Theor Appl Genet (1997) 94: 279–286

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Genetic relationships within avocado (*Persea americana* Mill) cultivars and between *Persea* species

Received: 3 August 1996 / Accepted: 23 August 1996

Abstract The origin of avocado (*Persea americana* Mill) cultivars, as well as the genetic relationships between *Persea* species, are not well defined and are based mainly on morphological parameters. Minisatellite DNA markers were used to analyze 24 *P. americana* cultivars in an attempt to define their racial allocation. DNA mixes representing the three races were evaluated and used for analysis. The allocation of 19 of the above cultivars was substantiated by the DNA markers, while new suggestions were offered regarding the remaining five. Eight cultivars, of unknown racial origin, were also examined, and a phylogenetic tree suggesting their origin is offered. Selfing progeny of five cultivars were analyzed for six morphological traits which differentiate the three races, and were compared to their parents in order to assess their origin. Eleven *Persea* species were analyzed, using DNA fingerprint patterns and SSR (simple sequence repeat) alleles, in order to identify the genetic relationships among the *Persea* species, and between them and the three *P. americana* races. The phylogenetic tree obtained is presented. The high value of variation between the avocado cultivars and *Persea* species observed in this work, suggests that the validity of race and species definition within *Persea* be treated with caution.

Communicated by R. Hagemann

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Key words Avocado · DNA fingerprints · Microsatellite · Minisatellite · Phylogenetic tree

Introduction

The commercial avocado (*Persea americana* Mill.) belongs to the *Persea* subgenus of the genus *Persea*, which also contains the subgenus *P. drymifolia*. Kopp (1966) described 81 *Persea* species belonging to the same subgenus and indicated that *P. schiedeana, P. floccosa* and *P. americana* are strongly related. However, subsequent observations (Bergh and Ellstrand 1986) suggested that all members except *P. schiedeana* may best be classified as belonging to a single polytypic species: *P. americana* Mill. This classification was recently supported by Ben-Ya'acov (1995) who argued that the various groups of plants belonging to the sub-genus *Persea* (excluding *P. schiedeana*) be best considered as sub-species, or botanical varieties, of *P. americana*. All *Persea* species examined, except *P. hintonii* (2n=48; Garcia 1975), have a chromosome number of 2n=24.

Based on their presumed centers of origin, the species *P. americana* has been divided into three distinguishable horticultural races (Popenoe 1941), known as the Mexican, Guatemalan and West-Indian ("Antillean") races and which have been respectively designated as *P. americana* var. *drymifolia*, *P. americana* var. *guatemalensis* and *P. americana* var. *americana* (Bergh and Ellstrand 1986). These taxonomic conclusions have been strengthened during the past decade by the analysis of genetic markers including isozymes, DNA fingerprints, and RFLPs (Goldring et al. 1985; Torres and Bergh 1980; Lavi et al. 1991; Furnier et al. 1990). Bergh and Lahav (1996) indicated that the three races are about equally distinct from each other. However, Kopp (1966) using morphological parameters, and Bufler and Ben-Ya'acov (1992) working with DNA markers, grouped the Guatemalan and West-Indian races into one botanical variety distinct from the Mexican race variety. Kopp (1966) also suggested that the species *P. lon-*

gipes, *P. schiedeana*, *P. cinerascens* and *P. floccosa* are closer to the Mexican race than to the other two races, and that *P. nubigena* and *P. steyermarkii* Allen have a similarity to the Guatemalan race. Williams (1977) proposed that *P. americana* var. *guatemalensis* was selected from *P. nubigena*. Using RFLP markers, Furnier et al. (1990) support this hypothesis and suggest that the Guatemalan race is a hybrid between *P. steyermarkii* Allen and *P. nubigena*. Williams (1977) was of the opinion that the West-Indian race was selected from the Mexican race.

There are no known sterility barriers between the three races of *P. americana*. Hence, hybridization occurs readily wherever trees of different races are growing in proximity, whether indigenously (Popenoe and Williams 1947) or planted by man (Bergh 1969).

