GENERATION AND APPLICATION OF SSR MARKERS IN AVOCADO

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Abstract

Simple Sequence Repeat (SSR) DNA markers were generated and applied to avocado. An SSR marker is based on a pair of primers which are synthesized on the basis of DNA sequences flanking a microsatellite. These markers are PCR based, quite polymorphic and abundant in several species. These are the markers of choice in the human genome. The number of SSR markers in the avocado genome was calculated to be about 45,000, with the A/T microsatellite being the most frequent (1 in 40 kb). SSR markers are quite expensive to generate due to the required multi-step procedure; Screening a genomic library, about 66% of the positive clones turned out after sequencing to be SSR containing clones. In only about 55% of these, was it possible to synthesize primers and, of this group, only about 50% of the markers were useful for typing a specific family. Typing of five avocado cultivars using 59 SSR markers results in one to eight alleles per locus, mean heterozygosity ranging between 0.51 and 0.66 and gene diversity ranging between 0.42 and 0.66. The SSR markers were used to estimate the genetic relationships between various *Persea* species. The number of alleles in these species ranged between five and twelve with heterozygosity levels between 0.11- 0.78 and gene diversity between 0.69-0.89. A preliminary genetic map, based on these SSR markers together with some DNA fingerprints (DFP) and randomly amplified polymorphic DNA (RAPD) markers, was drawn. The map consists of 12 linkage group having two to five markers each. Linkage analysis with several quantitative trait loci (QTLs) was performed by genetic typing and phenotypic assessment of the progeny of a controlled cross. The result of the interval mapping suggests that the gene(s) coding for the existence of fibers in the flesh, are probably linked to linkage group 3.

1. INTRODUCTION

Avocado (*Persea americana* Mill.) is a sub-tropical fruit-tree, with 24 chromosomes (2n=24) and haploid genome size of 8.83×10^8 bp. The classical avocado breeding programs are inefficient mainly because of a long juvenile phase, large tree size and limited genetic knowledge. These obstacles may be alleviated by applying marker assisted selection (MAS).

In the last few years, four types of genetic markers have been used in avocado. Degani *et al.* [1] used isozyme markers mainly to study the level of selfing and out-crossing in 'Fuerte'. Furneir *et al.* [2] used two restriction fragment polymorphism loci (RFLP) loci to study the relationships between Persea species and between avocado cultivars. DNA fingerprints (DFP), using multilocus minisatellite and microsatellite probes were applied for identification of cultivars and races [3] and for detection of linkage between a gene affecting fruit skin color and a specific DFP band [4].

Simple sequence repeat (SSR) markers have been shown to be highly polymorphic in human [5], animals [6] and plants. Preliminary studies of simple sequence repeat markers in fruit-trees were carried out: two SSR markers were isolated from a cross between lemon and orange and found to be polymorphic between citrus and related species [7]. Thomas and Scott [8] isolated five microsatellite loci from grapevine and found that the heterozygosity level of these loci ranged between 69% and 88%. Lavi *et al.* [9] generated 30 dinucleotide microsatellite markers and demonstrated the high level of polymorphism of two of them.

In this study, we present the generation of avocado-specific SSR and the application of these markers for genetic analyses as well as construction of the first integrated genetic linkage map of avocado.

2. MATERIALS AND METHODS

2.1. Plant material

Trees were grown at the Akko Experiment Station and the Agricultural Research Organization, Bet Dagan, Israel. Leaves were taken from 50 offspring of the cross 'Pinkerton'x'Ettinger' and from the following *Persea* species: *P. brobonia, P. cinerascens, P. floccosa, P. gigantea, P. indica, P. longipes, P. nubigena, P. schiedeana, P. schiedeana 05, P. schiedeana 06, P. steyermarkii Allen.*

2.2. DNA isolation

DNA was isolated from very young leaves using the CTAB (cetyltrimethyl ammonium bromide) method [10], following a few modifications [3].

2.3. DNA fingerprinting

Ten micrograms of DNA were digested with *Hinf*I restriction endonuclease. Electrophoresis and hybridization are detailed elsewhere [4].

