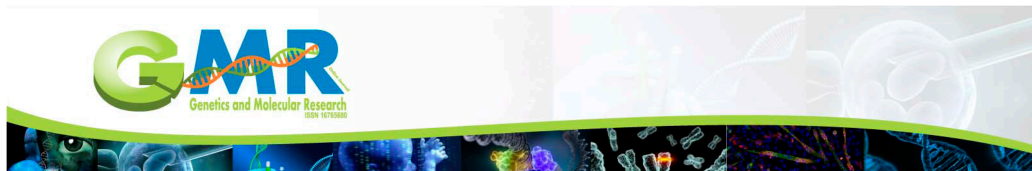

Assessment of genetic relationship in *Persea* spp by traditional molecular markers

J.C. Reyes-Alemán[1], E. Valadez-Moctezuma[2] and A.F. Barrientos-Priego[3]

[1]Centro Universitario UAEM Tenancingo, Universidad Autónoma del Estado de México, Tenancingo, Estado de México, México
[2]Laboratorio de Biología Molecular, Departamento de Fitotecnia, Universidad Autónoma Chapingo, Texcoco, Estado de México, México
[3]Programa de Fruticultura, Departamento de Fitotecnia, Universidad Autónoma Chapingo, Texcoco, Estado de México, México

Corresponding author: E. Valadez-Moctezuma
E-mail: evaladez@correo.chapingo.mx

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ABSTRACT. Currently, the reclassification of the genus *Persea* is under discussion with molecular techniques for DNA analysis representing an alternative for inter- and intra-specific differentiation. In the present study, the traditional random-amplified polymorphic DNA (RAPD) and the inter simple sequence repeat (ISSR) markers were used to determine the genomic relationship of different species and hybrids representative of the subgenera *Eriodaphne* and *Persea* in a population conserved in a germplasm bank. The data were analyzed statistically using multivariate methods. In the RAPD analysis, a total of 190 polymorphic bands were produced, with an average of 23.7 bands per primer, the percentage contribution of each primer was from 7.66 to 19.63; the polymorphic information content (PIC) ranged from 0.23 to 0.45, with an average of 0.35. In the ISSR analysis, a total of 111 polymorphic bands were considered, with an average of 18.5 bands per primer, the percentage contribution of each was from 11.83 to 19.57; the PIC ranged from 0.35 to 0.48, with an average of 0.42. The phenograms obtained in each technique showed the relationship among the accessions through the clusters formed. In general,

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both the techniques grouped representatives of the *Persea americana* races (*P. americana* var. drymifolia, *P. americana* var. guatemalensis, and *P. americana* var. americana). However, it was not possible to separate the species of *Persea* used as reference into independent clades. In addition, they tended to separate the representatives of subgenera *Eriodaphne* and *Persea*.

Key words: Avocado; PCR; Genetic resources; Differentiation; Genomic relationship; Molecular markers

INTRODUCTION

Avocado (*Persea americana* Mill.) has distinct nutritional properties and cultural importance. In Mesoamerica, it was widely used and domesticated by indigenous populations, long before the arrival of European explorers (Galindo-Tovar et al., 2007). Smith (1966) reported the oldest evidence of its use by people in caves located in the central part of Mexico and in the valleys of Oaxaca roughly between 7000-8000 BC. Later, in the mid-sixteenth century, avocado was cultivated by the residents of Mexico and Peru (Popenoe, 1963; Storey et al., 1986). Avocado belongs to genus *Persea*, which is currently divided into the subgenera *Persea* and *Eriodaphne* (Kopp, 1966). "True" avocados, belonging to the subgenus *Persea,* have larger fruits compared to those of *Eriodaphne*.

There are some differences among the races of *P. americana* Mill. (*P. americana* var. drymifolia, *P. americana* var. guatemalensis, and *P. americana* var. americana). They are widely distributed in Mesoamerica (Fiedler et al., 1998) and most of the commercial varieties known to date are found among them, although there are controversies regarding their differentiation owing to high variability in the progeny. It has been suggested that prior to the arrival of the Spanish people to America, the races were separated with little or no mobility because of topographical conditions, climatic barriers, and large seed size. Currently, the races in many regions of America have overlapped and numerous sample collections show a clear racial introgression (Gama-Campillo, 1994). The flowering habit and cross-pollination of avocado, coupled with its prolific form and a low fruit-set, are other factors that prevent the understanding of the lineage of current cultivars and races.

DNA markers have been used successfully to discern genealogies, design breeding strategies, and support systematization and conservation in germplasm banks (Zietkiewicz et al., 1994; Gilbert et al., 1999; Valadez-Moctezuma et al., 2014; Valadez-Moctezuma et al., 2015). Using these tools, races of *P. americana* and some hybrids have been distinguished (Davis et al., 1998; Clegg et al., 1999; Ashworth and Clegg, 2003). The aim of the present study was to determine the genomic relationship among different genotypes of avocado, including hybrids and an unidentified genotype (*Persea* sp), conserved *ex situ* in a germplasm bank. Two different traditional and molecular techniques were used to better characterize each genotype to ensure their best use.

