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Application of DNA Fingerprints for Identification and Genetic Analysis of Avocado

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Abstract. **Application of four DNA fingerprint probes to avocado *(Persea americana* Mill.) resulted in identification of various cultivars, characterization of the three avocado races, and a genetic analysis of family structure. Genomic DNA from 14 cultivars was probed with four DNA fingerprint probes. Three of the probes gave well-resolved bands. The individual-specific patterns obtained for each cultivar validate the use of this technique for definitive cultivar characterization, with the probability of obtaining a similar pattern for two different cultivars being 2×10^{-9}. DNA mixes representing either Mexican, Guatemalan, or West-Indian avocado races were hybridized with the DNA fingerprint probes, and a band pattern characteristic for each race was obtained. Progeny of a cross between the cultivars Ettinger and Pinkerton were analyzed. Their DNA fingerprints revealed one pair of linked bands and another band allelic to one of them. The application of these observations to identification, evolutionary studies, and breeding is discussed.**

Mini- and microsatellite loci have been found to be highly polymorphic in many species due to the presence of a variable number of tandem repeats (Dallas, 1988; Hillel et al., 1989; Jeffreys et al., 1985a, 1985b; Jeffreys and Morton, 1987; Nürnberg et al., 1989; Nybom and Schaal, 1990). DNA fingerprints obtained by the hybridization of mini- and microsatellite probes with genomic DNA are useful for cultivar and race identification, as well as for genetic analyses (Dunnington et al., 1991; Kuhnlein et al., 1989). Cultivar and race identification in avocado is currently limited to horticultural characteristics (Bergh, 1969). A more reliable method of identifying cultivars, rootstocks, and races is therefore needed for both horticultural and evolutionary studies.

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Avocado breeding programs are mainly based on screening many seedlings, an inefficient and expensive procedure. DNA markers could potentially alleviate this problem. The advantage of using DNA fingerprints rather than other genetic markers, such as isozymes and single locus restriction fragment length polymorphisms (Degani et al., 1989; Furnier et al., 1990; Torres and Bergh, 1980), for identification and genetic analyses stem from the simultaneous detection of many polymorphic loci, by one or a few multilocus probes.

In this study, DNA fingerprint information was applied to the identification and genetic analysis of avocado. The various uses of this system are discussed.

Materials and Methods

PLANT MATERIAL. Leaves were taken from the following cultivars as representatives of each race: Mexican: 'Akila', 'Azul', 'Gainesville', 'Glickson', and 'Schmidt'. West Indian: 'Fairchild', 'General Bureau', 'Maoz', 'Nahlat 7', and 'Simmonds'. Guatemalan: 'Anaheim', 'Benik', 'Comitan', 'Nabal', and 'Reed'. Other avocado cultivars used in this study included: 'Ardith', 'Ettinger', 'Fuerte', 'Gwen', 'Hass', 'Horshim', 'Pinkerton', 'Sharwill", 'TX531', 'Whitsell', and 'Wurtz'. Genetic analysis was carried out on 14 offspring from a cross, performed under a net cage (Lavi et al., 1991), between the female parent 'Pinkerton' and the pollen donor 'Ettinger'.

DNA ISOLATION. DNA was isolated from very young leaves as described by Murray and Thompson (1980), with the following modifications: 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 two volumes of precipitation buffer were added to the final aqueous phase. 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 l M CsCl, precipitated with ethanol, and redissolved 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 FINGERPRINTING. Ten micrograms of DNA was digested with Hinf I restriction endonuclease according to manufacturer's (New England Biolabs, Beverly, Mass.) recommendations. For race characterization, equal amounts of digested DNA from all cultivars representing a race were pooled to a total of 10 (µg/mix. Electrophoresis was carried out on a 20-cm-long 0.8% agarose gel in TBE (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, U.K.). Lambda Hind III Fragments (Biolabs) served as size markers.

Membranes were hybridized to double-stranded inserts of the minisatellite probes 33.6, 33.15 (Jeffreys et al., 1985a), 22.3 (a 7-kb probe that includes probe R18.1) (Haberfeld et al., 1991), and the whole single-stranded bacteriophage M13 (Vassart et al., 1987). Probes were labeled with ^{32}P-dCTP by random priming (Feinberg and Vogelstein, 1983). Prehybridization and hybridization were carried out at 65 °C for 3 to 5 h and 16

h, respectively, in 0.263 M Na-phosphate buffer with 7% sodium dodecyl sulfate (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 x saline sodium citrate (SSC), and 0.1% SDS for 20 min each and twice with 1 x SSC and 0.1% SDS for 20 min each. Washes were carried out at 65 °C. For the M13 probe, the first wash was as described above, followed by a wash in 3 x SSC and 0.1% SDS at room temperature for 20 min. Membranes were autoradiographed for 1 to 10 days at -80 °C with intensifying screens.

