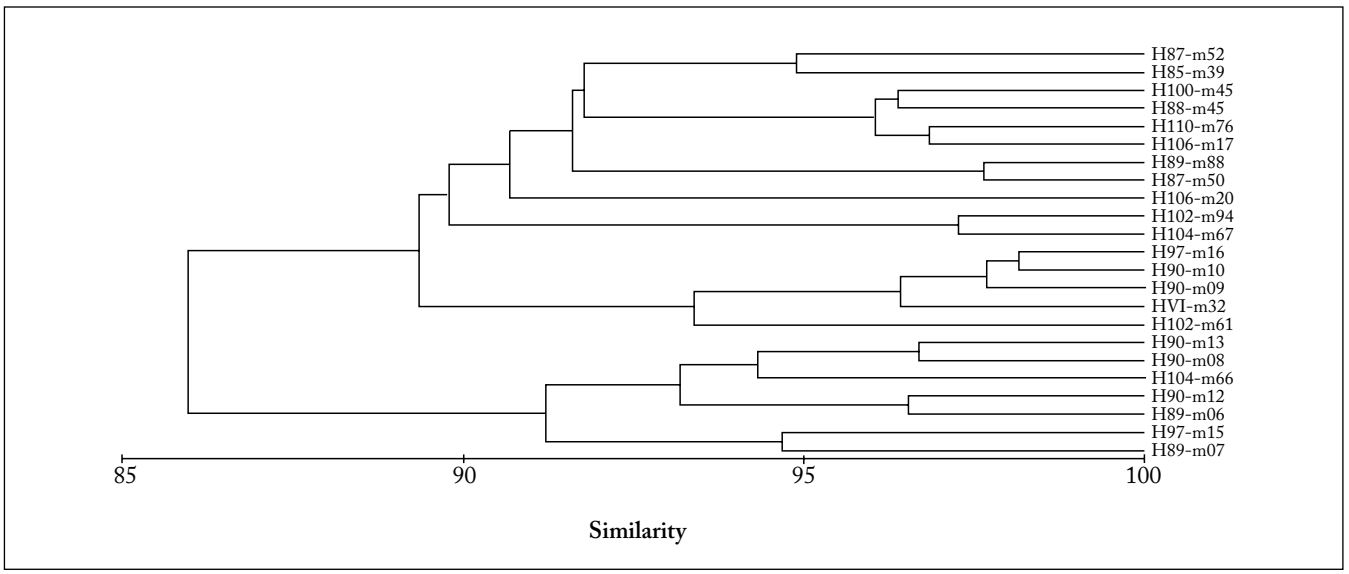

Fig. 4. Cladistics analysis based on morphological data, taking a similar genotype subset as shown in Fig. 3.
Fig. 4. Análisis cladístico basado en datos morfológicos, tomando un subconjunto de genotipos similares como se muestra en la Fig. 3.

