GENETIC VARIABILITY WITHIN MEXICAN RACE AVOCADO (*Persea americana* Mill.) GERMPLASM COLLECTIONS DETERMINED BY ISSRs

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ABSTRACT

The present study was undertaken in order to asses the existing genetic diversity within a germplasm collection of avocado (*Persea americana* Mill.) kept at the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) Campo Experimental Uruapan (CEFAP-Uruapan). The parental relationship among 77 accessions (231 plants) of the Mexican race was investigated by using seven Inter Simple Sequence Repeat microsatellites (ISSRs). A total of 154 loci were detected. Percentage polymorphism ranged from 82.3 to 95.4, with number of bands ranging from 17 to 25 within accessions. Genetic similarity analysis revealed formation of two major groups, one with eleven subgroups and the other one with three subgroups. Genetic similarity was highest between accession 237 (Atlixco, Puebla) and accession XTC01 (Uruapan, Michoacan), while accessions 532 (Atlixco, Puebla) and 369 (Chilchota, Michoacan) were the most dissimilar ones. None of the accessions were found to be duplicates. In general, the present study demonstrated the usefulness of ISSRs analysis for determination of genetic diversity in avocado.

ADDITIONAL KEY WORDS: microsatellites, germplasm, genetic similarity.

VARIABILIDAD GENÉTICA DENTRO DE LA RAZA MEXICANA DE AGUACATE (*Persea americana* Mill.) DETERMINADA POR ISSRs

RESUMEN

El presente estudio se efectuó para determinar la existencia de diversidad genética dentro de una colección de germoplasma de aguacate (*Persea americana* Mill.) perteneciente al Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental Uruapan (CEFAP-Uruapan). Se investigó la relación parental entre 77 accesiones (231 plantas) de la raza Mexicana con el uso de siete ISSRs (Inter Simple Sequence Repeat microsatellites, en inglés). En total se detectaron 154 *loci*. El porcentaje de polimorfismo varió de 82.3 a 95.4, con un número de bandas entre 17 y 25 dentro de las accesiones. El análisis de similitud genética reveló la formación de dos grupos principales, uno con once subgrupos y el otro con tres subgrupos. La similitud genética fue más alta entre las accesiones 237 (Atlixco, Puebla) y XTC01 (Uruapan, Michoacán), mientras que las accesiones 532 (Atlixco, Puebla) y 369 (Chilchota, Michoacán) fueron las más disímiles. No se encontraron duplicados en los genotipos analizados. En general, el presente estudio demostró la utilidad del análisis mediante ISSRs para la determinación de diversidad genética en aguacate.

PALABRAS CLAVE ADICIONALES: microsatélites, germoplasma, similitud genética.

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INTRODUCTION

Avocado (*Persea americana* Mill.) is grown under diverse environmental conditions all over the world. Mexico is the world's leading producer of this crop, where 105,477 ha and total production of 1.134 MMT were reported in 2006 with 84 % of avocado acreage occurring in the state of Michoacán. The several activities involved in the production chain of avocado generates over 40,000 permanent and over 60,000 seasonal jobs each year in Michoacán (Guillén-Andrade *et al*., 2007).

Since avocado is an outcrossing species, great genetic diversity has been generated throughout the evolution process of this crop. Natural selection first and man-driven selection later has lead to the development of genotypes adapted to diverse environments (Ben Ya'acov *et al*., 1992a, b and c). Therefore, the opportunities to exploit the existing genetic resources of avocado are almost endless (Bergh, 1992; Schieber and Zentmyer, 1992). Of the three major horticultural groups existing in avocado (Scora and Bergh, 1992), the Mexican race outstands for its high (15 to 20 %) oil content (Knight, 2002), making individuals within this race attractive for breeding purposes. Although the genetic diversity within the Mexican race is thought to be wide, the lack of information with regards to characterization and correct identification of germplasm collections somehow limits the efforts on genetic improvement of avocado in México.

Molecular markers have proven to be useful in clarifying genetic relationship among individuals in avocado germplasm. Techniques include both minisatellites (Davis *et al*., 1998; Fiedler *et al*., 1998; Lavi *et al*., 1991; Librada-Alcaraz and Hormaza, 2007; Mhameed *et al*., 1996) and microsatellites (Rodriguez *et al*., 2007; Shnell *et al*., 2003; Shnell *et al*., 2007). In addition to germplasm characterization, genetic maps have been constructed using molecular markers (Sharon *et al*., 1997; Viruel *et al*., 2007). In relation with other crops, however, the use of molecular marker technology for use in avocado is still scarce. Therefore, the objective of this study was to generate information at the DNA level regarding the genetic diversity of 77 Mexican race avocado accessions kept at the Field Experiment Station at Uruapan (CEFAP-Uruapan) germplasm collection belonging to the National Institute for Forestry, Livestock and Agricultural Research (INIFAP).