2.4. Randomly amplified polymorphic DNA (RAPD) markers

Reaction mixes for RAPD markers contained 30 ng of genomic DNA, 1.5 mM Mg^{2+}, 0.2 mM of primer, 250 mM of each nucleotide, 1X Taq buffer (containing 20 mM $(NH_4)SO_4$, 75 mM Tris-HCl, pH 9.0, 0.1% Tween) and 0.5 unit of Taq DNA polymerase in a total volume of 25ml. The reaction mixes were overlaid with 40ml of mineral oil. Reactions were denatured at 94°C for 3 min before cycling which consisted of a 1 min denaturation at 94°C, 1 min annealing at 36°C, and 1 min extension at 72°C for 44 cycles. Cycles were followed by an extension step for 5 min at 72°C. Amplification products were analyzed by electrophoresis in 1.5% agarose gels and detected by staining with ethidium bromide. RAPD primers used are from set #7 (numbered 601-700) of the University of British Columbia.

2.5. Genomic library

Genomic DNA of 'Pinkerton' was cut with the restriction enzyme *Sau*3AI. DNA fragments were size selected on agarose gels and fragments (400-600bp) were cloned in pBluescript II KS^+ and transformed into *E. coli* Inv-a strain. Transformants were grown in LB media with 50mg/ml Ampicillin. White colonies were transferred into microtiter plates and

stamped onto Hybond N+. Colonies were grown, lysed, denatured and neutralized. Membranes were hybridized to end-labeled g-^{32}p oligonucleotide probes. Probes were: $(A)_{30}$, $(CT)_{10}$, $(GT)_{10}$, $(CAC)_6$, $(TCC)_6$, $(TCT)_6$, $(GATA)_4$, $(GACA)_4$ and $(GGAT)_4$.

2.6. SSR definition

Sequences of positive clones were searched for any type of repeated sequences. A repeat was identified as an SSR if the number of repeats (n)>14 for mononucleotide repeat, (n)>6 for di-nucleotide repeats, (n)>3 for tri-nucleotide repeats and (n)>2 for tetra-nucleotide (or more) repeats.

2.7. Selection and synthesis of primers

SSR-containing clones were analyzed using the Primer Detective Software or PRIMER 0.5. Primers having melting temperatures of at least 60°C and which would amplify products with predicted lengths of 75-210bp were synthesized.

2.8. Genotyping

PCR reaction mixes for SSR markers contained 30 ng of genomic DNA, 1.5 mM Mg^{2+}, 0.15 mM of 3' and 5' end primers, 100 mM of each nucleotide, 200mg/ml BSA, 0.1 ml of 3,000 Ci/mmol a-^{32}P dCTP, 1X Taq buffer (containing 50 mM Tris-HCl, pH 9, 0.1% Triton X-100), and one unit of *Taq* DNA polymerase in a total volume of 10ml. The reaction mixes were overlaid with 20ml of mineral oil. Reactions were denatured at 94°C for 30 s before cycling which consisted of a 15 s denaturation at 95°C, 25 s annealing at 45°C or 50°C (depending on the primers), and 25 s extension at 68°C for 32 cycles. This was followed by an extension step for 2 min at 68°C. Ten ml of loading buffer (95% formamide; 0.02 M EDTA, pH 8; 1% bromophenol blue; 1% xylene cyanol; and 10 mM NaOH) were added and 3ml of the reaction were loaded on a DNA sequencing gel containing 6% polyacrylamide, 8 M urea and 1xTBE and electrophoresed at 50 Watt for three to four hours. Gels were dried and exposed over-night to an Fuji X-ray film. The length of the SSR alleles was determined using a sequence ladder of M13 as size marker.

2.9. Heterozygosity analysis

Gene Diversity (GD) was calculate according to [11]: $GD = 1-\Sigma pi^2$ where pi is the frequency of the i th pattern. Heterozygosity level was calculated as the proportion of heterozygote genotypes from all tested genotypes.

2.10. Evolutionary analysis

Phylogenetic trees was performed using the maximum parsimony method with the 'PAUP' software.

2.11. Map construction

For map construction, two-point analyses of all possible combinations were performed using MAPMAKER/EXP 3.0 (12) and JoinMap (13). Linkage was determined when the LOD score was >3.0. Linkage analysis of loci that showed significant deviation from Mendelian

expectation was tested using the c^2 test in addition to the LOD score calculation. The final map was drawn using MAPMAKER and was verified using several mapping programs.