MATERIAL AND METHODS

Persea genotypes

Five representative species of the subgenus *Eriodaphne,* five of the subgenus *Persea*, four genotypes of *P. americana* var. americana, 10 genotypes of *P. americana* var. guatemalensis, 34 of *P. americana* var. drymifolia, 4 hybrids of *Persea*, and an unidentified genotype called "*Persea*

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sp" were used as references (Table 1). Most of the materials used are preserved *ex situ* in the CICTAMEX germplasm bank property of the Fundación Salvador Sánchez Colín, CICTAMEX S.C. in Coatepec Harinas, Estado de México; however, some materials were collected *in situ*.

Table 1. *Persea* spp genotypes considered in the study, key genotype, origin, genotype name, number in the germplasm bank, specie/subgenus and unidentified genotype.

	Key genotype	Origin	Genotype name	Number in bank	Specie/subgenus
1	CH-Ver-1*	Veracruz, México	Persea sp	-	Probably *Eriodaphne*
2	CH-Ver-2	Veracruz-Chocamán	Parvifolia	-	*P. parvifolia/Eriodaphne*
3	CH-C-30	Veracruz	Cinerascens	-	*P. cinerascens/Eriodaphne*
4	CH-Ch5	Chile	Meyeniana	-	*P. meyeniana/Eriodaphne*
5	CH-Ch-4	Chile	Lingue 32	32	*P. lingue/Eriodaphne*
6	CH-Ver-3	Veracruz-Huatusco	Floccosa H	-	*P. longipes/Eriodaphne*
7	CH-I-3	Veracruz	Floccosa 10	10	*P. floccosa/Persea*
8	CH-GU-1	Guatemala	Shiedeana-Otrabanda	17	*P. schiedeana/Persea*
9	CH-I-4	Israel	Nubigena 1/7	11	*P. nubigena/Persea*
10	CH-Chis-1	Chiapas, México	Steyermarkii 155	232	*P. steyermarkii/Persea*
11	CH-I-2	Chiapas	Gigantea	3	*P. gigantea/Persea*
12	CH-I-6	Israel	Antigua 19	14	*P. americana* var. americana/*Persea*
13	CH-G-49	México	Tetiz 1	77	"
14	CH-CR-28	Costa Rica	Marichal	102	"
15	CH-G-48	México	Tetiz 2	79	"
16	CH-G-10	México	Olanca 2S3	80	*P. americana* var. guatemalensis/*Persea*
17	CH-GU-16	Guatemala	Miramundo	55	"
18	CH-G-9	México	Olanca	25	"
19	CH-GU-5	Guatemala	Palestre	34	"
20	CH-G-15	México	Larrainzar 1	54	"
21	CH-GU-17	Guatemala	Teni	56	"
22	CH-G-7	México	Sn Cr Mer 7s1	60	"
23	CH-C-43	México	Comcar 1	33	"
24	CH-G-4	México	Sn Cr Mer 4S2	61	"
25	CH-G-24	México	Amatenango S1	94	"
26	CH-C-62	Estados Unidos	M. Grande	19	*P. americana* (híbrido)/*Persea*
27	CH-C-58	México	230 ptb	51	"
28	CH-CR-44	Costa Rica	Cima de Copey	78	"
29	CH-C-60	México	24 ptb	87	"
30	CH-I-7	Israel	Ettinger	99	*P. americana* var. drymifolia/*Persea*
31	CH-C-61	México	La Meza	185	"
32	CH-Ch-3	Chile	Negra la Cruz	221	"
33	CH-Ch-2	Chile	Fuerte Negro	223	"
34	CH-C-63	México	Tepetl	46	"
35	CH-C-43	México	Lonjas	36	"
36	CH-C-17-b	México	Príncipe Negro 1	217	"
37	CH-C-57	México	Ixtapan del O	138	"
38	CH-P-31	México	Telez 1	38	"
39	CH-G-86	México	Mantequilla 1	37	"
40	CH-E-1	Ecuador	Guay1	41	"
41	CH-Crm-98	México	Portainjerto	98	"
42	CH-C-50b	México	S Ag Negro	96	"
43	CH-Crm-97	México	CRM (Criollo Coatepec)	97	"
44	CH-C-5	México	Temascaltepec 3S4	73	"
45	CH-C-2a	México	Malinaltenango	84	"
46	CH-C-38	México	Pintle 2	86	"
47	CH-C-43b	México	S Lonjas	90	"
48	CH-C-52b	México	S Parque Timb	91	"
49	CH-C-50-a	México	S Ag Negro (A. Negro)	115	"
50	CH-E-2	Ecuador	Guay III	157	"
51	CH-P-3	México	Tlacolula 3S2	161	"
52	CH-G-15	México	Larrainzar 2	172	"
53	CH-P-3	México	Tlacolula 86	177	"
54	CH-C-22	México	Almoloya	183	"
55	CH-C-50-b	México	Ag Negro	188	"
56	CH-C-19	México	Mantequilla 2	191	"
57	CH-G-26	México	Amatenango S4	202	"
58	CH-C-10b	México	Tochimilco 1	209	"
59	CH-C-12	México	Tochimilco 3	210	"
60	CH-C-13	México	Tochimilco 4	211	"
61	CH-C-14a	México	Tochimilco 5b	214	"
62	CH-C-17-a	México	Principe Negro 2	216	"
63	CH-C-18	México	Aquijic	220	"

*Unidentified genotype.