MATERIALS AND METHODS

Genetic material. Leaf material of a total of 231 plants representing 77 accessions of avocado (*Persea americana* Mill.) belonging in the Mexican race were used in the present study. Description of the plant material is presented in Table 1.

DNA isolation. DNA extraction was performed on 400 mg samples of leaf tissue according to the procedure

TABLE 1. Avocado (*Persea americana* Mill.) accessions within the mexican race included in the present study.

Núm.	ID	Location	Municipality, State
1	237	Atlixco	Atlixco, Puebla
2	247	San Pablo Etla	San Pablo Etla, Oaxaca
3	305	Huauchinango	Huauchinango, Puebla
4	308	Chiconautla	Huauchinango, Puebla
5	314	San Miguel	Zacatlán, Puebla
6	315	San Miguel	Zacatlán, Puebla
7	320	Ahuacatlán	Ahuacatlán, Puebla
8	321	Xalacapan	Zacapoaxtla, Puebla
9	322	Xalacapan	Zacapoaxtla, Puebla
10	326	San Juan Tetexco	Teziutlán, Puebla
11	327	San Sebastián	Teziutlán, Puebla
12	331	Jalacingo	Jalacingo, Veracruz
13	332	Jalacingo	Jalacingo, Veracruz
14	334	Altotonga	Altotonga, Veracruz
15	336	Altotonga	Altotonga, Veracruz
16	337	Altotonga	Altotonga, Veracruz
17	356	Aguascalientes	Aguascalientes, Aguascalientes
18	362	Calvillo	Calvillo, Aguascalientes
19	369	Cañada	Chilchota, Michoacán
20	370	Cañada	Chilchota, Michoacán
21	371	Cañada	Chilchota, Michoacán
22	435	Comonfort	Comonfort, Guanajuato
23	506	Valle de Bravo	Valle de Bravo, México
24	508	Tepalcatepec	Tenancingo, México
25	509	Tecomatlán	Tenancingo, México
26	511	Acazingo	Tenancingo, México
27	513	Malinalco	Malinalco, México
28	514	Malinalco	Malinalco, México
29	516	Tetela del Volcán	Tetela del Volcán, Morelos
30	519	Tlalama	Ozumba de Zárate, México
31	520	Tlalama	Ozumba de Zárate, México
32	524	Comonfort	Comonfort, Guanajuato
33	525	Comonfort	Comonfort, Guanajuato
34	532	Atlixco	Atlixco, Puebla
35	533	Atlixco	Atlixco, Puebla
36	534	Atlixco	Atlixco, Puebla
37	535	Atlixco	Atlixco, Puebla
38	538	Mariscala	Tepexi de Rodríguez, Puebla
39	541	Xalostepec	Tepexi de Rodríguez, Puebla
40	542	Santa Cruz	San Vicente Coyote, Puebla
41	543	Santa Cruz	San Vicente Coyote, Puebla
42	546	Totoltepec	Totoltepec, Puebla
43	551	Ocotlán	Ocotlán, Oaxaca
44	583	Patamban	Tangancicuaro, Michoacán
45	588	Puebla	Puebla, Puebla
46	632	Aramberri	Aramberri, Nuevo León
47	633	Aramberri	Aramberri, Nuevo León
48	635	Aramberri	Aramberri, Nuevo León
49	640	San Felipe	Zitácuaro, Michoacán

50	642	Santa María P.	Valle de Bravo, México
51	643	Tenancingo	Tenancingo, México
52	644	Tenancingo	Tenancingo, México
53	645	San José Tenería	Tenancingo, México
54	646	Malinalco	Malinalco, México
55	647	Ozumba	Ozumba de Zarate, México
56	648	Tetela del Volcán	Tetela del Volcán, Morelos
57	649	Teposcolula	Teposcolula, Oaxaca
58	650	Achiutla	San Juan Achiutla, Oaxaca
59	652	San Pablo Etla	San Pablo Etla, Oaxaca
60	666	San Pedro Chenalho	Chenalho, Chiapas
61	667	San Juan Chamula	Chamula, Chiapas
62	667	San Juan Chamula	Chamula, Chiapas
63	670	Huixtla	Huixtla, Chiapas
64	677	Carapan	Chilchota, Michoacán
65	678	Carapan	Chilchota, Michoacán
66	679	Carapan	Chilchota, Michoacán
67	680	Tingambato	Tingambato, Michoacán
68	681	Uruapan	Uruapan, Michoacán
69	687	El Diez	Tuxtla Chico, Chiapas
70	690	El Diez	Tuxtla Chico, Chiapas
71	724	La Mixtequilla	Alvarado, Veracruz
72	725	La Mixtequilla	Alvarado, Veracruz
73	728	Cunduacán	Cunduacán, Tabasco
74	729	Cunduacán	Cunduacán, Tabasco
75	740	Tacámbaro	Tacámbaro, Michoacán
76	XTCO1	Uruapan	Uruapan, Michoacán
77	XTRO2	Uruapan	Uruapan, Michoacán