2.12. Detection of association between DNA markers and loci controlling fruit traits

One way analysis of variance was performed between offspring genotypes (of each marker locus) and each of the nine fruit traits using the JMP computer program. The Contrast-Analysis was used in order to search for possible allelic interaction affecting the content of fibers in the flesh. The interval mapping method was performed between the 12 avocado linkage groups and each of the nine fruit traits. The paternal or maternal interval mapping analysis of the 12 linkage groups was made using MAPMAKER/QTL software [12]. The combined interval mapping analysis between linkage group 3 and the content of fibers in the flesh was made using MapQTL software [14].

3. RESULTS

3.1. Abundance of microsatellites in the avocado genome

An avocado genomic library containing about 10,000 clones with a mean insert size of 500 bp was screened with various SSR repeats (Table I). This library represents about 0.57% of the avocado genome ($5x10^6$bp out of 883Mbp). The frequency assessment of SSRs, based on positive clones, suggests that $(A/T)_n$ and $(TC/AG)_n$ sequences are the two most frequent microsatellites: 1 in 40kb and 1 in 68kb respectively. A total of 236 positive clones were identified suggesting about 45,000 microsatellite sequences in the avocado genome (Table I).

3.2. Generation of SSRs

Two hundred and twenty five positive clones were sequenced (from one side), of which 172 gave reliable sequence data (Table II). Of these 172 clones: 12 (7%) did not contain any type of repeat; the sequences of 47 clones (27%) contained short repeats; and 113 clones (66%) contained an SSR. Suitable primers could not be selected in 51 (45%) of the SSR-containing clones (Table III) due to location of the repeat near the cloning site (41 clones) or low melting temperature (10 clones), leaving 62 (55%) SSR primer pairs.

TABLE I. FREQUENCY OF NINE REPEAT TYPES IN THE AVOCADO GENOMIC LIBRARY

Repeat type	No. of screened clones	No. of positive clones (%)	Predicted ratio of SSRs per kb	Predicted No. of SSRs in the genome
(A/T)n	10,000	125(1.25)	1: 40	~22,000
(TC/AG)n	10,000	74 (0.74)	1: 68	~13,000
(GT/CA)n	10,000	27 (0.27)	1: 185	~ 4,800
(CAC/GTG)n	5,000	3 (0.06)	1: 833	~ 1,050
(TCC/AGG)n	5,000	2 (0.04)	1:1250	~ 700
(TCT/AGA)n	5,000	2 (0.04)	1:1250	~ 700
(GATA/CTAT)n	3,000	0 (0)	<1:1500	< 590
(GACA/CTGT)n	3,000	0 (0)	<1:1500	< 590
(GGAT/CCTA)n	3,000	3 (0.1)	1: 500	~ 1750
Total	**10,000**	**236(2.36)**		**~45,200**

TABLE II. FROM POSITIVE CLONES TO SSR-CONTAINING CLONES

	TC/AG	GT/CA	Others	Compound	Percent
No. of positive clones	66	21	9	10	
Good sequence	51	15	9	8	100%
No repeat	2	2	5	1	7%
Short repeat	10	5	1	2	27%
SSR-containing clones	39	8	3	5	66%

TABLE III. FROM SSR-CONTAINING SEQUENCES TO PRIMERS

	A/T	TC/AG	GT/CA	Others	Compound	Percent
SSR-containing sequences	51	33	6	9	14	100%
SSR near the cloning site	15	14	2	4	6	36%
Primers not found	6	2	2	0	0	9%
Primers were found	**30**	**17**	**2**	**5**	**8**	**55%**

The 62 SSR markers which were developed in this study, combined with 29 from a previous avocado library [9], and one from the 5' region of the cellulase gene (GenBank data) were used to analyse the avocado genome.

3.3. Polymorphism and heterozygosity levels in SSR loci

Fifty nine avocado SSR loci were used to analyse the polymorphism and heterozygosity level of the avocado genome. The dinucleotide repeats $(AG)_n$ are the most polymorphic SSR loci (of those examined) in avocado and thus presented the highest mean number of alleles per locus and highest Gene Diversity value (Table IV). The mean heterozygosity in the five cultivars was estimated to be 62%, and varied between 58% and 70%. 'Horshim' was the cultivar with the highest level of heterozygosity (Table V). In summary, the analysis of 59 loci in five cultivars indicated an average of 3.4 alleles per locus and a mean heterozygosity of 62%.