Development of molecular markers

Genomic DNA was extracted from young leaves (200 mg) using a slightly modified cetyltrimethylammonium bromide method (Saghai-Maroof et al., 1984). Genetic markers were developed using random-amplified polymorphic DNA (RAPD) and the inter simple sequence repeat (ISSR) (with anchor) techniques, both of which scan the whole genomes (Fontaine et al., 2004; Valadez-Moctezuma et al., 2014; Valadez-Moctezuma et al., 2015). Polymerase chain reaction (PCR) for RAPD comprised of 100 ng genomic DNA, 1.5 U Taq DNA polymerase (Fermentas, USA), 200 mM dNTPs, 1X Taq buffer, 2.0 mM $MgCl_2$, and 20 pM primer in a 25-μL total volume. The same components were used for ISSR but the amount of DNA and concentration of $MgCl_2$ was 50 ng and 3.0 mM, respectively. PCR was performed in a thermocycler Gene Amp PCR System 2700® (Applied Biosystems, USA) using the following programs: for RAPD, 1 min at 94°C followed by 35 cycles at 94°C for 30 s, 40°C for 30 s, and 72°C for 1.5 min, and a final extension at 72°C for 2.5 min; for ISSR, 1 min at 94ºC followed by 38 cycles at 94°C for 30 s, 48°C for 30 s, and 72°C for 2 min, and a final extension of 72°C for 2.5 min. The PCR primers used are shown in Table 2. PCR products were electrophoresed on 8% polyacrylamide gels (29:1); using1-kb DNA molecular weight marker (Gibco, USA) as a reference. The gel electrophoresis was done at 280 volts for approximately 2.5 h in 1X TBE buffer; the gels were stained with 0.2% $AgNO_3$ according to the standard protocol (Sambrook et al., 1989) and documented using the Kodak Digital Science 1D V.2.0.3 system (Eastman Kodak Company, New Haven, CT, USA).

Table 2. Primers used for DNA markers in *Persea* spp, type marker, primer key, locus/DNA bands, PIC, and RP.

Type marker	Primer key	Locus/DNA bands	PIC	RP
RAPD	A15	17	0.44	9.33
	C8	26	0.45	16.6
	C10	30	0.23	8.19
	C13	17	0.33	6.76
	E7	29	0.42	13.9
	E14	22	0.41	11.04
	E16	25	0.26	0.38
	E18	24	0.29	6.57
	Total	190	2.83	72.77
	Average	23.75	0.35375	9.09625
ISSRs*	$AC(GACA)_4$	18	0.47	9.71
	DBDA $(CA)_7$	15	0.48	8.1
	$(AC)_8YG$	20	0.42	8.16
	$(GA)_8YC$	18	0.42	9.81
	$(TCC)_5RY$	18	0.35	6.16
	$(GACA)_4$**	22	0.4	10.22
	Total	111	2.54	52.16
	Average	18.5	0.42	8.69

*B = G, T, C; D = G, A, T; Y = C, T; R = A, G anchored primers, **primer without an anchor.

Statistical analysis

Only the consistent and reproducible RAPD and ISSR amplified bands were considered for the analysis, in order to ensure the exclusion of gel artifacts. Polymorphic DNA bands were scored as discrete variables, "1" for the presence of a band and "0" for its absence, which were transformed into a binary character matrix. Subsequently, the data were processed with the FreeTree software (Version 0.9.1.5) (Pavlíèek et al., 1999) to produce a genetic distance matrix using Nei and Li/Dice similarity coefficient. The resulting matrix was computed using the unweighted pair group method

with arithmetic mean (UPGMA) to construct the phenogram with 1000 bootstrap replicates. Tree View 1.6.6 software was used to display the obtained tree (Hampl et al., 2001). The Mantel test was employed to compare the correlation between the distance matrices obtained with the RAPD and ISSR.

The ability of the most informative primers to differentiate between the accessions was assessed by the estimation of their PIC and resolving power (Rp). The PIC was calculated using the following formula described by Roldán-Ruiz et al. (2000):

$$PICi = 2fi\ (1\text{-}fi) \qquad \text{(Equation 1)}$$

where PICi was the polymorphic information content of the primer i, fi was the frequency of the bands present, and (1 - fi) was the frequency of the absent bands. The maximum value of PIC for dominant markers was 0.5 (De Riek et al., 2001). Rp was calculated (Prevost and Wilkinson, 1999) according to the following formula (Gilbert et al., 1999):

$$Rp = \sum \mathbf{I}b \qquad \text{(Equation 2)}$$

where **I**b represented the information band and was calculated using the formula:

$$\mathbf{I}b = 1\text{-}\ (2x\ |\ 0.5\text{-}p|) \qquad \text{(Equation 3)}$$

where p was the proportion of accessions containing B and **I** was the percentage of polymorphic bands.

