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Association between DNA Markers and Loci Controlling Avocado Traits

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ABSTRACT. The detection of association between DNA markers and traits of interest in an outbred population is complicated and requires highly polymorphic markers. A genetic linkage map of avocado *(Persea americana* Mill.) recently generated consists of simple sequence repeat (SSR) markers as well as DNA fingerprint (DFP) and randomly amplified polymorphic DNA (RAPD) markers. These markers were used to detect putative quantitative trait loci (QTLs) of eight avocado fruit traits. Two statistical methods were used: one-way analysis of variance and interval mapping. Six traits were found to be associated with at least one of the 90 DNA markers. Based on the two statistical approaches, a putative QTL associated with the presence of fibers in the flesh, was found to be located on linkage group 3. This putative QTL was found to be associated with the SSR marker AVA04 having a high significant value *(P = 4.4 x 10^{-3})*. The haplotype analysis of linkage group 3 showed a putative dominant interaction between the alíeles of this locus.

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Avocado (*Persea americana* Mill.). is a subtropical fruit tree with unique characteristics. Its taste can be either mild, nutty or sweet (Bergh, 1975). The avocado is an important part of the human diet in some parts of the world due to its mineral, vitamin and protein contents (Slater et al., 1975). The world avocado production has increased by 500,000 metric tons during the past 15 years and was estimated to be >2 t in 1995 (FAO, 1995). Mexico is the main avocado producer (740,000 t) and the United States is the main avocado importer (24,000 t), while four European countries (France, United Kingdom, Netherlands, and Germany) import together 105,000 t annually.

Lavi et al. (1993a) studied the inheritance of three avocado traits: fruit color, flowering group and anise scent, and found that these traits are controlled by several loci each having few alleles. A high level of nonadditive genetic variance and a low level of additive genetic variance were found in the analysis of the variance components of nine agriculturally important traits (Lavi et al., 1993b) suggesting that in breeding programs, highly divergent cultivars should be used.

The leading avocado cultivars, 'Hass' and 'Fuerte', have some disadvantages and therefore, an avocado breeding program aiming to select better cultivars is being carried out in Israel. In this project, many crosses using various cultivars have been performed (Lavi et al., a997). The classical avocado breading programs are inefficient mainly because of a long juvenile phase, large tree size and limited genetic knowledge. Marker assisted selection (MAS) has the potential to improve the efficiency of tree breeding programs (Hospital and Charcosset, 1997.

Recently, DNA markers were applied to analysis of the avocado genome (reviewed by Lavi et al., 1994b). Restriction fragment length polymorphism (RFLP) markers were used to study genetic relationships in the *Persea* genus (Furnier et al., 1990), DNA fingerprint analysis (Lavi et al., 1991a) as well as detecting a genetic association between DFP bands and agronomically important traits (Mhameed et al., 1995). Simple sequence repeat (SSR) markers were recently applied for identification purposes and genetic analysis in avocado (Lavi et al., 1994a). The heterozygosity level of the avocado genome was studied using DFP bands and SSR alleles (Mhameed et al., 1996). Using these markers, analysis of genetic relationships in the *Persea* genus was carried out (Mhameed et al., 1997).

The main application of a genetic map is linkage analysis for the detection of important loci. Paterson et al. (1991) detected in tomato (*Lycopersicon esculentum* Mill.) linkage between RFLP markers and quantitative trait loci (QTL) for several fruit traits using the interval mapping approach. In potato (*Solanum tuberosum* L.) a *Phytophthora* resistance QTL was detected using diploid plants that are not inbred (Leonards-Schippers et al., 1994) QTL's for a few traits were located on a genetic linkage map of rice (*Oryza sativa* L.) (McCouch and Doerge, 1995). In apple (*Malus domestica* Borkh.), a randomly amplified polymorphic DNA (RAPD marker linked to apple-scab resistance QTL was recently reported (Yang and Korban, 1996).

The optimal population for detecting associations between markers and traits using interval mapping or linear model methods are F_2 or back cross (BC_1) in which the parents are inbred lines. In these populations, the alleles of the QTLs and the markers segregate, and the parental allele-phases are known. Although such populations are not available in most subtropical fruit trees, a cross of two outbred parents can be performed. The linkage

analysis using this family structure is more complicated and requires markers with high heterozygosity level, QTL's having a significant effect on the trait and a large number of offspring (Haley et al., 1994; Leonards-Schippers et al., 1994).