described by Clarke *et al*. (1989). Isolated DNA was quantified on a DR/4000 U spectrophotometer (HACH™) by using 40 µL DNA and 1960 µL TE solution of each sample. Readings were made at 260 and 280 nanometers length waves. DNA was standardized at 200 µM and then stored at 4 ºC until use.

Microsatellite markers and DNA amplification. Seven inter simple sequence repeat (ISSR) markers (Zietkiewickz *et al*., 1994) were used in the present study. Microsatellite number and primer sequence are listed in Table 2. PCR amplification reactions were carried out in a total volume of 20 µL which consisted of 4.0 µL genomic DNA (40 ng), 1.5 mL dNTP mixture (187 mM each dNTP), 2.0 µL ISSR primer (1.0 mM), 2.2 µL 1X Taq buffer, 0.2 µL DNA Taq polymerase (1 U·mL^{-1}), 0.8 µL $MgCl_2$ (2.0 mM), and 9.3 µL ddH_2O. Thermal cycling profile consisted of the following: initial heating at 94 ºC for 4 min, 35 cycles of denaturing at 94 ºC for 1 min, annealing at 52 ºC for 1 min, extension at 72 ºC for 2 min, and a final extension for 5 min at 72 ºC. PCR was carried out on a MJ Research (DNA Engine Tetrad®) thermoclycer. Primer pairs were run individually.

Electrophoresis. Polyacrylamide gels (37.5:1) at 6 % were use on a 38 x 50 cm vertical Sequio-Gen GT (BIO-RAD®) electrophoresis system. Buffer solution consisted of 1X TBE (0.09 M Tris-boric acid and 2 mM EDTA). As polymerizing agents, 25 % ammonium persulfate and 0.11 % TEMED® were added to achieve a final concentration of 0.028 %. For every 20 µL amplification product 5 µL 5X sample gel buffer-xilene cyanole staining solution (1M Tris pH 8.0, 0.5 M EDTA pH 8.0, 25 % sucrose, bromophenol blue, 2 mg·mL^{-1}, and xilene cyanole 2 mg·ml^{-1}) were added; approximately 7 µL of each sample were loaded and then run at 120 W for 3 h. Detection of amplified fragments was achieved by staining with silver nitrate.

Data analysis. Band number (alleles) and degree of polymorphism, revealed by each microsatellite marker (locus) on the polyacrylamide gels, for each avocado accession were scored on the basis of the presence (1) or absence (0) of band at each locus of the sample. A 0 and 1 matrix was created for computing Nei and Li distances (Nei and Li, 1979). Molecular data were recorded by the HyperMap Data software (Hoisington, 1993). Genetic similarity coefficients were calculated by using the Numerical Taxonomy and Multivariate Analysis System (NTSYS-PC) software, version 2.02 (Rohlf, 1993). Genetic distances were calculated with the help of the Similarity Genetic Data (SIMGEND) procedure. Dendrograms were constructed with the Sequential, Agglomerative, Hierarchical, and Nested (SAHN) clustering method (Sneath and Sokal, 1973).

RESULTS AND DISCUSSION

Due to the high level of polymorphism observed in preliminary studies, only seven ISSR markers were used in the analysis of seven avocado accessions collected in seven states of the Mexican Republic. There were 154 polymorphic fragments (alleles) in total; the number of bands and the polymorphism information content (PIC) produced by each primer ranged from 17 to 25 and 82 to 95 %, respectively (Table 2). A polymorphic primer was one which presented at least one different band among the 77 genotypes; that

TABLE 2. Level of polymorphism obtained with seven ISSR markers in 231 individuals of 77 Mexican race avocado (*Persea americana* Mill.) accessions.