RESULTS

The DNA extracted from the studied samples was determined to be appropriate for RAPD and ISSR analyses. The photometric measurement quotient, $OD_{260/280}$, of the DNA varied between 1.6 and 1.8, indicating acceptable quality of genomic DNA. Moreover, the DNA yield from 200 mg young leaf tissue was approximately 50 ng/μL. Both the employed DNA marker systems were sufficiently informative. The size of the PCR products considered for the RAPD analysis ranged between 350 and 2500 bp. In this analysis, a total of 190 amplified bands were considered, all of which were polymorphic; an average of 23.7 polymorphic bands were obtained per primer (Table 2) and the percentage contribution of each primer varied from 7.66 for the primer C13 to 19.63 for C8. The PIC ranged from 0.23 for the primer C10 to 0.45 for C8, with an average of 0.35. Among the RAPD primers used, C8 was the most informative. The estimated Rp values exhibited a total rate of 72.77 and ranged from 0.38 (E16) to 16.6 (C8), with an average of 9.0 (Table 2). Moreover, C8 primer seemed to be the most efficient for assessing the genetic diversity as it presented the highest Rp value. The six ISSR primers produced 111 bands; all the bands were polymorphic and were amplified at an average of 18.5 bands per primer and the percentage contribution of the primers varied from 11.83 for the $(TCC)_5$ RY primer to 19.57 for the AC $(GACA)_4$ primer (Table 2). The size of the PCR products considered in the analysis ranged between 350 and 2500 bp. The PIC ranged from 0.35 for $(TCC)_5$ RY to 0.48 for DBDA$(CA)_7$ with an average of 0.42. Among the ISSR primers used, the DBDA $(CA)_7$ primer was the most informative. The Rp values estimated for this marker system had a total rate of 52.16 and ranged from 6.16 for $(TCC)_5$ RY to 10.22 for $(GACA)_4$, with an average of 8.69 (Table 2). Also, the primer $(GACA)_4$ (the primer without an

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anchor) seemed to be the most efficient for assessing the genetic diversity as it presented a high rate of Rp (Table 2).

Genetic similarities and relationships among accessions

Estimates of genetic relationships between the accessions were obtained from the marker data using the Dice similarity coefficient (Pavlíèek et al., 1999). The UPGMA analysis confirmed the genetic divergence described above (Figures 1 and 2). The phenograms exhibited seven main groups (A, B, C, D, E, F, and G). Furthermore, all the genotypes could be distinguished from each other suggesting a high level of genetic variation among the studied *Persea* accessions. The phenogram constructed with the RAPD markers (Figure 1) provided information equivalent to that obtained by using the ISSR markers (Figure 2). Reference species and races dispersed within the clade of the subgenus *Persea* are usually held together but also distributed throughout the clade, indicating the presence of DNA fragments common between the species and races representative of the subgenus. The 63 genotypes in the phenogram of RAPD were distributed as follows: group A comprised of the genotypes of the Mexican race (*P. americana* var. drymifolia), group B of the Guatemalan and Antillean races (*P. americana* vars. americana and guatemalensis), P. *nubigena* (Nubigena 1/7) and *P. steyermarkii* (Steyermarkii 155), group C comprised of a small group of Mexican races; the genotype Malinaltenango, an individual of the Mexican race without predefined taxonomic position, which joined the group of *P. americana* var. drymifolia (Mexican) based on ISSR, was located in group D. Group E was formed by four of the five species representative of the subgenus *Eriodaphne* namely, *P. lingue* (Lingue32), *P. longipes* (Floccosa H), *P. cinerascens* (Cinerascens), and *P. meyeniana* (Meyeniana); the F group was formed by *P. parvifolia* (Parvifolia) subgenus *Eriodaphne* and *P. schiedeana* (Shiedeana-Otrabanda), subgenus *Persea*. The last group (G) was represented by the unidentified *Persea* sp.

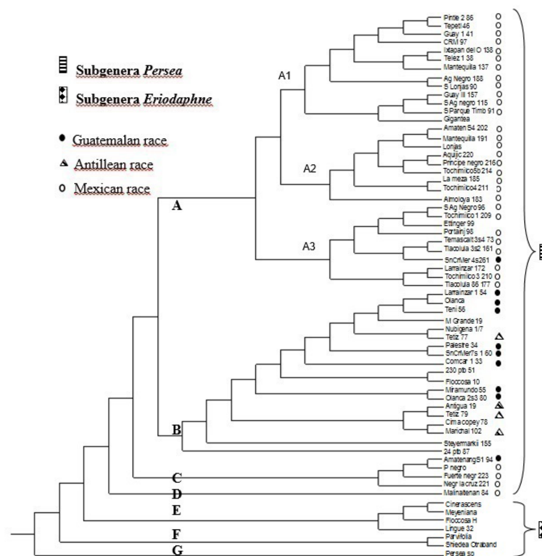

Figure 1. UPGMA grouping of accessions of *Persea* spp analyzed with RAPD profiles, distancing Nei and Li/Dice and bootstrapping 1000 repetitions.