SSR markers were found to have high heterozygosity level in the human genome (an average of 70%) and thus were chosen for human genome mapping (Hudson et al., 1995). SSR markers in plants were applied mainly for identification and mapping proposes (Akkaya et al., 1995; Bell and Ecker, 1994; Roder et al., 1995). In avocado, SSR markers were used for genetic analysis (Lavi et al, 1994a; Mhameed et al., 1996; 1997). An integrated avocado genetic linkage map consisting mainly of SSR markers with the addition of DFP and RAPD markers was generated (Sharon et al, 1997). The map contains 12 avocado linkage groups having two to five markers on each, and covering together 353 cM.

In the current study, we screened for associations between DNA markers and eight avocado fruit traits.

Materials and Methods

Plant Material

Trees were grown at the Akko Experiment Station and the Agricultural Research Organization, Bet Dagan, Israel. The cross between the female parent 'Pinkerton' and the pollen donor 'Ettinger' was performed by caging the two parents, during the flowering season, under a net in the presence of a beehive. Young leaves from 60 offspring of this cross were used for DNA extraction.

Trait Evaluations

The fruit of the two parents ('Pinkerton' and 'Ettinger') and 60 offspring were evaluated for eight fruit traits.

After picking, the fruit were weighed and stored at room temperature until softening. After softening, skin color and skin gloss were evaluated, the fruit were opened and the following traits were evaluated: skin thickness, skin peeling, seed size, fibers in the flesh and flesh taste. The fruit were tested by a panel of three avocado experts throughout the harvest season of each seedling during 2 to 4 years. The evaluated traits are detailed here and presented in Table 1.

Fruit Weight. The fruit were weighed immediately after picking. The mean fruit weight of 'Pinkerton' was 290 g and of 'Ettinger' was 310g.

Skin Color. Skin color was assessed after fruit softening. 'Pinkerton' had a green skin color (rank 2), while 'Ettinger' had light green color (rank 1).

Skin Gloss. Four degrees of skin gloss were determined. 'Pinkerton' had matte gloss (rank 4) and 'Ettinger' had a medium level of skin gloss (rank 2).

Skin Thickness. Skin thickness was evaluated relative to that of several well known commercial cultivars. 'Ettinger' had a thin skin (rank 2) and 'Pinkerton' had a medium skin thickness (like 'Hass', rank 4).

Skin Peeling. The skin peeling of 'Ettinger' was difficult (rank 4) and that of 'Pinkerton' was medium (rank 3).

SEED SIZE. The seed size was measured as percentage of the fruit weight. The seed size of 'Pinkerton' was 11% to 15% and of 'Ettinger' was 16% to 20%.

FIBERS IN THE FLESH. The flesh of 'Pinkerton' did not contain fibers (rank 1) while the flesh of 'Ettinger' contained a few colorless fibers (rank 2).

TASTE. 'Ettinger' had a poor taste, while 'Pinkerton' had a good flesh taste.

DNA EXTRACTION

DNA was isolated using the cetyltrimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980), following a few modifications (Lavi et al., 1991a).

DNA FINGERPRINTING MARKERS

Ten micrograms of DNA were digested with *Hinf* I restriction endonuclease according to the manufacturer's recommendations (New England Biolabs, Beverly, Mass.). Electrophoresis, Southern blots and hybridizations are detailed elsewhere (Mhameed et al., 1995). The probe used for hybridization is 22.3, which was isolated from a genomic library of cattle (Haberfeld et al., 1991).

RAPD MARKERS

PCR amplifications of RAPD markers were performed in a total volume of 25 µL containing 30 ng genomic DNA, 1.5 mM Mg^{2+}, 0.2 mM primer, 250 mM of each nucleotide, l x Taq buffer (containing 200 mM $(NH_4)_2SO_4$,750 mM Tris HC1 pH = 9,0.1% Tween), and 0.5 unit of Taq DNA polymerase (Advanced Biotechnologies, Leatherhead, Surrey, U.K.). Forty microliters of mineral oil were placed on top of the reaction. The 44 PCR cycles started with a preliminary step of 94 °C for 3 min and consisted of denaturation at 94 °C for l min, annealing at 36 °C for l min and extension at 72 °C for l min, followed by an extension step at 72 °C for 5 min. Amplification products were analyzed by electrophoresis on 1.5% agarose gels and detected by staining with ethidium bromide. The RAPD primers were from set 7 (numbered 601 to 700) of the University of British Columbia.