Most of the commercial avocado cultivars are racial hybrids (Popenoe and Williams 1947) and identification in terms of racial origin is difficult. However, the agricultural differences between the races remain of great horticultural importance. The two most similar races, in terms of fruit characteristics, are the Mexican and West-Indian races, but they are the most dissimilar in terms of general climatic adaptation. Hence, the main problem is in distinguishing between West-Indian and Guatemalan germ plasm in tropical regions, and between Mexican and Guatemalan germ plasm in less tropical areas (Williams 1977). In both cases, probably the most useful morphological criterion is maturity season. Other quite helpful criteria include, anise scent, colored lenticels on young flush, skin thickness and surface, and seed size (Bergh and Lahav 1996). All of these characters appear to be polygenically controlled (Lavi et al. 1993; Bergh and Lahav 1996). The determination of racial origin can be based not only on morphological features, but also on isozymes (Torres and Bergh 1980), RFLPs (Furnier et al. 1990), DNA fingerprints (Lavi et al. 1991), and SSR markers (Lavi et al. 1994).

In the present study, VNTR (Variable Number of Tandem Repeats) markers and genetic analysis were used to assess the genetic relationships between *P. americana* cultivars and races in order to obtain a phylogenetic tree of the *Persea* species.

Materials and methods

Plant material

Leaves were collected from the following trees grown at the Akko Experiment Station and the Agricultural Research Organization, Bet Dagan, Israel:

Mexican types – 'Aquila 1', 'Aquila 2', 'Azul', 'Banos', 'Gainsville', 'Guayabamba', 'Km 145', 'Shiller 1', 'Tezuitlan'. West-Indian types- 'Antigua', 'Galvan', 'Orizaba 1', 'Orizaba 4', 'San Sebastian 10', 'Tela 2'. Guatemalan types – 'Benik', 'Comitan 2', 'Comitan 3', 'Cuevas', 'N-151–2', 'Nabal', 'Reed', 'Shomrat'. Guatemalan×West-Indian hybrid – 'Semil 34'. (The above cultivars were morphologically allocated to the three races by the two avocado experts Dr. E. Lahav and Dr. A. Ben Ya'acov). Persea species- *P. borbonia*, *P. cinerascens*, *P. floccosa*, *P. gigantea*, *P. indica*, *P. longipes*, *P. nubigena*, *P. schiedeana*, *P. schiedeana* 05, *P. schiedeana* 06, *P. steyermarkii* Allen. Cultivars of unknown origin- 'Ettinger', 'Fuerte', 'Hass', 'Horshim', 'Maoz', 'No Race', 'Pinkerton', 'Rincon'.

Performance analysis

The selfing progeny of five cultivars, 'Anahiem', 'Ettinger', 'Nabal', 'Reed' and 'Rincon', were analyzed. Seeds were collected from trees that were caged under a net in the presence of a bee hive. The harvested seeds were sown in a nursery, and 1 year later were transplanted to the breeding plots. In all cases, seedlings were analyzed only when proved by isozyme analysis to be self progenies (Goldring et al. 1987). All selfing progenies, except 'Rincon', included a low percentage of outcrossing. In the case of 'Rincon' the rate of outcrossing was high. The following isozyme loci, each homozygous for a certain cultivar, were used. In 'Anahiem': glutamate oxaloacetate transaminase (GOT-1; EC 2.6.1.1), leucine aminopeptidase (LAP-2; EC 3.4.11.1), malate dehydrogenase (MDH-1; EC 1.1.1.37), phosphoglucomutase (PGM-1; EC 2.7.5.1), and triosephosphate isomerase (TPI-1; EC 5.3.1.1). In 'Ettinger': GOT-1 and MDH-1. In 'Nabal': LAP-2, PGM-1, MDH-1, and TPI-1. In 'Reed': LAP-2, PGM-1, and TPI-1. In 'Rincon': LAP-2, PGM-1, and MDH-1. Each seedling was characterized by 41 traits of which six were selected as the most useful criteria for race identification. These were: anise scent of the leaves, colored lenticels on young shoots, start of harvest season, skin thickness, skin surface, and seed weight (percent of fruit weight).

DNA isolation

DNA was isolated from very young leaves as described by Murray and Thompson (1980), with the following modifications (Lavi et al. 1991): extraction buffer 2% cetyltrimethyl ammonium bromide (CTAB), 0.1 M Tris-HCl pH=8, 0.02 M ethylenediamine tetra acetic acid (EDTA), 1.4 M NaCl and 1% 2-mercaptoethanol. Chloroform extractions were carried out three times and 2 vol of precipitation buffer were added to the final aqueous phase. The precipitation buffer was: 1% CTAB, 0.05 M Tris-HCl pH=8, 0.01 M EDTA, and 1% 2-mercaptoethanol. Nucleic acids were dissolved in 1 M CsCl, precipitated with ethanol, and re-dissolved in TE (10 mM Tris-HCl pH=8, 1 mM EDTA). Three additional extractions were made with phenol, phenol-chloroform, and chloroform. The DNA was precipitated with ethanol and dissolved in TE. DNA concentration was evaluated by a spectrophotometer at 260 nm or viewed on an ethidium bromide-stained agarose gel. DNA mixes were made by combining equal amounts of DNA from each sample.