Núm.	[z]Primer	Number of total bands	Number polymorphic bands	PIC
1	HVH$(CA)_7$T	19	19	0.928893
2	$(CT)_8$RC	17	17	0.934349
3	$(AG)_8$G	22	22	0.94285
4	$(CA)_8$[a]	22	22	0.948197
5	$(GA)_8$C	25	25	0.953975
6	$(AG)_8$C	25	25	0.953927
7	$(AG)_8$T	24	24	0.822833

[z]R = purine residue anchor, Y = irimidine, B = any but adenine, D = any but cytosine, H = any but timine, C = cytosine y G = guanine PIC= polymorphism information content.

is, polymorphic bands were those that were missing in at least one of the genotypes analyzed. An example of the allelic diversity is given in Figure 1 for the $(AG)_8C$ primer. Highest band number (25) and percentage polymorphism (95.4 %) were obtained by both $(AG)_8C$ and $(GA)_8C$ primers. On the other hand, primer $(CT)_8RC$ produced the lowest band number (17) while primer $(AG)_8T$ had the lowest PIC value (82.3 %). In general, the high PIC value (92.64 %) indicates, on one hand, the existence of great genetic variability within the analyzed avocado accessions; in the other hand, these findings also indicate the usefulness of ISSRs as a tool in distinguishing genotypes with diverse origins. However, the loci detected in this study were not associated with any morphological trait, an approach that would be worth taking into consideration for future studies.

The level of polymorphism observed in this work was in agreement with results found in other studies (Fang and Roose, 1997; Guillén-Andrade *et al*., 2000; Salimath *et al*., 1995; Wolff *et al*., 1995; Yamamoto *et al*., 2007) in which the same type of primers have been used. Wang *et al.* (1994) point out that in plants the most common microsatellite sequences are the dinucleotides $(AT/AT)_n$, followed by the sequences $(GT/TG)_n$. In grapes, the most common sequences were the dinucleotides $(CA/GT)_n$ (Thomas and Scott, 1993). In the present work, based on the number of primers and the number of bands generated by them, the most common sequences found in the avocado accessions belonging in the Mexican race were the dinucleotides $(AG/AC)_n$ and $(CT/RC)_n$; the number of bands found in this work, however was lower in relation with the number of bands found in other studies (Fang and Roose, 1997; Prevost and Wilkinson, 1999).

Genetic differentiation among and relatedness of accessions

ISSR data were used to generate a genetic distance matrix. Genetic distance values among accessions ranged from cero (closely-related material) to one (none-related material). From the genetic distance (Nei and Li, 1979) matrix a dendrogram was constructed (Figure 2). Two major groups were clearly distinguished. Group I was composed of 11 subgroups: subgroup I_a encompassed accessions collected in Puebla, Guanajuato, Veracruz, Michoacán, Oaxaca, and the State of Mexico; subgroup I_b was formed