ANALYSIS OF DNA FINGERPRINT PATTERNS. Band-sharing (BS) levels (twice the number of common bands divided by the total number of bands) and the determination of obligate linkage and allelic relationships between bands were obtained according to Hillel et al. (1989). Analyses were carried out on samples run on the same gel only. The probability (P) of two individuals having the same DNA fingerprint (DFP) pattern was calculated as described by Jeffreys et al. (1985b): $P = (BS)^n$, where BS is the average of the BS levels between pairs detected by probes 33.6, M13, and 22.3, and n is the average number of bands per individual detected by these probes. Transmission frequency was calculated as described by Jeffreys et al. (1986). The total number of potentially detectable loci was calculated according to Hillel et al. (1989).

Results

Table 1. Number of scoreable bands per individual (N), number of specific bands (S) and level of band sharing (BS) between 14 avocado cultivars for three probes.

	Probes			
Criterion	22.3	33.6	M13	Total
Avg no. N ±SE	15.2 ± 0.7	11.8 ± 1.9	10.0 ± 0.7	37.0 ± 1.5
No. S ±SE	12.6 ± 0.9	9.7 ± 2.3	7.3 ± 0.5	29.6 ± 1.9
Range in S	4–21	3–17	3–11	11–38
BS (%) ±SE	55.9 ± 4.2	59.5 ± 2.8	63.4 ± 2.8	

POLYMORPHIC DNA FINGERPRINTS OF AVOCADO CULTIVARS. Hinf I-digested DNA from 14 avocado cultivars was hybridized to minisatellite probes 33.6, 33.15, M13, and 22.3. Probes 22.3, 33.6, and M13 revealed resolvable complex polymorphic patterns. Probe 22.3 detected the highest level of polymorphism (Fig. 1), with an average of 12.6 specific bands and an average BS level of 55.9% between cultivars (Table 1). The DNA fragments detected by this probe ranged in size from 2 to 20 kb (fragments smaller than 2 kb were not analyzed). Related cultivars such as 'Gwen' and 'Whitsell', which are probably progeny of 'Hass', showed a relatively high level of BS (average BS between 'Hass', 'Gwen', and 'Whitsell' was 81%).

Probe 33.6 detected fewer specific bands than 22.3 and had an average BS of 59.5%. Only small (2 to 5 kb) fragments were detected by this probe (data not shown). Bacteriophage M13 probe was the least efficient, revealing a low average number of specific bands and the highest level of BS. Fragments ranging from 2 to 20 kb were detected. Probe 33.15 yielded nonresolvable patterns due to high background (data not shown). DNA restricted with DraI and hybridized with probes 22.3, 33.15, and 33.6 resulted in significantly fewer polymorphic patterns (data not shown).

CHARACTERIZATION OF AVOCADO RACES. To characterize the avocado races, we analyzed individual DFP patterns from five representatives of each race and DNA mixes of those five representatives (Fig. 2). DNA fingerprints of DNA mixes were used to dilute out

cultivar-specific bands and to emphasize race-specific ones (Dunnington et al., 1991). DNA was digested with Hinf I and hybridized to probes 22.3, M13, and 33.6.

In each race, specific bands were present in all cultivars representing that race, but not in the other two races. These bands were in addition to those characterizing each individual. The DFP patterns of the Guatamalan and Mexican races obtained from hybridization with probe 22.3 were composed of strong, easily distinguishable bands, while the DFP of the West-Indian cultivars was characterized by fewer and fainter bands (Fig. 2). Probes 33.6 and M13 also yielded race-specific DFP patterns (data not shown).