DNA fingerprinting (DFP)

Ten micrograms of DNA were digested with *Hinf*I restriction endonuclease according to the manufacturer's recommendations (New England Biolabs, Beverly, Mass.). Electrophoresis was carried out on a 20-cm long 0.8% agarose gel in TBE buffer (0.045 M Tris borate, 0.001 M EDTA pH=8.0) at 1.5 V/cm for 40 h. Gels were Southern-blotted onto Hybond N^+ membranes (Amersham International, Amersham, UK). Lambda *Hin*dIII Fragments (New England Biolabs, Beverly, Mass.) served as size markers.

Membranes were hybridized to the double-stranded probe 22.3, a 2-kb probe that includes probe R18.1 (Haberfeld et al. 1991) labelled with α^{32}P-dCTP by random priming (Feinberg and Vogelstein 1983). Pre-hybridization and hybridization were carried out at 65°C for 3 h to 5 h and 16 h, respectively, in 0.263 M Na-phosphate buffer with 7% sodium dodecyl sulphate (SDS), 1 mM EDTA, and 1% bovine serum albumin (BSA).

Membranes were washed with 0.263 M Na-phosphate and 1% SDS for 20 min, then twice with 2×saline sodium citrate (SSC) and 0.1% SDS for 20 min each, and twice with 1×SSC and 0.1% SDS for 20 min each. Washes were carried out at 65°C. Membranes were autoradiographed for 1 to 10 days at –80°C with intensifying screens.

PCR amplifications

PCR amplifications were performed in a total volume of 10 µl, containing 30 ng DNA, 1.5 mM Mg^{2+}, 0.15 µM of each of the two primers, 200 mM of each of the four nucleotides, 2 mM of dUTP, 1× *Taq* buffer (containing 50 mM Tris HCl pH=9, 0.1% Triton X–100) and 1 unit of *Taq* DNA polymerase (Promega, Madison, WIS., USA). Thirty microliters of mineral oil were placed on top of the reaction. After a pre-warming step of 95°C for 30 s, 32 cycles of denaturation at 95°C for 15 s, annealing at 45°C or 50°C (depending on the primers) for 25 s, and extension at 68°C for 25 s were performed, followed by an extension stage of 2 min. A 1-µl aliquot of each PCR reaction was mixed with loading buffer containing 6 fmol of internal size standard TAMRA (Applied Biosystems International, ABI, Foster City, Calif., USA) and loaded on a 6% polyacrylamide gel, containing 8 M urea and 1×TBE. Fluorescent PCR products were automatically sized using the associated GENESCAN™ software (Applied Biosystems International, ABI, Foster City, Calif., USA). In a few cases a radioactive SSR reaction was performed as well, using 0.1 µl of 3000 Ci/mmol α^{32}P-dCTP. Ten microliters of stop solution (95% formamide, 0.02 M EDTA pH=8, 1% bromophenol blue, 1% xylene cyanol 10 mM NaOH) were added at the end of the reaction, and 3 µl were loaded on a DNA sequencing gel containing 6% polyacrylamide, 8 M urea and 1×TBE at 50 W for 3–4 h. Gels were dried and exposed over-night to a Fuji X-ray film.

Data analyses

Only DNA fragments that could be reliably and consistently evaluated in each sample were analyzed. DNA fingerprint fragments range between 2 and 22 kb, and SSR fragments range between 97 and 182 bp. Genetic distances between cultivars and races were evaluated by two methods: first we calculated the 'd' value (Nei and Li 1979), which is based on the average number of nucleotide changes in a restriction site, and formed UPGMA trees using the software 'd-value' (Gentzbittel and Nicolas 1991). Second, we formed phylogenetic trees using the maximum parsimony method with the 'PAUP' software (Swofford and Begle 1993) based on a matrix in which '1' represented the existence of a band, and '0' the absence of a band.