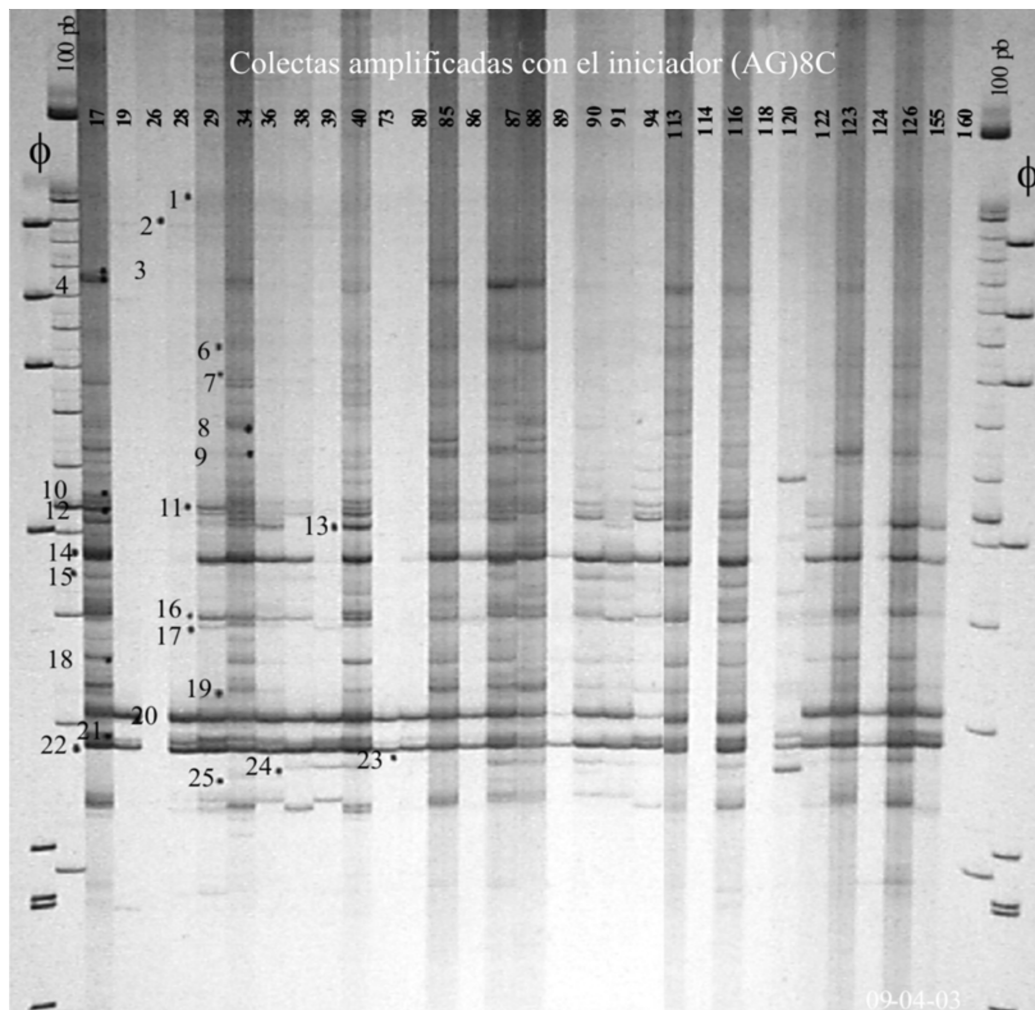

FIGURE 1. Genetic profile of Mexican race avocado accessions obtained by the $(AG)_8C$ inter simple sequence repeat primer.