TABLE IV. IDENTIFICATION OF SSR MARKERS IN FIVE AVOCADO CULTIVARS

Gene Diversity	Average No. of alleles (range)	No. of markers	Repeat element
0.66	4.1 (1-8)	29	$(AG)_n$
0.42	3.0 (1-6)	19	$(A)_n$
0.56	2.5 (1-5)	7	Compound
0.55	2.6 (1-4)	4	Others
0.56	**3.4**	**59**	**Sum**

TABLE V. HETEROZYGOSITY LEVEL (HL) OF FIVE AVOCADO CULTIVARS

Simple repeat	'Ettinger'	'Pinkerton'	'Bacon'	'Horshim'	'XX102'	Mean
AG	68	68	67	82	63	70
A	65	79	67	60	65	67
Mix	30	30	43	60	33	39
Others	40	20	20	60	50	38
Total	59	62	58	70	58	62

3.4. Evolution of the *Persea* genus

Nine *Persea* species were chosen to detect genetic distances between *Persea* species using ten SSR markers. Fig. 1 represents the phylogenetic tree constructed by PAUP software. The three avocado races are located on close branches although the Mexican race seems to be closest to the other *Persea* species which can be divided into four main groups.

3.5. Polymorphism within family

Each parent of the mapping population ('Pinkerton' and 'Ettinger') was screened with each of the 92 primer pairs. Five SSRs gave no PCR product (Table VI), 14 gave non-reliable or multiple products, 25 gave non-informative patterns (non-polymorphic, or both parents homozygous for different alleles). Forty eight of 92 analysed SSRs were used for typing 50 offspring of the mapping population. Two primer pairs amplified products from two different loci each and thus a total of 50 loci were scored.

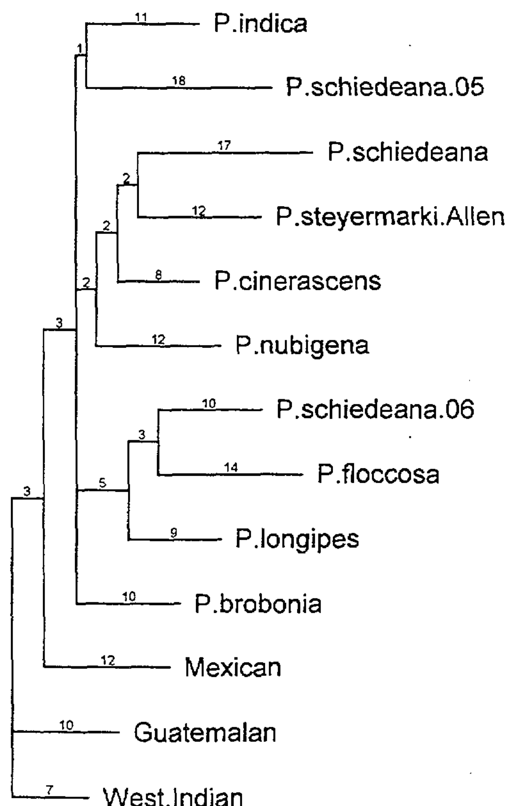


FIG.1. Evolution tree of Persea genus.

TABLE VI. TYPING THE PROGENY OF THE CROSS 'PINKERTON' X 'ETTINGER'

	TC/AG	GT/CA	Others	Compound	Total	Percent
Currently available	36	3	5	19	92	100%
No PCR product	3	0	1	1	5	5%
Non-reliable product	4	0	0	3	14	15%
Non-informative	7	2	1	6	25	27%
Typed	22	1	3	9	48	52%

3.6. Avocado linkage map

The genotypes of the two parents ('Pinkerton' and 'Ettinger') and 50 offspring were determined using 50 SSRs, 17 RAPDs and 23 DFP bands [4]. All 90 loci were used for two point linkage analysis using the following programs: CRI-MAP, JoinMap, LINKAGE, LINKEM and MAPMAKER. The two-point analysis of LINKAGE and CRI-MAP programs resulted in identical LOD scores and *theta* values and the two-point analysis of LINKEM and MAPMAKER resulted in identical LOD scores and *theta* values. The last two programs gave higher values of LOD score (about 10% higher compared to the results obtained by LINKAGE and CRI-MAP). The two point analysis of JoinMap and MAPMAKER resulted in similar LOD (Log of the Odds) score values (except for markers showing segregation distortion).