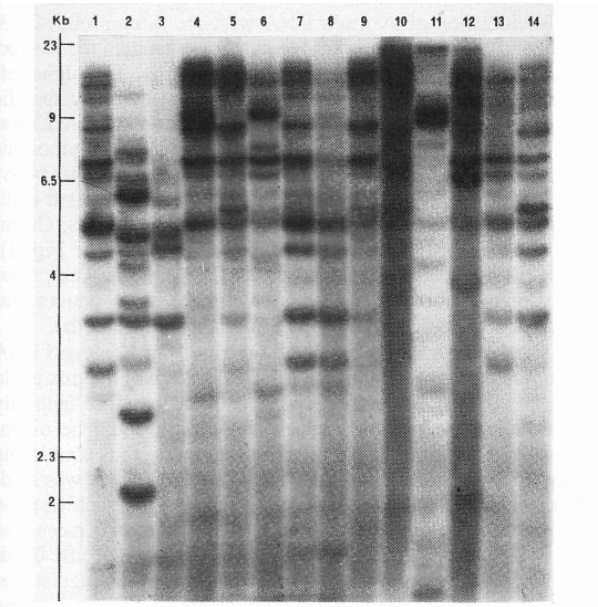

Fig. 1. DNA fingerprints of avocado cultivars following digestion (Hinf I) and hybridization with probe 22.3. Size of markers is given in kilobase. Lanes were as follows: 1, 'Horshim'; 2, 'Ettinger'; 3, 'Pinkerton', 4, 'Fuerte'; 5, 'Wurtz'; 6, 'Sharwill'; 7, 'Whitsell'; 8, 'Hass'; 9, 'Gwen', 10, 'TX531'; 11, 'Ardith'; 12, 'Nabal'; 13, 'Benik'; 14, 'Reed'.

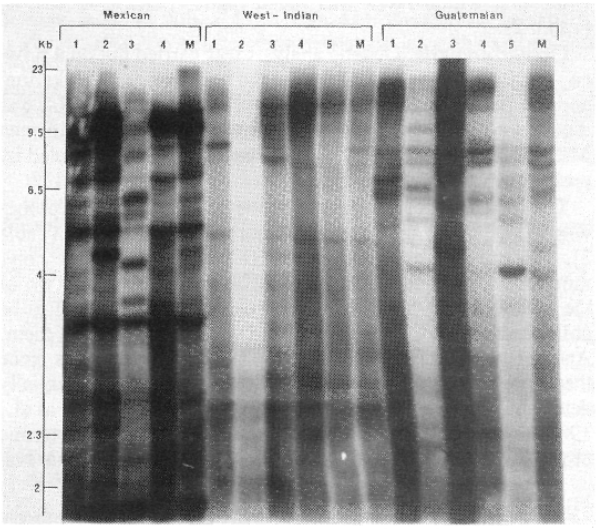

Fig. 2. DNA fingerprints of avocado races following digestion (Hinf I) and hybridization with probe 22.3. Size markers are given in kilobase. Lanes were as follows. Mexican cultivars: 1, 'Schmidt'; 2, 'Gainesville', 3, 'Glickson'; 4, 'Akila'; M-Mix (contains the cultivars above + 'Azul'). West-Indian cultivars: 1, 'General Bureau'; 2, 'Simmonds'; 3, 'Maoz'; 4, 'Nahlat 7'; 5, 'Fairchild'; M-Mix. Guatemalan cultivars: 1, 'Comitan'; 2, 'Reed'; 3, 'Nabal'; 4, 'Anaheim'; 5, 'Benik'; M-Mix.

Table 2. Number of specific bands and level of band sharing between (off diagonal) and within (diagonal) three avocado races. Individual samples of Hinf I-digested DNA from five representatives per race, as well as the DNA mix of the five race representatives were hybridized to probes 33.6, 22.3, and M13. Analysis within races was based on pairwise comparison between cultivars. Analysis between races was based on comparisons of DNA mixes.

Race[z]	Mexican	West-Indian	Guatamalan
Mexican			
S ± SE	11.3 ± 0.5		
BS ± SE	61.7 ± 2.3		
West-Indian			
S ± SE	22.7 ± 2.6	7.3 ± 0.5	
BS ± SE	26.3 ± 6.9	69.9 ± 2.6	
Guatamalan			
S ± SE	17.0 ± 1.0	13.7 ± 0.7	10.3 ± 0.5
BS ± SE	45.0 ± 5.0	48.7 ± 5.2	59.2 ± 3.5

[z]S, specific bands; BS, band sharing.

The average number of race-specific bands ranged between 7.3 and 11.3 within races and between 13.7 and 22.7 between races. The level of BS between races was low (26.3% to 48.7%) while that within races was much higher (59.2% to 69.9%) (Table 2).

GENETIC ANALYSIS. Genetic analysis was carried out on a progeny resulting from a cross between the female parent 'Pinkerton' and the pollen donor 'Ettinger' (Lavi et al., 1991). DNA was digested with Hinf I and hybridized to probes 22.3, 33.6, and M13 (Table 3). The DFP of 10 of the 14 offspring revealed by probe 22.3 are shown in Fig. 3.