Results

DNA fingerprint patterns of 24 *P. americana* cultivars, allocated morphologically to the three races, were analyzed using the PAUP software. Each cultivar's band pattern was compared to the representative pattern of each race (obtained by the DNA mix of the cultivars morphologically allocated to the race). The results are shown in Table 1. The nine cultivars of supposed Mexican origin have a low number of changes (0–8) from the Mexican mix. However, 'Aquila 2' is closer to the Guatemalan mix (0.8 changes), and 'KM 145' is similar to the West-Indian mix (0.4 changes). Five of the six assumed West-Indian cultivars – 'Galvan', 'Orizaba 1', 'Orizaba 4', 'San Sebastian 10' and 'Tela 2' – showed no changes from the West-Indian mix. On the other hand, 'Antigua', allocated by its morphological traits to the West-Indian race, showed a close relationship with the Guatemalan mix (0 changes). Six of the eight

Table 1 Number of DFP band changes between 24 avocado cultivars and the three avocado races; M- Mexican, G- Guatemalan, WI- West-Indian

Race[a]	Cultivar	Number of DFP band changes[b] of each cultivar from:		
		Mexican mix	West-Indian mix	Guatemalan mix
M	'Aquila 1'	0	6.4	6.8
	'Aquila 2'	5	18.4	0.8
	'Azul'	8	20.4	28.8
	'Banos'	0	9.4	17.8
	'Gainsville'	3	15.4	21.8
	'Guayabamba'	1	13.4	19.8
	'Km 145'	0	0.4	15.8
	'Shiller 1'	1	13.4	21.8
	'Tezuitlan'	1	13.4	7.8
WI	'Antigua'	30	42.4	0
	'Galvan'	16	0	40.8
	'Orizaba 1'	23	0	47.8
	'Orizaba 4'	15	0	39.8
	'San Sebastian 10'	12	0	36.8
	'Tela 2'	3	0	27.8
G × WI	'Semil 34'	4	0	28.8
G	'Benik'	21	33.4	0
	'Comitan 2'	21	0	45.8
	'Comitan 3'	26	38.4	0
	'Cuevas'	0	0	23.8
	'N-151-2'	25	37.4	0
	'Nabal'	15	27.4	0
	'Reed'	13	25.4	0
	'Shomrat'	9	21.4	0

[a] The supposed racial origin was identified by two avocado experts (see Materials and methods) based on morphological assessment

[b] The average number of DFP band changes of all the cultivars from the race mix was subtracted from the number of changes of each cultivar from the mix; values were rounded up

cultivars of supposed Guatemalan origin, 'Benik', 'Comitan 3', 'N-151–2', 'Nabal', 'Reed' and 'Shomrat', showed no changes from the Guatemalan mix. However, 'Comitan 2' is closest to the West-Indian mix (0 changes from the West-Indian mix, 21 changes from the Mexican mix, and 45.8 changes from the Guatemalan mix), and 'Cuevas' shows no changes from either the Mexican or the West-Indian mixes, whereas it is 23.8 changes away from the Guatemalan mix. The cultivar 'Semil 34', which was thought to be a hybrid of the West-Indian and Guatemalan races, was found to be closer to the West-Indian and Mexican races (4 changes from the Mexican mix, 0 changes from the West-Indian mix, and 28.8 changes from the Guatemalan mix).

The DFP of eight cultivars who's racial affiliation is unknown were examined with the PAUP-derived phylogenetic tree (Fig. 1). The cultivars 'Ettinger', 'Fuerte', 'Hass', 'Horshim', 'Pinkerton' and 'Rincon' are in a cluster closest to the Mexican mix (one node away from the Mexican mix, and three nodes away from both the West-Indian mix and the Guatemalan mix). The distance from the common node of the above cluster to each of the mixes is: 17 changes to the Mexican mix, 26 changes to the West-Indian mix, and 28 changes to the Guatemalan mix. The cultivar 'Maoz' (actually a rootstock) branches from the same node as the Guatemalan mix (24 changes away), while it is three nodes away from the West-Indian mix (32 changes away) and three nodes away from the Mexican mix (33 changes away). The cultivar 'No Race' is branched from the same node as the West-Indian mix (23 changes away), while it is three nodes away from the Mexican mix (30 changes away) and three nodes away from the Guatemalan mix (31 changes away).