The paternal, maternal and combined maps are presented in Fig. 2. The combined map consists of 12 linkage groups and 34 markers. The number of markers per linkage group varies between two to five and the length of the 12 linkage groups varies from 14.7cM to 68.7cM. A specific region on linkage group 3 varies significantly between the paternal and maternal maps. The maternal distance between locus AVA13 and locus AVA04 is almost twice the parental distance (14.7cM versus 7.6cM). Although, in other linkage groups only minor differences can be detected between paternal and maternal maps, the maternal distances are higher than the paternal ones (21% difference on the average). Analysis of marker distribution on the linkage groups did not reveal any deviation from the Poisson distribution, indicating no significant deviation from randomness. The total map length is 352.6cM.

3.7. Association between avocado fruit traits and DNA markers

Sixty offspring of the cross 'Pinkerton' X 'Ettinger' were analysed to detect associations between 90 DNA markers (50 SSRs, 23 DFP bands and 17 RAPDs) and loci controlling nine avocado fruit traits. Each marker was analysed against each of the nine fruit traits. To avoid a false positive result, association was declared only if P was equal or less than 0.01. The positive associations are presented in Table VII. For seven out of the nine traits, positive associations were found for at least one marker.

The location of loci controlling fruit traits was determined by the interval mapping procedure while each trait was analysed against each linkage group and the results are shown in Table VIII. In three cases, high LOD score levels (>2.0) were identified. A locus controlling skin gloss was found to be in association with linkage group 6, which is composed of two