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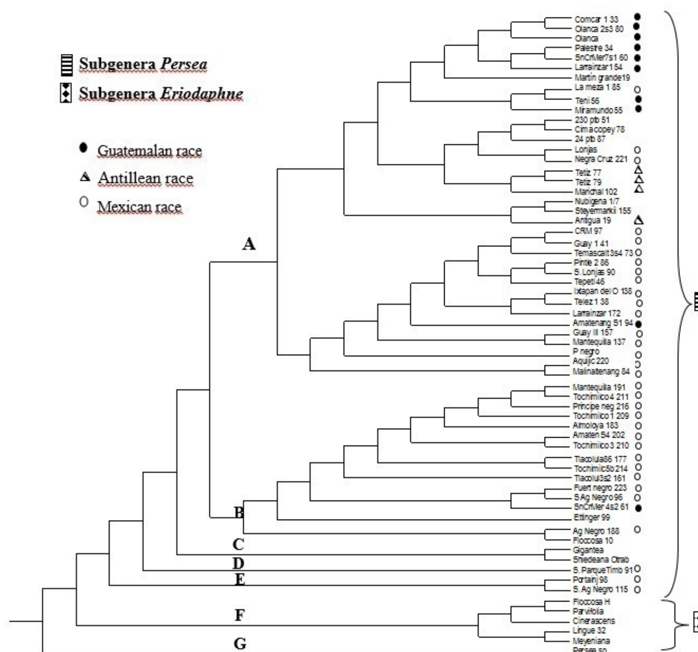

Figure 2. UPGMA grouping of accesions of *Persea* spp analyzed with ISSR profiles, distancing Nei and Li/Dice and bootstrapping 1000 repetitions.

In the phenogram constructed using the ISSR data (Figure 2), the 63 genotypes were also distributed into seven major groups. Group A comprised the Guatemalan and Antillean races, and some Mexican race genotypes as well as *P. nubigena* (Nubigena 1/7) and *P. steyermarkii* (Steyermarkii 155). Group B comprised of most of the genotypes of the Mexican race (*P. americana*); *P. gigantea* (Gigantea) and *P. schiedeana* (Shiedeana-Otrabanda) were located in group C; in groups D and E, the Timb 91, Porta injerto 98, and S Ag negro-115 genotypes were located. Group F consisted of the five species of the subgenus *Eriodaphne*, *P. lingue* (Lingue32), *P. longipes* (Floccosa H), *P. cinerascens* (Cinerascens), *P. meyeniana* (Meyeniana), and *P. parvifolia* (Parvifolia). Group G was represented only by *Persea* sp, as in the RAPD analysis.

DISCUSSION

The ISSR technique is known to detect conserved amplicons located between the microsatellites of the same type; if the fragments correspond in size in individuals being compared, the likelihood of those individuals having a close relationship increases. Using this technique, it was also evident that the three horticultural varieties of *P. americana* usually stayed together throughout the clade; the closeness between the Antillean and Guatemalan races was also demonstrated by the representative species used.

The hybrid genotypes, M. Grande (G755), obtained by a cross between *P. schiedeana* and the Guatemalan race, had a marked resistance to root rot (Ellstrand et al., 1986), genotypes 230 ptb (obtained by segregation of the commercial variety Colin V-33) and 24 ptb (obtained by segregation

of *P. americana* var. drymifolia) that were both tolerant to soil salinity (López et al., 1993), and the hybrid, Costa Rica Cima de Copey were all generated by selections within *P. americana* and were pooled between the Guatemalan and Antillean races, with both the marker types (Figures 1 and 2). In the study, the Guatemalan accessions, Olanca 2S3, Miramundo, Olanca, Palestre, Larrainzar 1, Teni, SnCrMer 7s1, Comcar 1, SnCrMer 4S2, and Amatenango S1, were also located near those. The Mantel test indicated a significant and positive correlation (r = 0.201, P < 0.009) between the linear RAPD and ISSR individual pairwise genetic distance matrices.