Selfing progeny of five cultivars were assessed for six morphological traits which differentiate the three races (Table 2). Three of these cultivars are considered to be of the Guatemalan race, whereas the other two are believed to represent a Gutemalan Mexican cross (with about 70–90% Mexican "blood" in them). All cultivars show some traits atypical for their race. The results of the three Guatemalan cultivars ('Nabal', 'Reed' and 'Anaheim') showed typical Guatemalan performance for the progeny of noncolored lenticels on young shoots, anise scent, and skin thickness (Table 3). 'Anaheim' and 'Nabal' had only

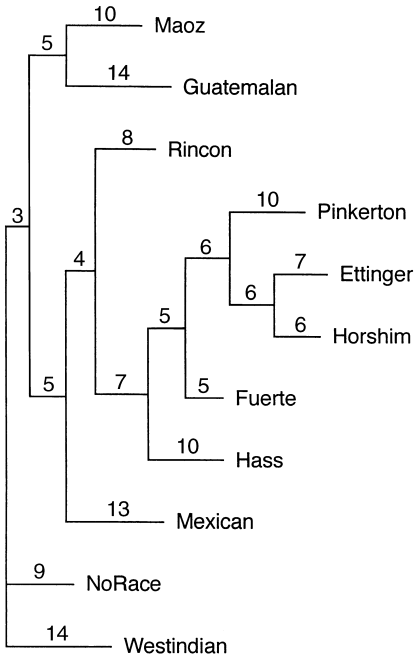


Fig. 1 PAUP-derived phylogenetic tree of eight avocado cultivars of unknown racial origin, and the three avocado races. *Branch lengths* represent the number of DFP band changes between nodes

Table 2 Distribution (in %) of the performance of selfing progeny of five cultivars in respect of six traits, including the racial classification of the traits

A. Colored lenticels on young shoots

Parent	Race	Numerous	Medium	Few	None
'Ettinger'	M × G	11.8	26.5	35.3	26.5
'Rincon'	M × G (?)	0	29.2	33.3	37.5
'Nabal'	G	2.4	3.6	12.1	81.9
'Reed'	G	4.3	0	17.4	78.3
'Anaheim'	G	0	5.5	12.7	81.8
Race classification		M			G, WI

B. Anise scent of leaves

Parent	Race	Strong	Medium	Weak	None
'Ettinger'	M × G	0	0	57.1	42.9
'Rincon'	M × G (?)	0	20.8	29.2	45.8
'Nabal'	G	0	0	17.6	82.4
'Reed'	G	0	0	13.0	87.0
'Anaheim'	G	0	0	5.2	94.8
Race classification		M			G, WI

Table 2 Continued

C. Start of harvest season

Parent	Race	Early	Early-Mid	Mid season	Mid-late	Late
'Ettinger'	M × G	0	42.9	42.9	14.3	0
'Rincon'	M × G (?)	63.6	0	18.2	9.1	9.1
'Nabal'	G	13.6	18.2	45.5	4.6	18.2
'Reed'	G	0	20.0	60.0	20.0	0
'Anaheim'	G	0	27.8	5.6	33.3	33.3
Race classification		M	WI			G

D. Skin thickness

Parent	Race	Mexican type	Like 'Fuerte'	Like 'Tova'	Like 'Hass'	Like 'Nabal'	West Indian type
'Ettinger'	M × G	42.9	57.1	0	0	0	0
'Rincon'	M × G (?)	0	50.0	40.0	10.0	0	0
'Nabal'	G	0	0	31.8	31.8	27.3	9.1
'Reed'	G	0	0	40.0	40.0	20.0	0
'Anaheim'	G	0	0	16.7	0	55.6	27.8
Race classification		M				G	WI

E. Seed weight (% of fruit weight)

Parent	Race	6–10	11–15	16–20	21–25
'Ettinger'	M × G	0	57.1	28.6	14.3
'Rincon'	M × G (?)	10.0	0	60.0	30.0
'Nabal'	G	9.1	50.0	36.4	4.6
'Reed'	G	0	20.0	60.0	20.0
'Anaheim'	G	0	22.2	66.7	11.1
Race classification		G			M, WI

F. Skin surface

Parent	Race	Smooth	Slightly-rough	Rough	Slightly pimpled
'Ettinger'	M × G	71.4	14.3	14.3	0
'Rincon'	M × G (?)	20.0	40.0	30.0	10.0
'Nabal'	G	0	45.5	45.5	9.1
'Reed'	G	20.0	60.0	20.0	0
'Anaheim'	G	0	44.4	55.6	0
Race classification		M		WI	G

Table 3 Similarity to designated race of the three "Guatemalan" cultivars based on the performance of selfing progeny in respect of six traits

Selfing progeny of	Lenticels	Anise	Date of harvest	Skin thickness	Seed size	Skin surface
'Nabal'	–	–	+	–	–	–
'Reed'	–	–	+	–	+	+
'Anaheim'	–	–	–	–	+	–