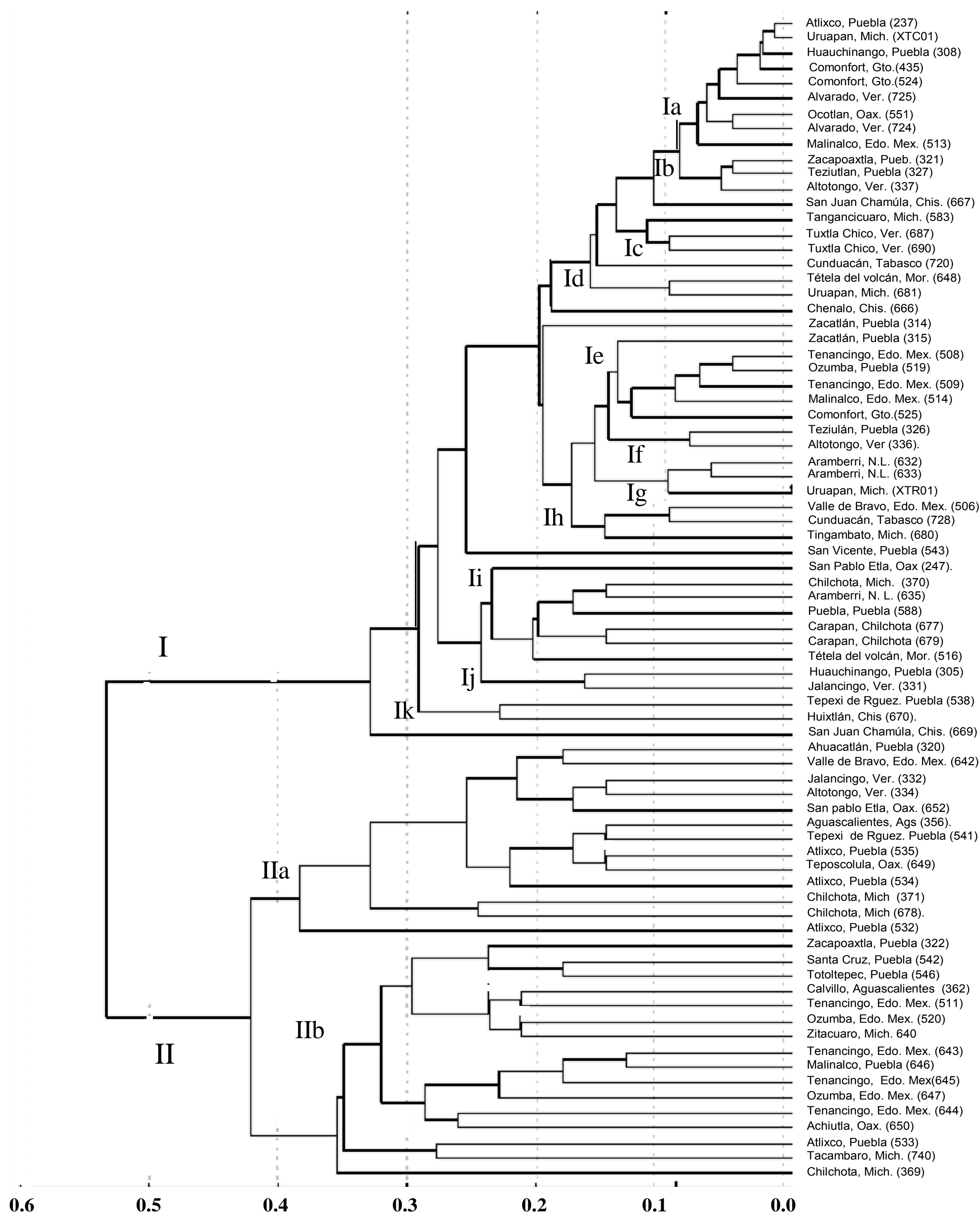

FIGURE 2. Dendrogram of 77 avocado (*Persea americana* Mill.) accessions within the Mexican race generated by seven inter simple sequence repeat primers.

by accessions of Veracruz and Puebla; subgroup I_c shared a Chiapas collection with subgroup I_b and was formed by Michoacán and Veracruz collections; subgroup I_d grouped two accessions, one collected in Morelos and the other one in Michoacán and it also was related to I_c by a Chiapas collection; in subgroup I_e two collections of Puebla, three of the State of México and one of Guanajuato were associated; subgroup I_f was made up by one collection each of Puebla and Veracruz; subgroup I_g grouped two collections of Nuevo Leon and one of Michoacán; in subgroup I_h two collections of Michoacán and one of the State of México were involved; subgroup I_i was made up with three collections of Michoacán,

one of Oaxaca, one of Nuevo Leon, one of Puebla, and one of Morelos, and also shared a collection with Puebla; subgroup I_j was formed by Puebla and Veracruz collections; and subgroup I_k grouped one collection each of Puebla and Chiapas. We must point out that in subgroup I_a the greatest genetic similarity between genotypes was for accessions 237 (Atlixco, Puebla) and XTC01 (Uruapan, Michoacán).

In Group II a more clearly distinguishable association among accessions was observed; three subgroups were formed. Subgroup II_a grouped accessions collected in Chiapas, Puebla, State of México, Veracruz, Oaxaca, Aguascalientes, and Michoacán. In this subgroup highest genetic similarity was for accessions 332 (Jalancingo, Veracruz), 334 (Altotongo, Veracruz), 356 (Aguascalientes, Aguascalientes), 541 (Xalostepec, Puebla), 535 (Atlixco, Puebla), and 649 (Teposcolula, Oaxaca). Subgroup II_b was made up with accessions of Puebla, Aguascalientes, State of México, Michoacán, and Oaxaca, with all seven accessions collected in the State of Mexico being the most predominant ones. In the end, accessions 532 (Atlixco, Puebla) and 369 (Chilchota, Michoacán) had the greatest genetic dissimilarity.

The usefulness of ISSR microsatellites to detect genetic variation, even among closely related individuals, was confirmed in this research and was in agreement with other works in avocado (Clegg *et al*., 1999; Librada-Alcaraz and Hormaza, 2007; Rodríguez *et al*., 2007; Schnell *et al*., 2007) and other crops (Nybom and Hall, 1991; Yamamoto *et al*., 2007). It is confirmed that ISSRs overcome technical limitations of RFLP and RAPD markers (Prevost and Wilkinson, 1999; Ratnaparkhe *et al*., 1998), with results similar to those obtained with AFLPs (Lanham and Brennam, 1999; Viruel *et al*., 2007).

The polymorphism detected in this work with the ISSR markers is an indication of the high genetic variation existing among avocado accessions within the Mexican race. This can be explained by the high interest in exploiting avocado germplasm for commercial use, mainly in central and southern México, generating a constant movement of this species throughout the Country in seek of new production areas. The diversifying soil and climatic conditions of this Country plus the fact that avocado is an outcrossing species have all favored a certain degree of genetic variation within his species. These results clearly indicate the wide genetic variation existing in the avocado germplasm collection at the CEFAP-Uruapan experiment station. In addition, none of the accessions were found to be duplicates, therefore there is no need to discard any of the individuals; these should be kept and used for breeding purposes.

LITERATURE CITED

BEN YA'ACOV, A.; BUFLER, G.; BARRIENTOS-PRIEGO, A. F.; DE LA CRUZ TORRES, E.; LÓPEZ LÓPEZ, L. 1992a. A study of avocado germplasm resources, 1988-1990. I. General description of the international project and its findings. Proc. 2nd World Avocado Congress. Orange, Cal., USA. pp. 535-541.