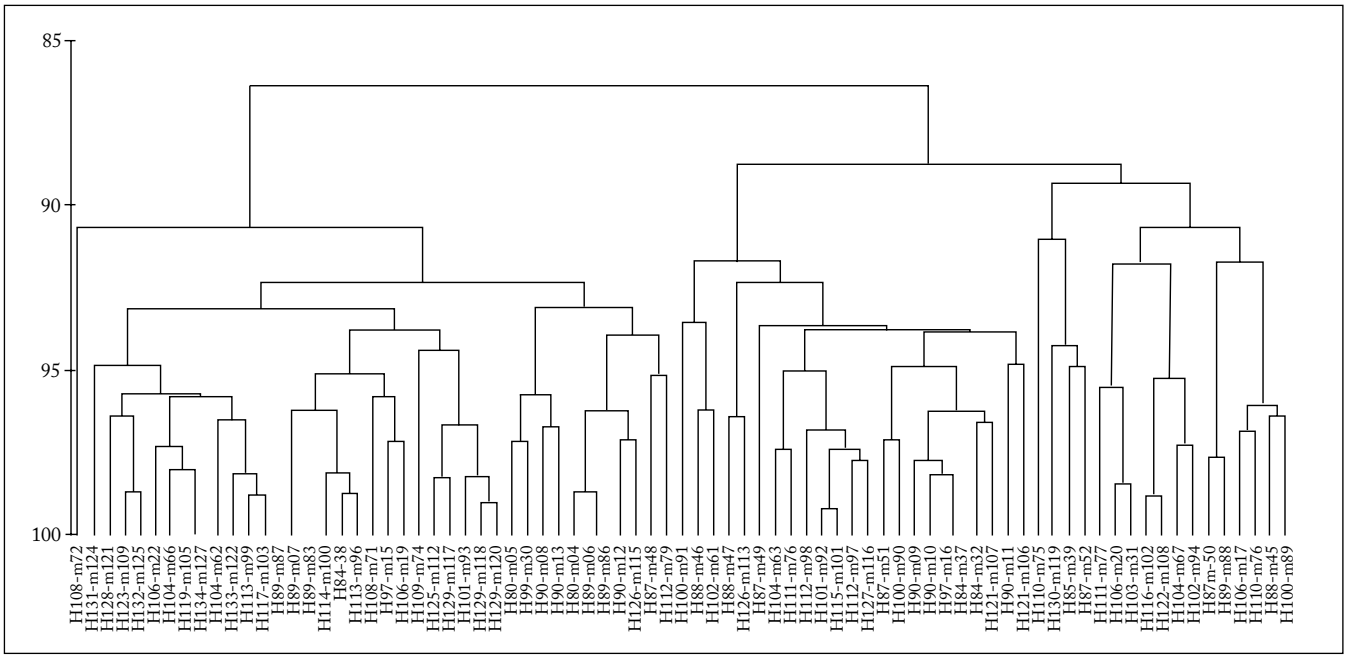

Fig. 5. Cladistics analysis of morphological data of guava genotypes from commercial orchards located in Calvillo, Aguascalientes (Central Mexico). Grouping tags: A= Genotypes with rounded fruits, A1= Fruits with four carpels and medium thickness, A2= Fruits with four carpels, round end, pulp with medium-thick flesh, A3= Five carpels, whitish-colored pulp, B= Pear-shaped fruits with acute and truncated end, C= Pear-shaped with necked end of fruits and white flesh.
Fig. 5. Análisis cladístico y morfológico de genotipos de guayaba de huertas comerciales localizados en Calvillo, Aguascalientes (Centro de México). Etiquetas de agrupación: A= Genotipos con frutos redondos, A1= Frutos con carpelos and grosor medio, A2= Frutos con cuatro carpelos, extremo redondo, pulpa con carne de grosor medio, A3= Cinco carpelos, pulpa de color blanco, B= Frutos en forma de pera con punta aguda y truncada, C= Frutos en forma de pera extremo de cuello y pulpa blanca.

whitish colored pulp and oblong leaves. Groups B and C contained variations of pear-shaped fruits with 4-5 carpels and white color pulp for most of the genotypes. In group B the predominant shape of the fruit end was acute and truncated, and the flesh thickness ranged between medium and thick. In contrast, Group C showed necked end of the fruits and pulp color mostly white.

Fruit size (taking into account both polar and equatorial diameters) ranged from 3.5 to 10.7 centimeters. These sizes were mostly influenced by genotype and water availability on summer (the later not measured, but mentioned by the growers as the main limiting factor for both fruit set and size). Shape patterns of guava fruit showed a distribution as follows: 34% pear-shaped fruits, 50% rounded fruits, and the rest were either ellipsoid or ovoellipsoid. Considering the shape of the base of the fruit, the following was found: 32% round, 18% very round, 20% necked, 16% truncated and 14% angular. The neck itself was found on these fruits as: 19% wide, 13% narrow, 19% medium and 49% absent in the rest of the fruits. Pulp color was distributed as follows: 37% white, 23% creamy, 19% spotted pink and the rest were pale pink. Both types of pink pulp are not considered a good characteristic for fresh consumption in the Mexican market. Thickness of pulp was recorded as: 52% medium and 48% thick.