– Performance typical of the designated race. + Performance untypical of the designated race. Typical performance was declared when at least half of the progeny showed similarity to the expected racial performance (Table 2)

one atypical trait. The 'Anaheim' progeny had larger-than-expected seed size, and the 'Nabal' progeny had a late harvest season, with seedlings bearing fruit in mid-season which is not expected of the Guatemalan race. 'Reed' had three atypical traits: date of harvest, seed size and skin surface. On the other hand, most of the characteristics of the progeny of the two Mexican-Guatemalan cultivars, 'Ettinger' and 'Rincon', were not typical of their race. In the 'Ettinger' progeny, only skin surface (smooth in 71.4% of the seedlings) was typical of the Mexican race, and in

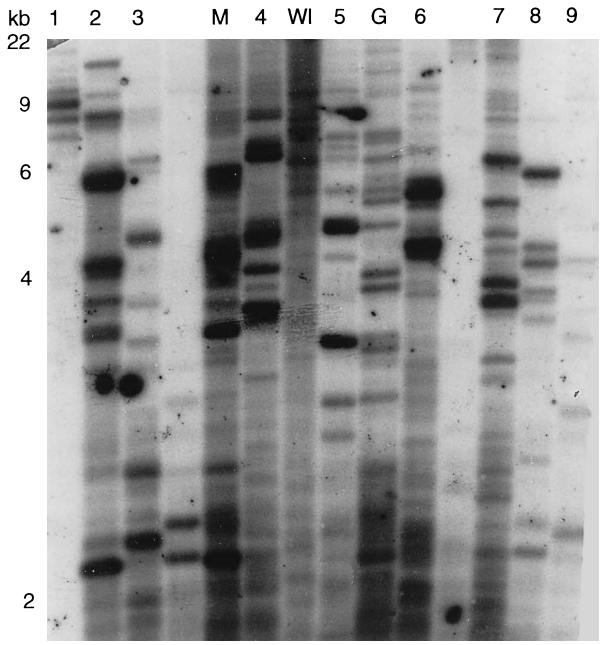


Fig. 2 DFP patterns of eight *Persea* species and three DNA mixes representing the three *Persea americana* races. *Persea* species: *1 P. indica, 2 P. gigantea, 3 P. steyermarkii Allen, 4 P. longpipes, 5 P. nubigena, 6 P. floccosa, 7 P. schiedeana 05, 8 P. schiedeana 06, 9 P. cinerascens. P. americana* races: *M*=Mexican, *WI*=West-Indian, *G*=Guatemalan. The DNA samples were restricted with enzyme *Hinf*I, and hybridized with probe 22.3

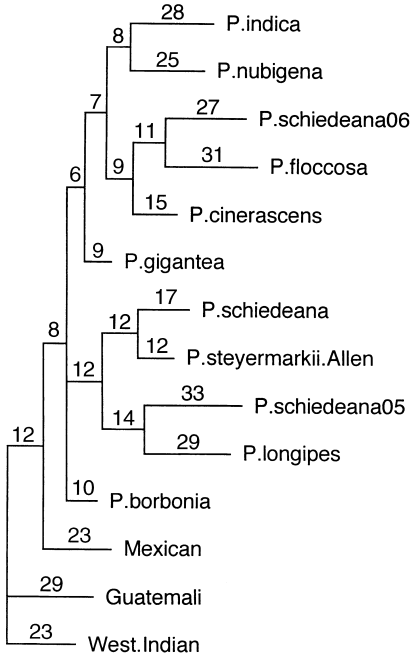
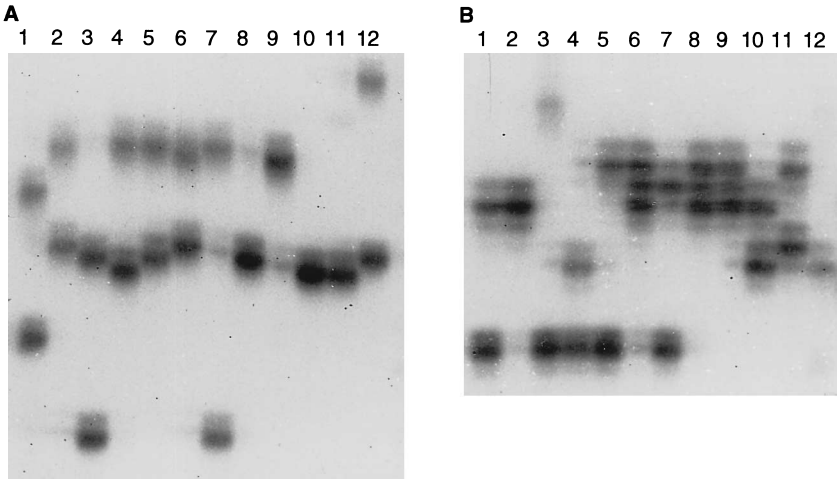