Recent studies on the genetic diversity of the genus *Persea* have shown the efficacy of molecular techniques in estimating the genetic relationships among avocado genotypes (Gutiérrez-Díez et al., 2009; Cuiris-Pérez et al., 2009; Galindo-Tovar et al., 2011; Abraham and Takrama, 2013; Reyes-Alemán et al., 2013). Results obtained with the techniques used in this study, applied to the representatives of the subgenera *Persea* and *Eriodaphne* revealed a tendency to separate the genotypes of both the subgenera; this separation was more evident with the ISSR when compared to the RAPD technique. Remarkably, 301 DNA fingerprints were considered for the analyses of which 221 were shared by the 63 genotypes used. Furthermore, all of those were polymorphic. Representatives of the *Eriodaphne* group showed 67 monomorphic fingerprints in their own group while those of *Persea* showed 13, characteristic of the respective groups (data not shown). These few monomorphisms caused the differences that separated the genotypes into different clades. Based on the morphological characters, *Eriodaphne* and *Persea* have been reported as two subgenera within the genus *Persea* (Kopp, 1966). In the last decade, separating the genus based on differences in morphological as well as in the ITS sequences has been proposed (Campos et al., 2007). However, its taxonomic nomenclature is yet considered difficult because of the differences in ITS sequences, which are not entirely exclusive (Rohwer et al., 2009). The latter observation was also reflected in our results, because even though representatives of both the subgenera demonstrated a tendency to be separate in the phenograms, the amount of shared fragments (221) was higher than those stored for each subgenus (67 and 13). Another clear observation in this study was that *P. floccosa*, *P. schiedeana*, *P. nubigena, P. steyermarkii*, and *P. gigantea* were not separated in specific clades but were distributed in a large clade of *P. americana*. This behavior assumes that differences at the DNA level were not sufficient between these species to keep them in independent groups. The other genotypes (hybrid) were also distributed throughout the *Persea* clade, suggesting that they also maintained close genetic relationship. Differences between the races have been discussed by several authors (Furnier et al., 1990; Fiedler et al., 1998; Ashworth and Clegg, 2003). The present study indicates a close relationship between the Guatemalan and Antillean races, as reported by Bufler and Ben- Ya'acov (1992) and Mhameed et al., (1997) However, there was a trend of mixing between the races due to the proximity of the genotypes capable of interracial hybridization. This observation was also made by Chen et al. (2008). It has been suggested that *P. americana* has contributions from other species of *Persea* (Williams 1977a; Campos et al., 2007). This was also evident from the clusters in Figures 1 and 2, where the horticultural races could be observed to mix with *P. steyermarkii* and *P. nubigena*, which are wild species of the mountainous region of Chiapas (Mexico) and share morphological similarities, such as rudimentary little fruit pulp, leathery leaves, erect habit, and large-sized tree. Both the species were closely related to the Guatemalan race, based on the observations made earlier (Williams, 1977b; Furnier et al., 1990). This genetic contribution from the currently recognized *Persea* species is one of the reasons why its classification is not clear.

The genotype *Persea* sp, collected from Veracruz (Mexico), has not yet been identified, but

shares morphological characteristics with *Eriodaphne*, such as lanceolate, leathery leaf, strong aroma on crushing, and “aguacatillo” type fruit, indicating the possibility of wild and unknown new species or hybrids, as reported by Lorea-Hernández (2005). This accession could be related to *P. primatogena* and *P. parvifolia*, two native *Persea* species also found in Veracruz and described by Williams (1977b).

P. schiedeana is a species with dense pubescence on their structures (Williams, 1977b). Its most distinctive morphological features include rigid and wide scales on its buds and short, straight, rigid, and persistent pubescence of gineceo (Scora et al., 2002). This species is capable of hybridizing with *P. americana* because of sexual and vegetative compatibility. Based on the analysis and location in the phenograms, there was a similarity with *Eriodaphne* genotypes, although, it is currently classified within *Persea*. Both the subgenera share the same distribution in America as indicated by Rohwer et al. (2009) and this is, perhaps due to the likeness in the two subgenera.

There are genotypes related to avocado that still need to be defined, such as “aguacatillos” and/or “cascarudos” from warm southern regions of Estado de México. Efforts for breeding could unravel genes saved in native populations, because if a local population represents a contribution to the genus diversity, then the population conserved in a germplasm collection, originated at different times and in different places, gains a higher value. This study helped in identifying the genomic relationships that germplasm accessions maintain and also their diversity. The genotypes used have different origins, and it could be possible to infer differences between the groups based on geographical confinement due to its origin as was suggested by Chen et al. (2009). In the past, this argument forced the designation of specific sites for the conservation of avocado collections from temperate climate and other warm weather in the CICTAMEX SC gene bank which are known sites of high and low elevation (López-López et al., 1997).

RAPD and ISSR markers were efficacious in demonstrating that the genotypes of *Persea* species and races of *P. americana* preserved in the germplasm bank were interrelated and had the ability to form new combinations. The RAPD and ISSR markers provided data that grouped the studied genotypes. DNA fingerprints separated the genotypes and classified them into *Persea* and *Eriodaphne*. The dispersed location of the genotypes of drymifolia, americana, and guatemalensis varieties within the clade of *P. americana* suggested a constant hybridization between the horticultural races. The unidentified genotype, referred to in this work as *Persea* sp, was kept separate from the genotypes of the subgenera *Persea* and *Eriodaphne*.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Abraham JD and Takrama JF (2013). Morphological and genetic diversity of *Persea americana* Mill. (Avocado) in two regions of Ghana. Conference on International Research on Food Security, Natural Resource Management and Rural Development. Tropentag, Stuttgart.