CONCLUSIONS

RAPDs were useful on finding polymorphic banding patterns on guava genotypes from commercial orchards of Central North Mexico. Further, cladistics showed different grouping and divergence rates for RAPD data compared to morphological characters of these guava genotypes. Since these two groups of markers did not correspond one to each other, it is suggested their use in a complementary form for local genotype discrimination. The use of biotechnology (linkage maps) for assisted selection and propagation of guava genotypes having good vegetative characters and high fruit quality both internal and external has been proposed by Valdés-Infante Herrero et al. (2012b). Our work, using RAPD, is a step toward the selection and preservation of good genotypes readily present in Central Mexico. Local guava producers are aware of some variability inside their orchards, and they know their best genotypes. Hopefully, propagation of genotypes of interest could be traced to avoid undesirable variation checking their RAPD pattern. Nevertheless, the use of RFLPs may help on the claims to certificate the origin of guava from Calvillo (Central Mexico), since producers from other regions pack guava with fake origin labels for export.

DISCLOSURE POLICY

The authors declare that there is no conflict of interests regarding the publication of this paper.

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REFERENCES

Ahmed, B., M.A. Mannan & S.A. Hossain (2011). Molecular characterization of guava (*Psidium guajava* L.) germplasm by RAPD analysis. *International Journal of Natural Sciences* 1: 62-67.

Bajpai, A., R. Chandra, S. Rajan & N. Srivastava (2008). RAPD and minisatellite markers for genetic diversity and relationship in guava varieties. *Indian Journal of Genetics* 68: 441-445.

Brody, J.R. & S.E. Kern (2004). Sodium boric acid: a Tris-free, cooler conductive medium for DNA electrophoresis. BioTechniques 36: 214-216.

Chen, T-W., C-C. NG, C-Y. WANG & Y-T. Shyu (2007). Molecular identification and analysis of *Psidium guajava* L. from indigenous tribes of Taiwan. *Journal of Food and Drug Analysis* 15: 82-88.

Cheng, Y-J., W-W. Guo, H-L. Yi, X-M. Pang & X. Deng (2003). An efficient protocol for genomic DNA extraction from citrus species. *Plant Molecular Biology Reporter* 21: 177a-177g.

Clarke, K.R. & R.N. Gorley (2005). PRIMER v6: User Manual/ Tutorial. PRIMER-E, Plymouth.

Doyle, J.J. & J.L. Doyle (1987). A rapid DNA Isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry* Bull 19: 11-15.

Feria-Romero, I.A. (2008). Clonación y caracterización de fragmentos RAPD y loci microsatelite, asociados a *Psidium guajava* L. cultivada en 4 Estados de la República Mexicana. (Cloning and characterization of RAPD fragments and microsatellite loci, from *Psidium guajava* L. cultivated in four Mexican states). Ph.D. Thesis. Universidad Autónoma Metropolitana, Mexico, 271 p.

Grattapaglia, D., R.E. Vaillancourt, M. Shepherd, B.R. Thumma, W. Foley, C. Külheim, B.M. Potts & A.A. Myburg (2012). Progress in Myrtaceae genetics and genomics: *Eucalyptus* as the pivotal genus. *Tree Genetics & Genomes* 8: 463-508.

ISAAA (2013). Pocket K No. 19: Molecular breeding and marker-assisted selection http://www.isaaa.org/resources/publications/pocketk/19/default.asp . Visited on Nov. 24, 2013.

Kujal, S., S.K. Chakrabarti, S.K. Pandey & S.M.P. Khurana (2005). Genetic divergence in tetraploid potatoes (*Solanum tuberosum* subsp. *tuberosum*) as revealed by RAPD *vis-à-vis* morphological markers. *Potato J.* 32: 17-27.

Liu, X. & G. Yang (2012). Assessment of clonal fidelity of micropropagated guava (*Psidium guajava*) plants by ISSR markers. *AJCS* 6: 291-295.

Nogueira, A.M., J.H.S. Guilhen, É. Mangaravite, A. Ferreira & M.F.S. Ferreira (2012). Discriminant analysis of wild guava tree by morphological descriptors and microsatellites. Proc. 3rd International Symposium on Guava and Other Myrtaceae. 15 p.