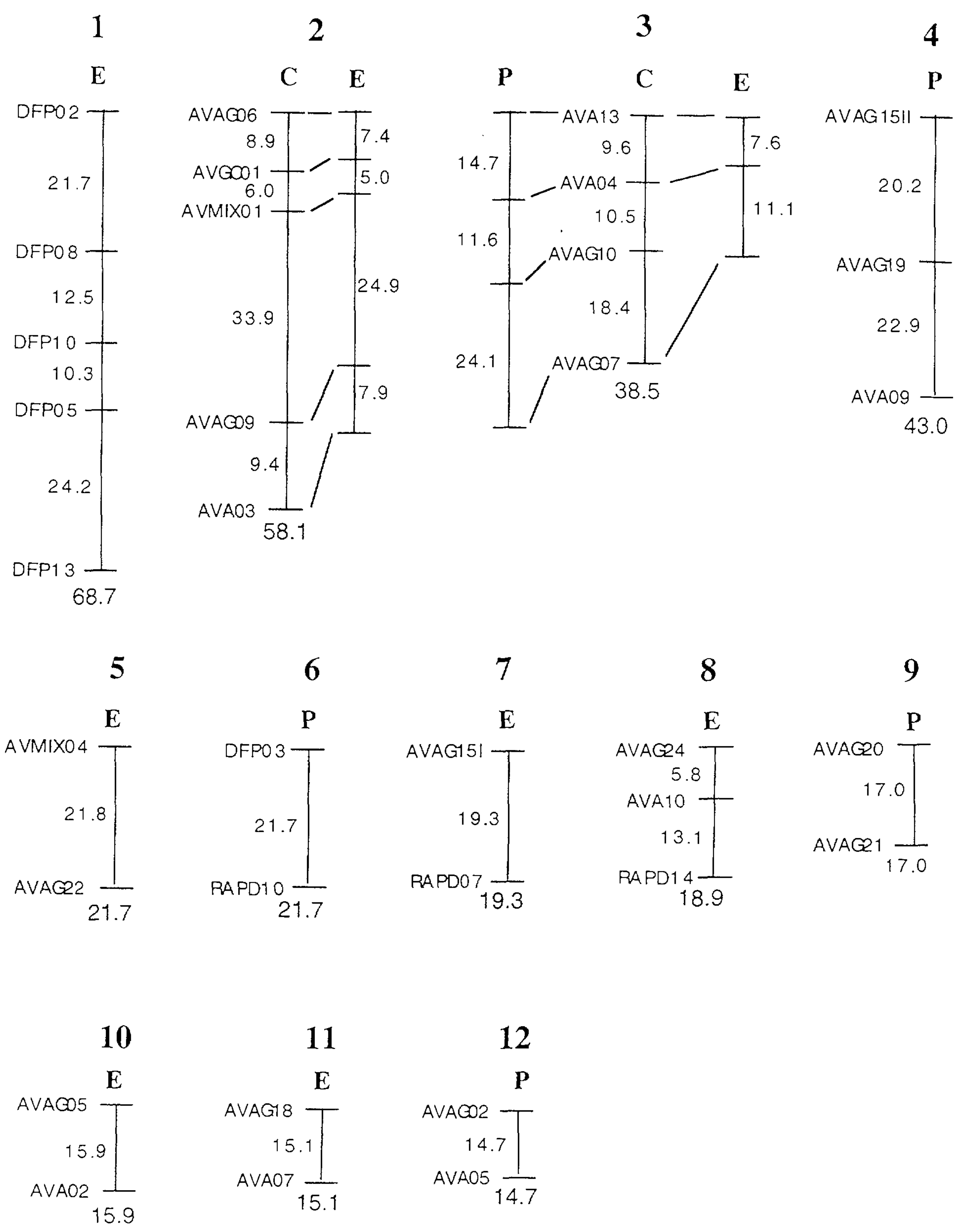


FIG. 2. Avocado genetic linkage groups. E- 'Ettinger' map, P- 'Pinkerton' map, C- Combined map. Distances between markers are in CentiMorgan.

TABLE VII. RESULTS OF ONE-WAY ANALYSIS OF VARIANCE OF AVOCADO FRUIT TRAITS

Trait	Marker	Marker's location	Polymorphism source	P value
Skin gloss	AVAG11	Un-mapped	'Ettinger'	0.0014
	AVAG13	Un-mapped	'Ettinger'	0.01
	AVA08	Un-mapped	'Ettinger'	0.0014
	DFP03	Group 6	'Pinkerton'	0.005
	RAPD10	Group 6	'Pinkerton'	0.004
Skin surface	AVAG15II	Group 4	'Pinkerton'	0.004
	AVAG23	Un-mapped	'Pinkerton'	0.009
	AVA07	Group 11	'Ettinger'	0.01
Skin thickness	AVAG14	Un-mapped	'Ettinger'	0.009
Seed size	AVAG24	Group 8	combined	0.0006
	AVAG12	Un-mapped	'Pinkerton'	0.007
Skin peeling	AVAG01	Un-mapped	'Ettinger'	0.006
	AVAC01	Un-mapped	'Ettinger'	0.003
	AVMIX06	Un-mapped	'Ettinger'	0.005
Fibers in the flesh	AVAG07	Group 3	combined	0.007
	AVA04	Group 3	combined	0.00001
	AVA13	Group 3	combined	0.01
Flesh taste	AVMIX04	Group 5	'Ettinger'	0.01
	DFP05	Group 1	'Ettinger'	0.008

markers, each having two alleles in 'Pinkerton'. The effect of this association on the progeny skin surface explains 25% of the trait variance. A locus controlling skin surface was found in association with linkage group 9, which is composed of two SSR markers each having two alleles in 'Pinkerton'. The effect of the locus on the skin surface progeny average explains 38% of the trait variance.

3.8. A locus(i) controlling fibers in the flesh

A locus controlling fibers in the flesh was found to be located on linkage group 3. This linkage group is composed of four SSR markers, and locus(i) controlling fibers in the flesh was

TABLE VIII. RESULTS OF THE INTERVAL MAPPING ANALYSIS TO DETECT LINKAGE BETWEEN LOCI CONTROLLING FRUIT TRAITS AND MARKER LOCI

Trait	Linkage group	LOD score value	Detected map	Effect (in SD values)	% variance
Skin gloss	6	2.9	'Pinkerton'	1.17	25
Skin surface	4	1.7	'Pinkerton'	-0.78	16
	9	2.6	'Pinkerton'	-1.20	38
	11	1.7	'Ettinger'	-0.81	17
Fibers in the flesh	3	5.4	Combined	0.62	37.5
Flesh taste	5	1.5	'Ettinger'	-0.81	16

located close to AVA04, having a LOD score value of 5.4. The locus effect on the progeny average explains 37.5% of the trait variance.

Fig. 3a presents the parental phases of the marker alleles on linkage group 3 and the parental haplotypes marked as P1 and P2 for 'Pinkerton' and E1 and E2 for 'Ettinger'. The offspring are thus divided to various haplotype combinations where the four parental groups are the majority. Fig. 3b presents contrast analysis between the alleles of the markers located on the haplotype combination E1+P1 and the alleles of the other haplotype combinations. The association between the locus(i) controlling fibers in the flesh and the alleles of each marker on E1+P1 haplotype is significant (except for the marker AVAG10). This result indicates that a locus controlling fibers in the flesh is located within this linkage group close to the marker AVA04 and having an extremely high significance level ($P=4.4x10^{-8}$). Furthermore, this locus controls high level of fibers in the flesh only when specific allele combinations are present (interaction between alleles). Namely, only when P1 alleles are present in the genome in combination with E1 alleles is the level of fibers in the flesh significantly high. The association between the locus(i) controlling fibers in the flesh and the marker AVAG10 is not significant because only 'Pinkerton' is a heterozygote for this marker and thus using this marker one can not distinguish between offspring having E1 haplotype and offspring having E2 haplotypes.