Fig. 4 PAUP-derived phylogenetic tree of nine *Persea* species (*P. schiedeana* is represented by three individuals), and the three *P. americana* races. *Branch lengths* represent the number of DFP band changes between nodes

Fig. 3A, B SSR alleles of eight *Persea* species and a member of each of the three *P. americana* races. *Persea* species: *1 P. indica, 2 P. schiedeana 05, 3 P. schiedeana 06, 4 P. steyermarkii Allen, 5 P. cinerascens, 6 P. nubigena, 7 P. floccosa, 8 P. longipes, 9 P. brobonia. P. americana* races: *10* Mexican, *11* West-Indian, *12* Guatemalan.
A- SSR marker AVAG21.
B- SSR marker AVAG22.



the 'Rincon' progeny only harvest season (63.6% early as in the Mexican race, and 9.1% late as in the Guatemalan race) was typical for the two races. All other four traits were very variable in progeny performance, and not typical of the parent races. In several traits of the two Mexican-Guatemalan cultivars, especially 'Ettinger', no Guatemalan characteristics were found.

The genotypes of eight *Persea* species and one member of each of the *P. americana* races were typed using the minisatellite probe 22.3, producing 93 DFP bands (Fig. 2), and ten SSR markers, revealing 128 alleles (Fig. 3). The phylogenetic tree obtained is presented in Fig. 4. Two of the three *P. americana* races (Guatemalan and West-Indian) branch off from the same node, while the Mexican race is located two branches away. All the other nine *Persea* species originate from one node, which is closer to the Mexican race than to the other two races. Among the nine *Persea* species there are three clear clusters: the first con-

tains only *P. borbonia*, the second contains three species on two branches (*P. longipes*, *P. schiedeana*, *P. schiedeana* 05, and *P. steyermarkii* Allen), while the third cluster contains six species on two branches, *P. gigantea* on one branch and five species on three sub-branches (*P. cinerascens*, *P. floccosa*, *P. indica*, *P. nubigena*, *P. schiedeana* 06). It is important to note that *P. schiedeana* is represented by three samples which are not clustered together.

Discussion

The characterization of cultivars, races and species of avocado is mainly based on morphological characters of the tree and fruit. However, due to the low number of such characters, their limited polymorphism, and the difficulty of defining and quantifying them, horticulturists are looking for an alternative approach. VNTR markers reveal polymorphic genetic loci that enable the characterization of each individual with a specific band pattern. These markers have been applied to many plants (Adato et al. 1995; Dallas 1988; Nybom and Schaal 1990; Tzuri et al. 1991; Sharon et al. 1992), including avocado (Lavi et al. 1994; Mhameed et al. 1995), for the purpose of identification and for the estimation of genetic relatedness.

In the present study we examined 32 *P. americana* cultivars, attempting to allot their origin to one of the three races of *P. americana*. Of these 32 cultivars, 24 have been attributed to races using morphological traits. The DFP data support the allocation of eight of the nine Mexican cultivars (except 'Km 145' which is almost equally close to the West-Indian race). A similar result was found concerning five of the six cultivars allocated to the West-Indian race, and six of the eight cultivars assigned to the Guatemalan race. The other five cultivars ('Aquila 2', 'Antigua', 'Cuevas', 'Comatin 2', and 'Semil 34') were found to be closer to races that they were not thought to originate from. However, since the DFP results of 19 of the 24 cultivars examined match the morphological analysis, it can be assumed that the three DNA mixes do represent the three avocado races. Analysis of these mixes allows a quantitative comparison of the three races.

The results of the morphological analysis of the progeny of five selfings indicates that the distribution within each cultivar is wide, imposing question marks on the definition of races. The progeny of Mexican-Guatemalan cultivars ('Ettinger' and 'Rincon') were less true to race than were the progeny of the three Guatemalan cultivars ('Reed', 'Nabal' and 'Anaheim'). The fact that atypical characteristics were found in all traits, indicates that the race determination of the five cultivars is not definitive, and that each of the cultivars contain "foreign blood". Another possible hypothesis is that the high variation in the offspring phenotypes suggests the existence of dominant alleles in the trait genes, that determine the parent characteristics. According to the six traits tested, it seems that 'Anaheim' and 'Nabal' are closer to the Guatemalan race (with only one trait atypical for the race) than 'Reed' (three atypical traits). This is in contradiction to the DFP results, where 'Nabal' and 'Reed' are equally close to the Guatemalan race.