BEN YA'ACOV, A.; LÓPEZ LÓPEZ, L.; DE LA CRUZ TORRES, E.; BARRIENTOS P., A. F. 1992b. A study of avocado germplasm resources, 1988-1990. II. Findings from the central part of México. Proc. 2nd World Avocado Congress. Orange, Cal., USA. pp. 543-544.

BEN YA'ACOV, A.; MICHELSON, E.; ZILBERSTAINE, M.; BARKAN, Z.; SELA, I. 1992c. Selection of clonal avocado rootstocks in Israel for high productivity under different soil conditions. Proc. 2nd World Avocado Congress. Orange, Cal., USA. pp. 521-526.

BERGH, B. O. 1992. The origin, nature, and genetic improvement of the avocado. Calif. Avocado Soc. Yearbook 76: 61-75.

CLARKE, B. C.; MORAN, L. B.; APPELS, R. 1989. DNA analysis in wheat breeding. Genome 32: 334-339.

CLEGG, M. T.; KOBAYASHI, M.; LIN, J. Z. 1999. The use molecular markers in the management and improvement of avocado (*Persea americana* Mill.). Revista Chapingo Serie Horticultura 5: 227-231.

DAVIS, J.; HENDERSON, D.; KOBAYASHI, M.; CLEGG, M. T; CLEGG, M. T. 1998. Genealogical relationships among cultivated avocado as revealed through RFLP analysis. J. Hered. 89(4): 319-323.

FANG, D. Q; ROOSE, M. L. 1997. Identification of closely related citrus cultivars whit inter-simple sequence repeat markers. Theor. Appl. Genet. 95: 408-417.

FIEDLER, J.; BUFLER, G.; BANGERTH, F. 1998. Genetic relationships of avocado (*Persea americana* Mill.) using RAPD markers. Euphytica 101: 249-255.

GUILLÉN-ANDRADE, H.; MONTALVO H., L.; MONTERO T., V.; MEDERO S., O. A.; ACOSTA G., J. A. 2000. Etiquetado del gen de respuesta al fotoperiodo en frijol común mediante ISSRs y RAPDs. Memoria del XVIII Congreso Nacional de Fitogenética. Zavala, G. F.; ORTEGA, P. J.; MEJÍA, J. A.; BENITEZ, C. I.; GUILLÉN-ANDRADE, H. (eds). 2000. Notas Científicas. SOMEFI. Chapingo, México. p. 308.

GUILLÉN-ANDRADE, H.; LARA-CHÁVEZ, B. N.; GUTIÉRREZ-CONTRERAS, M.; ORTÍZ-CATÓN, M.; ÁNGEL-PALOMARES, M. E. 2007. Cartografía Agroecológica del Cultivo del Aguacate en Michoacán. Morevallado Editores de Morelia, Michoacán, México 141 p.

HOISINGTON, D. A.; GONZÁLEZ DE LEÓN, D. 1993. HyperMapData. A HyperCard stack for entry and analysis of molecular genetic segregation data. Agronomy abstracts. Annual meetings, Cincinnati, Ohio, USA. November 7-12. 1993.

KNIGHT, R. J. 2002. History, distribution and uses, pp. 1-15. *In*: The Avocado, Botany, Production and Uses. A. WHILEY, B. SCHAFFER, AND B. WOLSTENHOLME (eds.). CAB Intl., New York. p. 1-14.

LANHAM, P. G.; R. M. BRENNAN. 1999. Genetic characterization of gooseberry (*Ribes grossularia* subgenus *grossularia*) germplasm using RAPD, ISSR and AFLP markers. J. Hort. Sci. Biotech. 74(3): 361-366.

LAVI, U.; HILLEL, J.; VAINSTEIN, A.; LAHAV, E.; SHARON, D. 1991. Application of DNA fingerprints for identification and genetic analysis of avocado. J. Amer. Soc. Hort. Sci. 116:1078-1081.

LIBRADA-ALCARAZ, M.; HORMAZA, J. I. 2007. Molecular characterization and analysis of genetic diversity in 75 avocado accessions using SSRs. Proc. VI World Avocado Congress,

Viña del Mar, Chile. 12-16 November 2007. 54 p.

MHAMEED, S.; SHARON, D.; HILLEL, J.; LAHAV, E.; KAUFMAN, D.; LAVI, U. 1996. Level of heterozygosity and mode of inheritance of variable number of tandem repeat loci in avocado. J. Amer. Soc. Hort. Sci. 121: 778-782.

NEI, M.; LI, W. H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Nat. Acad. Sci. 76: 5269-5273.