4. DISCUSSION

4.1. Generation and polymorphism of SSR in avocado

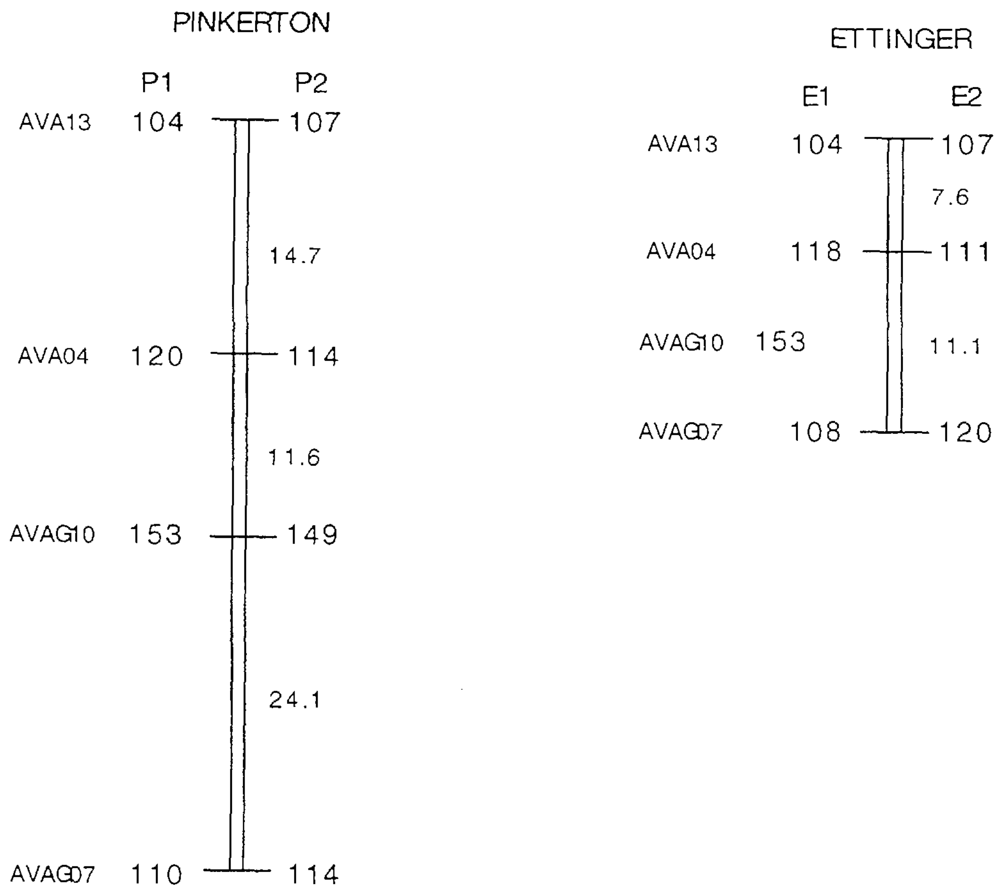
While in the human genome [5], $(GT/CA)_n$ repeats are the most frequent, the $(TC/AG)_n$ and $(AT/TA)_n$ repeats seem to be the most frequent in plants genomes, while in avocado we found that the two most frequent repeats are $(A/T)_n$ and $(TC/AG)_n$. The high number of predicted microsatellites in the avocado genome (~45,200, Table I) indicates that an SSR based map with high resolution can be obtained.

RFLP and isozyme markers show low heterozygosity levels in avocado compared to the results of this study. This is due to the high level of polymorphism in VNTR loci compared with RFLP and isozymes. For breeding purposes, a high degree of heterozygosity among cultivars can be an advantage, providing a high degree of variation among seedlings that might be exploited by selection. On the other hand, this situation limits the ability of a breeder to predict offspring performance.