Padilla-Ramírez, J.S., E. González-Gaona, F. Esquivel-Villagrana, E. Mercado-Silva, S. Hernández-Delgado & N. Mayek-Pérez (2002). Caracterización de germoplasma sobresaliente de guayabo de la región Calvillo-Cañones, México. (Characterization of outstanding guava germplasm from Calvillo-Cañones, Mexico). *Revista Fitotecnia Mexicana* 25: 393-399.

Padilla-Ramírez, J.S., E. González-Gaona, M.A. Perales- de la Cruz, H.R. Reyes-Phillips & E.S. Osuna-Ceja (2007). Variabilidad del fruto de la guayaba (*Psidium guajava* L.) mexicana. (Fruit variability of Mexican guava *Psidium guajava* L.). INIFAP-CIRNOC-CEPAB. Publicación especial No. 31. Mexico, 68 p.

Perales- de la Cruz, M. A., J. S. Padilla-Ramírez, E. González-Gaona & H. Reyes-Phillips (2005). Manual para la producción Integral del Cultivo de la Guayaba. (Guidelines for integrated production of guava). México. INIFAP. Pabellón de Arteaga, Ags. 9 p.

Provan, J., W.T.B. Thomas, B.P. Forster, & W. Powell (1999). Copia-SSR: A simple marker technique which can be used on total genomic DNA. *Genome* 42: 363-366.

Quesada-Parga, M.L., M.A. Perales-de la Cruz, F.J. Consuelo-Tamayo, F.F. González-Gutiérrez & R.R. Reyes-Phillips (2005). Manual para la producción integral del cultivo de la guayaba. (Guidelines for integrated production of guava). Fundación Produce Aguascalientes A.C, Campo Experimental Pabellón, INIFAP y Consejo Nacional Mexicano de la Guayaba, A.C. 61 p (Booklet version).

Rai, M.K., M. Phulwaria, Harish, A.K. Gupta, N.S. Shekhawat & U. Jaiswal (2012). Genetic homogeneity of guava plants derived from somatic embryogenesis using SSR and ISSR markers. *Plant Cell Tissue and Organ Culture* 111: 259-264.

SAGARPA (2012). Plan rector del sistema producto guayaba Estado de Aguascalientes actualización 2012. http://dev.pue.itesm.mx/sagarpa/estatales/EPT%20COMITE%20SISTEMA%20PRODUCTO%20GUAYABA%20AGUASCALIENTES/PLAN%20RECTOR%20QUE%20CONTIENE%20PROGRAMA%20DE%20TRABAJO%202012/PR_GUAYABA_AGUASCALIENTES_2012.pdf

Sánchez-Urdaneta, A.B. & C.B. Peña-Valdivia (2011). Morphological descriptor for genus *Psidium* characterization. *Revista de la Facultadad Agronomía* (LUZ) 28: 303-343.

UPOV (1987). Guidelines for the conduct of tests for distinctness, homogeneity and stability. Guava (*Psidium guajava* L.), 27 p.

Valdés-Infante Herrero, J., N.N. Rodríguez-Medina, M. Bautista-Alor, M.M. Ortíz-García, A. Quiroz-Moreno, L.F. Sánchez Teyer, A.M. Risterucci & W. Rohde (2012a). Microsatellites developed in guava *Psidium guajava* L. and their usefulness in evaluating diversity in the Myrtaceae family. *Revista Colombiana de Biotecnología* 12: 1-9.

Valdés-Infante Herrero, J., N.N. Rodríguez-Medina, L. González, J.B. Velázquez-Palenzuela, D. Rivero-Rodríguez, D.G. Sourd-Martínez, F. Martínez-González & J.A. Rodríguez-Rodríguez (2012b). La biotecnología como herramienta para la propagación, conservación y el mejoramiento genético del guayabo. (Biotechnology as a tool for propagation, conservation and breeding of guava). *Revista Colombiana de Biotecnología* 14: 7-19.