Ashworth VE and Clegg MT (2003). Microsatellite markers in avocado (*Persea americana* Mill.): genealogical relationships among cultivated avocado genotypes. *J. Hered.* 94: 407-415. http://dx.doi.org/10.1093/jhered/esg076

Bufler G and Ben-Ya'acov AD (1992). Study of avocado germplasm resources, 1988-1990. Ribosomal DNA repeat unit polymorphism in avocado. Proceedings of the 2nd World Avocado Congress, Orange.

Campos RE, Terrazas T and López-Mata L (2007). *Persea* (avocados) phylogenetic analysis based on morphological characters: hypothesis of species relationships. *Genet. Resour. Crop Evol.* 54: 249-258. http://dx.doi.org/10.1007/s10722-005-3808-x

Chen H, Morrell PL, de la Cruz M and Clegg MT (2008). Nucleotide diversity and linkage disequilibrium in wild avocado (*Persea americana* Mill.). *J. Hered.* 99: 382-389. http://dx.doi.org/10.1093/jhered/esn016

Chen H, Morrell PL, Ashworth VE, de la Cruz M, et al. (2009). Tracing the geographic origins of major avocado cultivars. *J. Hered.* 100: 56-65. http://dx.doi.org/10.1093/jhered/esn068

Clegg MT, Kobayashi M and Lin JZ (1999). The use of molecular markers in the management and improvement of avocado (*Persea americana* Mill.). Proceedings of the IV World Avocado Congress, México. *Rev. Chapingo Ser. Hortic.* 5: 227-231.

Cuiris-Pérez H, Guillen-Andrade H, Pedraza-Santos ME, López-Medina J, et al. (2009). Genetic variability within mexican race avocado (*Persea americana* Mill.) germplasm collections determined by ISSRs. *Rev. Chapingo Ser. Hortic.* 15: 169-175. http://dx.doi.org/10.5154/r.rchsh.2009.15.023

Davis J, Henderson D, Kobayashi M, Clegg MT, et al. (1998). Genealogical relationships among cultivated avocado as revealed through RFLP analysis. *J. Hered.* 89: 319-323. http://dx.doi.org/10.1093/jhered/89.4.319

De Riek J, Calsyn E, Everaert I, Bocksteal VE, et al. (2001). AFLP based alternatives for the assessment of the distinctness, uniformity and stability of sugar beet varieties. *Theor. Appl. Genet.* 103: 1254-1256. http://dx.doi.org/10.1007/s001220100710

Ellstrand NC, Lee JM, Bergh BO, Coffey MD, et al. (1986). Isozymes confirm hybrid parentage for 'G755' selections. *Calif. Avocado Soc. Yearbook* 70: 199-203.

Fiedler J, Bufler G and Bangerth F (1998). Genetic relationships of avocado (*Persea Americana* Mill.) using RAPD markers. *Euphytica* 101: 249-255. http://dx.doi.org/10.1023/A:1018321928400

Fontaine C, Lovett PN, Sanou H, Maley J, et al. (2004). Genetic diversity of the shea tree (*Vitellaria paradoxa* C.F. Gaertn), detected by RAPD and chloroplast microsatellite markers. *Heredity (Edinb)* 93: 639-648. http://dx.doi.org/10.1038/sj.hdy.6800591

Furnier GR, Cummings MP and Clegg MT (1990). Evolution of the avocados as revealed by DNA restriction fragment variation. *J. Hered.* 81: 183-188.

Galindo-Tovar ME, Arzate-Fernández AM, Ogata-Aguilar N and Landero-Torres I (2007). The avocado (*Persea americana*, Lauracea) crop in Mesoamerica: 10,000 years of history. *Harv. Pap. Bot.* 12: 325-334. http://dx.doi.org/10.3100/1043-4534(2007)12[325:TAPALC]2.0.CO;2

Galindo-Tovar ME, Milagro-Pérez PA, Alejandre-Rosas JA, Leyva-Ovalle OR, et al. (2011). Relaciones genéticas del aguacate (*Persea americana* Mill.) en siete municipios del centro de Veracruz, caracterizadas con microsatélites. *Trop. Subtropical Agroecosystems* 13: 339-346.

Gama-Campillo L (1994). Studies on the systematic and ethnobotany of the subgenus *Persea*. Doctoral thesis, University of California, Riverside.

Gilbert JE, Lewis RV, Wilkinson MJ and Caligari PDS (1999). Developing an appropriate strategy to assess genetic variability in plant germplasm collections. *Theor. Appl. Genet.* 98: 1125-1131. http://dx.doi.org/10.1007/s001220051176

Gutiérrez-Díez A, la Cerda JM, García-Zambrano EA, Iracheta-Donjuan L, et al. (2009). Estudio de diversidad genética del aguacate nativo en Nuevo León, México. *Rev. Fitotec. Méx.* 32: 9-18.