4.2. Mapping the avocado genome

Of the 90 SSR, RAPD and DFP markers that were analyzed, 34 were mapped in linkage groups and 56 markers remain un-linked. The proportion of un-linked loci (62%) is higher than in other maps. In soybean, 40 SSRs were integrated into a known map using 60 F_2 plants (15). When only the 40 SSRs were analyzed, 18 of them (45%) remained un-linked. Poisson distribution analysis, indicated that the SSR markers are randomly dispersed in the avocado genome. Marker distribution analyses in other species has shown similar results (15). Linkage was found between a RAPD marker and a DFP band (linkage group 6) but not between SSR markers and DFP bands. The lack of linkage between SSR and DFP markers could be explained based on the finding that DFP loci are not randomly distributed but rather clustered in centromeric and telomeric regions. The differences in recombination frequencies between the paternal and maternal genomes are in agreement with [16]. They detected differences in

a



b

FIG.3. a. Parental haplotypes of linkage group 3.b. Contrast analysis between SSR markers on linkage group 3 and fibers in the flesh.

recombination frequency in tomato using the same cultivar as either the female or the male parent.

None of the computer mapping programs available to date can efficiently map the genome of an out-crossing species using polymorphic markers. The mapping approach which we used to map the avocado genome consists of transforming the avocado F1 mapping data to BC1 data which meet the MAPMAKER requirements. The result of this analysis was verified using other computer programs and found to be reliable. The loblolly pine genome, which is another out-crossing species, was mapped using the JoinMap computer [17]. This computer program is capable of analysing this data set, except for markers with segregation which deviates from Mendelian expectations.

4.3. Avocado fruit traits linked to DNA markers

The detection of association between genetic markers and QTLs is complicated in families resulting from a cross between heterozygote parents [18]. The main problems includes type I error in which a QTL is falsely detected as linked to a marker, and type II error in which no linkage is detected even though such linkage does exist [19]. Type I error is caused, in most of the cases, by using a high number of loci and a high number of traits and a type II error results from the demand of only high LOD score and P values. Thus QTLs which are far from the marker or have a small effect on the trait would not be detected. In the present study, 90 markers and nine traits (total of 810 pairwise comparisons), were detected. A significant value of 6.2×10^{-5} should be used to avoid type I error. This value should be corrected due to the existence of 12 linkage group having 34 linked markers (612 pairwise comparisons) resulting in a significant value of 8.2×10^{-5}. However, this high P value can cause a type II error and thus association between loci controlling fruit traits and DNA markers having a P value of P=0.01 or less were presented. The only association in which this P value was less than 8.2×10^{-5} is the one between linkage group 3 and the locus controlling fibers in the flesh on which we focused for further studies.

To verify the results, we used two statistical approaches: one-way analysis of variance and interval mapping. In most cases when association was found in the one-way analysis of variance it was also detected by the interval mapping except for two cases: the association between the marker AVAG24 and locus controlling seed size, and between the DFP marker DFP05 and locus controlling flesh taste. In these two cases there were only two alleles of the markers in the family, and the LOD score values of the interval mapping analysis were between 0.5 and 1. In one case, skin surface on linkage group 9, an association was detected by the interval mapping but could not be detected in the one-way analysis of variance. Although, a significant P value ($P<0.05$) could be detected, we did not consider this value as an indication for association.

It is noteworthy that no association was detected between fruit weight and any of the marker loci using both statistical approaches (P values of 0.02 and 0.03 were detected with two markers). On the other hand, there was a significant difference in the mean fruit weight between the offspring and the parents. This negative result can be explained by the finding that the trait is highly affected by the environment and heritability is quite low (20). Mhameed *et al.* [4] reported that no linkage was found between DFP bands and fruit weight even for ($0.01<P<0.05$) in this family. This result is compatible with the results obtained in this work and marked the difficulty in detecting markers which are linked to a locus controlling fruit weight.

In the case of the existence of fibers in the flesh, the offspring average is slightly higher than the parents performance, suggesting that a dominant component is involved in the determination of the trait. The Contrast Analysis suggested that three out of the four genotype groups of the marker AVA04, showed a low level of fibers in the flesh while the fourth group, having haplotypes E1 and P1, showed a high level of fibers in the flesh. This result can be explained by a negative dominant factor which is located on the haplotypes E2 and P2 and thus the fibers level of the offspring having one of those haplotypes is low compared with the offspring that lack these haplotypes.

The results presented in this study emphasize the importance of using highly polymorphic markers especially for map construction and detection of linkage with QTLs. The SSR markers which were used in this study, allow better detection of association with QTL compared with RFLP, RAPD or DFP markers.

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