Eight cultivars having an unknown racial attribution were compared to the DFP pattern of the three mixes. The cultivar 'No Race', formerly not allotted to a race, was found to be closest to the West-Indian race. The cultivars 'Ettinger', 'Fuerte', 'Horshim' and 'Rincon', as expected, were found to be closest to the Mexican race. However, 'Hass' and 'Pinkerton' were also closest to the Mexican race, though they are thought to be Guatemalan. In the case of 'Ettinger' and 'Rincon', both thought to have 10–30% Guatemalan "blood", these results are more consistent than the morphological results, where both seem to have more Guatemalan traits. The rootstock 'Maoz' was found to be closer to the Guatemalan race, though it has been assumed to belong to the West-Indian race. Based on these findings, the DFP patterns may support the assumption that these cultivars resulted from crosses between the races. The second parent for each cultivar can not be determined on the basis of the current results.

Three DNA mixes representing the three races were compared in order to estimate genetic relationships among them. The study of Bergh and Lahav (1996), which focused on morphological characters, showed that each one of the races is equally different from the other two. This fact is substantiated by isozyme studies (Goldring et al. 1985), as well as by RFLP (Furnier et al. 1990) and DFP markers (Lavi et al. 1991). Kopp (1966) suggested that the Guatemalan and West-Indian races belong to the same botanical group which is different from the Mexican race. Bufler and Ben-Ya'acov (1992) argue that the distance between the Guatemalan and West-Indian races is smaller than each of their distances from the Mexican race. The phylogenetic tree presented in the present study (Fig. 4) supports the hypothesis that the Guatemalan and West-Indian races are closer to each other than to the Mexican race.

DFP and SSR markers were used for the estimation of relationships among *Persea* species. Some researchers divide these *Persea* species into two groups:

1) ***Persea persea*** which includes *P. gigantea*, *P. nubigena*, *P. floccosa*, *P. steyermarkii* Allen, and *P. schiedeana*. The fruit of this group is edible and group members can be grafted on each other. The stock of this group is sensitive to avocado root rot caused by *Phytophthora cinammomi*.
2) ***Persea drymifolia*** which contains *P. cinerascens*, *P. indica* and *P. longipes*. The fruit of this group is very large and they cannot be grafted on each other. Some of the members of the group are resistant to avocado root rot (Kopp 1966).

Ben-Ya'acov (1995) divides the genus *Persea* into two groups of species with *P. schiedeana* in one group, and all other *Persea* species in the other. This division is based on the fact that the variance within species is not smaller than the variance between the *Persea* species. *P. schiedeana* is a separate group based on unique characteristics, such as fibrous leaves, fibrous branches and orange inflorescence. Based on the VNTR markers we found no groupings among

the *Persea* species. However, it is worth mentioning that in this study the variance within the species was not determined since only one tree from each species was available for typing, except for *P. schiedeana* in which the three accessions were located on different branches of the phylogenetic tree (Fig. 4). This suggests that the genetic variation within a species may be greater than that between species. Comparing the *Persea* samples to the DNA mixtures representing the races, we found that the *Persea* species tested are closer to the Mexican race. Furnier et al. (1990) suggested, on the basis of RFLP analysis, that the Guatemalan race may have originated from a cross between *P. steyermarkii* Allen and *P. nubigena*. According to our data, both species seem to have a low relationship to the Guatemalan race.

In this study we have examined the known genetic relations within the genus *Persea* and within the species *P. americana*. In most cases, the current results coincided with the findings of the other studies quoted above. It is noteworthy that most DNA markers probably originate from non-coding regions of the DNA; hence, it is not surprising to find differences between the morphological and the DNA marker results. Both the genetic analysis of the selfing progeny and the VNTR analysis suggest a high level of heterozygosity (as was suggested by Lavi et al. 1991, and by Mhameed et al. 1996). The large variation observed within the selfing progeny, as well as within species and races, raise questions concerning the validity of race and species definition in the *Persea* species.

Acknowledgements The authors are grateful to Dr. A. Ben-Ya'acov for his significant contribution through thorough discussions. We also appreciate the excellent technical assistance of D. Sa'ada, D. Zamet and A. Chapnick.

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