Hampl V, Pavlícek A and Flegr J (2001). Construction and bootstrap analysis of DNA fingerprinting-based phylogenetic trees with the freeware program FreeTree: application to trichomonad parasites. *Int. J. Syst. Evol. Microbiol.* 51: 731-735. http://dx.doi.org/10.1099/00207713-51-3-731

Kopp L (1966). A taxonomic revision of the genus *Persea* in the Western Hemisphere (Persea-Lauraceа). *Mem. N. Y. Bot. Gard.* 14: 1-117.

López LL, Saavedra GC and Rubí AM (1993). Selecciones de segregantes de aguacate (*Persea americana* Mill) bajo condiciones de salinidad. In. Memoria de actividades Fundación Salvador Sánchez Colín CICTAMEX-SC, México, 167-179.

López-López L, Rubí-Arriaga M, Ben-Ya'acov AD and Barrientos-Priego AF (1997). La biodiversidad del género *Persea* y especies afines preservados en los bancos de germoplasma del Estado de México. *Rev. Chapingo Ser. Hortic.* 3: 51-54.

Lorea-Hernández FG (2005). Nuevas especies de *Licaria, Ocotea* y *Persea* (Laurácea) de México. *Acta Bot. Mex.* 71: 61-87.

Mhameed S, Sharon D, Kaufman D, Lahav E, et al. (1997). Genetic relationships within avocado (*Persea americana* Mill.) cultivars and between *Persea* species. *Theor. Appl. Genet.* 94: 279-286. http://dx.doi.org/10.1007/s001220050411

Pavlíèek A, Hrdá S and Flegr J (1999). FreeTree-freeware program for construction of phylogenetic trees on the basis of distance data and bootstrap jackknife analysis of the tree robustness. Application in the RAPD analysis of genus *Frenkelia. Folia Biol-Prague*. 45: 97-99.

Popenoe W (1963). Early history of the avocado. *Calif. Avocado Soc. Yearbook* 47: 19-24.

Prevost A and Wilkinson MJ (1999). A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theor. Appl. Genet.* 98: 107-112. http://dx.doi.org/10.1007/s001220051046

Reyes-Alemán JC, Valadez-Moctezuma E, Simuta-Velázco L, Barrientos-Priego AF, et al. (2013). Distinción de especies del género *Persea* mediante RAPD e ISSR de ADN. *Rev. Mex. Cienc. Agríc* 4: 517-529.

Rohwer JG, Li J, Rudolph B, Schmidt SA, et al. (2009). Is Persea (Lauraceae) monophyletic? Evidence from nuclear ribosomal ITS sequences. *Taxon* 58: 1153-1167.

Roldán-Ruiz I, Dendauw J, Van Bockstaele E, Depicker A, et al. (2000). AFLP Markers reveal high polymorphic rates in Ryegrasses (*Loium* spp). *Mol. Breed.* 6: 125-134. http://dx.doi.org/10.1023/A:1009680614564

Saghai-Maroof MA, Soliman KM, Jorgensen RA and Allard RW (1984). Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. *Proc. Natl. Acad. Sci. USA* 81: 8014-8018. http://dx.doi.org/10.1073/pnas.81.24.8014

Sambrook J, Fritsch EF and Maniatis T (1989). Gel electrophoresis of DNA. In: Molecular Cloning: A Laboratory Manual. 2nd edn., Vol 1. Cold Spring Harbor Laboratory Press, New York.

Scora RW, Wolstehholme BN and Lavi U (2002). Taxonomy and botany. In: The avocado: botany, production, and uses (Whiley AW, Shaffer B and Wolstenholme BN, eds.). CAB International, Wallingford, UK, 15-37.

Smith CE (1966). Archaeological evidence for selection in avocado. *Econ. Bot.* 20: 169-175. http://dx.doi.org/10.1007/BF02904012

Storey WB, Bergh B and Zentmyer GA (1986). The origin, indigenous range and dissemination of the avocado. *Calif. Avocado Soc. Yearbook* 70: 127-133.

Valadez-Moctezuma E, Ortiz-Vásquez Q and Samah S (2014). Molecular based assessment of genetic diversity of xoconostle accessions (*Opuntia* spp.). *Afr. J. Biotechnol.* 13: 202-210. http://dx.doi.org/10.5897/AJB2013.13350

Valadez-Moctezuma E, Samah S and Luna-Páez A (2015). Genetic diversity of *Opuntia* spp. varieties assessed by classical marker tools (RAPD and ISSR). *Plant Syst. Evol.* 301: 737-747. http://dx.doi.org/10.1007/s00606-014-1112-y

Williams LO (1977a). The avocados, a synopsis of the genus *Persea*, subg. *Persea Econ. Bot.* 31: 315-320. http://dx.doi.org/10.1007/BF02866883

Williams LO (1977b). The botany of the avocado and its relatives. Proceedings of 1st International Tropical Fruit Short Course (Sauls JW, Phillips RL and Jackson LK, eds.). University of Florida, Gainesville.

Zietkiewicz E, Rafalski A and Labuda D (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20: 176-183. http://dx.doi.org/10.1006/geno.1994.1151