NYBOM, H.; HALL, H. K. 1991. Minisatellite DNA “fingerprints” can distinguish *Rubus* cultivars and estimate their degree of relatedness. Euphytica 53: 107-114.

PREVOST, A.; WILKINSON, M. J. 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theor. Appl. Genet. 98: 107-112.

RATNAPARKHE, M. B.; TEKEOGLU, M.; MUEHLBAUER, F. J. 1998. Inter simple secuence repeat (ISSR) polymorphisms as useful for finding markers associate with disease resistance genes clusters. Theor. Appl. Genet. 97: 515-519.

RODRÍGUEZ, N. N.; FUENTES, J. L.; COTO, O.; FUENTES, V. R.; RAMÍREZ, I. M.; BECKER, D.; RODRÍGUEZ, I.; GONZÁLEZ, C.; XIQUÉS, S.; ROMÁN, M. I.; VELÁZQUEZ, B.; RHODE, W.; JIMÉNEZ, N. 2007. Comparative study of polymorphism level, discrimination capacity and informativeness of AFLP, ISTR, SSR and Isoenzymes markers and agro-morphological traits in avocado. Proc. VI World Avocado Congress, Viña del Mar, Chile. 12-16 November 2007. p 76.

ROHLF, J.F. 1993. NTSYS-pc. Numerical taxonomy and multivariate analysis system. Exceter Softwer, New York, USA. 157 p.

SALIMATH, S. S.; OLIVEIRA, A. C.; GODWIN, I. D.; BENNETZEN, J. L. 1995. Assessment of genome origins and genetic diversity in the genus *Eleusine* with DNA markers. Genomes 38: 757-776.

SCORA, R.W.; BERGH, B.O. 1992. Origin of and taxonomic relationship with the genus *Persea*. Proc. 2nd World Avocado Congress. Orange, Cal. 2: 505-514.

SCHIEBER, E.; ZENTMYER, G. A. 1992. Archeology of the avocado in Latin America. Proc. 2nd World Avocado Congress. Orange, Cal., USA, pp. 49.

SCHNELL, R. J.; BROWN, J. S.; OLANO, C. T.; POWER, E. J.; KROL, C. A.; KUHN, D. N.; MOTAMAYOR, J. C. 2003. Evaluation of avocado germplasm using microsatellite markers. J. Amer. Hort. Sci. 128: 881-889.

SCHNELL, R.J.; BROWN, J.S.; OLANO, C.T.; AYALA-SILVA, T.; KUHN, D.N.; MOTAMAYOR, J.C. 2007. Evaluation of avocado germplasm using microsatellite markers. Proc. VI World Avocado Congress, Viña del Mar, Chile. 12-16 November 2007. p. 48.

SHARON, P.; CREGAN, B.; MHAMEED, S.; KUSHARSKA, M.; HILLET, J.; LAHAV, E.; LAVI, U. 1997. An integrated genetic map avocado. Theor. Appl. Genet. 95: 911-921.

SNEATH, P.H.A.; SOKAL, R.R. 1973. Numerical Taxonomy - The Principles and Practice of Numerical Classification. W. H. Freeman, San Francisco. USA.

THOMAS, M.R.; SCOTT, N. S. 1993. Microsatellite repeats in grapevine reveal DNA polymorphism when analyzed as sequence-tagged sites (STSs). Theor. Appl. Genet. 86: 985-990.

VIRUEL, M. A.; GROSS, E.; BARCELÓ-MUÑOZ, A. 2007. Development of a linkage map with SSR and AFLP markers in avocado. Proc. VI World Avocado Congress, Viña del Mar, Chile, 12-16 November 2007. p. 52.

WANG, Z.; WEBER, J. L.; ZHONG, G.; TANKSLEY, S. D. 1994. Survey of plant short tandem DNA repeats. Theor. Appl. Genet. 88: 1-6.

WOLFF, K.; ZIETKIEWIKCZ, E.; HOFSTRA, H. 1995. Identification of chrysanthemum cultivars and stability of DNA fingerprint patterns. Theor. Appl. Genet. 91: 439-447.

YAMAMOTO, M.; TOMITA, T.; ONJO, M.; ISHIHATA, K.; KUBO, T.; TOMINAGA, S. 2007. Genetic diversity of white sapote (*Casimiroa Edulis* la Llave & Lex.) demonstrated by intersimple sequence repeat analysis. HortScience 42: 1329-1331.

ZIETKIEWICKZ, E.; RAFALSKI, A.; LABUDA, D. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchores polymerase chain reaction amplification. Genomics 20: 176-183.