Table 3. Pedigree analysis of polymorphic DNA fingerprint bands detected with three probes in 14 offspring of a cross between 'Pinkerton' as the female parent and 'Ettinger' as the pollen donor.

Component	Probe 22.3	33.6	M13	Total
Total parental bands	30	23	24	77
Parental polymorphic bands	18	11	14	43
Maternal specific bands	2	5	5	12
Paternal specific bands	16	6	9	31
Linked pairs	1	0	0	1
Allelic pairs	1	0	0	1
Total number of parental loci[z]	64			

[z]Total number of potentially detected parental loci was calculated according to Hillel et al. (1989).

The total number of parental bands detected by these probes was 77, of which 43% were polymorphic (BS = 44%) (Table 3). The paternal contribution was 31 bands (72% of the polymorphic bands) while 12 bands (28%) were maternal. Two of the bands (4.6%) hybridizing to probe 22.3 were found to be obligatorily linked, and another band was allelic to one of them. Another two paternal bands were probably homozygous since they appeared in all progeny. The total number of potentially detectable paternal loci was estimated to be 64 (Hillel et al., 1989). Each band in the offspring could be traced back to one of the parents demonstrating the reliable application of this system for paternity cases. Transmission frequency (Jeffreys et al., 1986) was 52.3% for paternal specific bands and 39.7% for maternal specific bands.

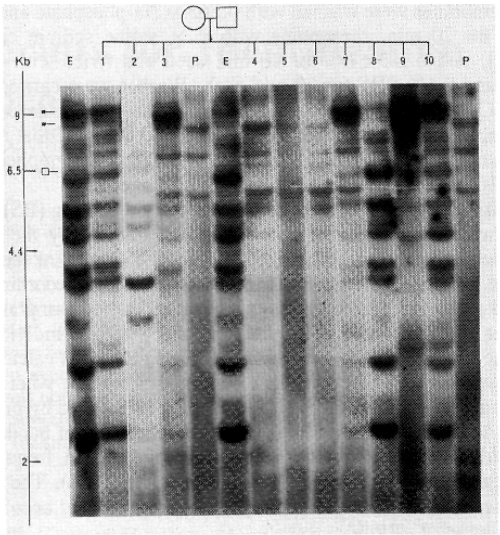

Fig. 3. DNA fingerprints of progeny from a cross between 'Pinkerton' as the female parent and 'Ettinger' as the male. Offspring are numbered 1–10. DNA was digested with Hinf I and hybridized to probe 22.3. Size markers are given in kilobase. ○ linked bands; □ a band allelic to one of the linked bands.

Discussion

Hybridization of avocado DNA with the three probes 22.3, 33.6, and M13 detecting variable number of tandem repeats loci resulted in polymorphic multiple band patterns. The higher efficiency of probes 22.3 and 33.6 is indicated by many specific bands and a low level of BS (Table 1). Bacteriophage M13 was less efficient as a probe but still provided some polymorphic patterns with low BS levels. The DFP pattern from the 14 avocado cultivars revealed high polymorphism in unrelated cultivars (average BS 58.8%), while related cultivars (Hass, Gwen, and Whitsell) exhibited a high level (81%) of BS (see Fig. 1). Each cultivar can be identified by its DFP pattern. The probability of two unrelated cultivars having the same pattern was calculated to be 2×10^{-9} (See Materials and Methods).

Avocado races were identified by mixing Hinf I-digested DNA from five race representatives. The DFP pattern of the mix could be found in most of the five representatives, therefore faithfully representing the race. Moreover, the pattern could be distinguished easily from the patterns of other races, thus identifying a specific pattern for each race. The West-Indian race was readily distinguishable by the very low-intensity DFP yielded upon hybridization to probe 22.3 (Fig. 2). The other two races also had distinguishable DFP patterns. The many race-specific bands and the BS levels provide useful guidelines to distinguish between races and to characterize relationships between races and cultivars.

The pedigree analysis consisted of 14 offspring of a cross between 'Pinkerton' and 'Ettinger'. Out of a total of 77 bands detected, 43 were polymorphic, and two of them were tightly linked. Another band was allelic to one of these linked bands. Overall, this inheritance pattern suggests that most minisatellite loci in avocado are not clustered.

These results suggest that DNA fingerprints in avocado will likely be useful not only for identification purposes but also to improve breeding through the detection of genetic linkage between DNA fingerprint loci and economically important traits. Selection could be performed for the genetic markers at an early age, rather than for the traits themselves on mature trees. Moreover, application of the multilocus genetic marker systems could reduce the number of backcrossed generations required for introgression of specific single gene traits into a commercial cultivar (Hillel et al., 1990).

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