SSR Markers

PCR amplifications of SSR markers were performed in a total volume of 10 µL containing 30 ng genomic DNA, l.5 mM Mg^{2+}, 0.15 mM of each primer, 100 mM of each nucleotide, 200 mg-mL^{-}1 bovine serum albumin (BSA), 0.1 µL of 3,000 Ci-$mmol^{-1}$ α -^{32}P dCTP, Ix Taq buffer (containing 50 mM Tris HC1 pH = 9, 0.1 % Triton X-100), and one unit of Taq DNA polymerase. Thirty microliters of mineral oil were placed on top of the reaction. The PCR reaction started with a denaturation step of 95 °C for 30 s, and was followed by 32 cycles consisting of denaturation at 95 °C for 15 s, annealing at 45 or 50 °C (depending on the primers) for 25 s and extension at 68 °C for 25 s followed by an extension step at 72 °C for 2 min. Ten microliters of stop solution (95% formamide, 0.02 M EDTA pH = 8, 0.1% bromophenol blue, 0.1% xylene cyanol, 10 mM NaOH) were added at the end of the reaction and 3 µL of the reaction were loaded on a DNA sequencing gel containing 6% polyacrylamide, 8 M urea and 1 x TBE at 50 W for 3 to 4 h. Gels were dried and exposed overnight to a Fuji X-ray film. The length of the SSR alleles was determined using a sequence ladder of M13 as size marker.

DETECTION OF ASSOCIATION BETWEEN DNA MARKERS AND LOCI CONTROLLING FRUIT TRAITS

The genotypes of 90 DNA markers (50 SSR's, 23 DFP bands, and 17 RAPD markers) were determined (Sharon et al., 1997) and used for further analyses. DNA fingerprint and RAPD markers were serially numbered (DFPO1 to DFP23 and RAPD01 to RAPD17, respectively). SSR markers were labeled with AV (for avocado) and then the repeat type (AG, AC, etc.) and a serial number (for example AVAG10). Each marker was classified into one of four groups (Groover et al., 1994). Maternal informative (MI) loci, which are informative for the maternal gametes only (the paternal genotype is homozygous), paternal informative (PI) loci, which are informative for the paternal gametes only (the maternal genotype is homozygous), both informative (BI) loci, which are heterozygous in both parents with the same two alíeles, and fully informative (FI) loci, where both parents are heterozygous and the number of alleles is three or four. One way analysis of variance was performed between offspring genotypes (of each marker locus) and each of the eight fruit traits using JMP (SAS Institute Inc., Gary, N.C.) computer program. For FI markers, the association between the presence of each allele and each trait was analyzed as well. The Contrast-Analysis method of the JMP computer program was used in order to search for possible allelic interaction affecting the content of fibers in the flesh. The level of heterozygosity of each offspring was calculated as the number of heterozygous loci divided by the total number of loci.

Detection Of Association Between Linkage Groups And Loci Controlling Fruit Traits

The interval mapping method was performed for the 12 avocado linkage groups (Sharon et al., 1997) and each of the eight fruit traits. For markers having two alleles in each parent, three different analyses were made based on paternal, maternal or combined linkage groups. The paternal or maternal interval mapping analysis of the 12 linkage groups was made using MAPMAKER/QTL software (Lander et al., 1987). The combined interval mapping analysis between linkage group 3 and the content of fibers in the flesh was made using MapQTL software (Van Ooijen and Moliepaard, 1996).

Table 1. Scoring levels and parents performance in eight avocado traits.

	Level									
Trait	1	2	3	4	5	6	7	Avg (SD)[z]	P[y]	E[y]
Fruit weight	Grams							245 (6.1)	290	310
Skin color	Light green	Green	Dark green	Black purple				2.0 (0.8)	2	1
Skin gloss	High	Medium	Slight	Matte				2.8 (0.7)	4	2
Skin thickness	Mexican type	Like Fuerte	Like Tova	Like Hass				2.4 (0.7)	4	2
Skin peeling	Excellent	Good	Medium	Difficult	Impossible			3.5 (1.2)	3	4
Seed size[x]	<5	6–10	11–15	16–20	21–25	26–30	>30	3.6 (0.9)	3	4
Fibers in the flesh	None	A few (colorless)	A few (colored)	Apparent (colorless)	Apparent (colored)	Numerous		1.7 (1)	1	2
Flesh taste	Excellent	Very good	Good	Poor	Bad			3.5 (0.6)	3	4

[z]Average performance and standard deviation of 60 offspring of the cross 'Pinkerton' x 'Ettinger'.
[y]Performance of the parents: E = Ettinger', P = 'Pinkerton'.
[x]As percent of fruit weight.

Results

Phenotypes Of Eight Avocado Fruit Traits. Scale grades of the eight traits and the parents' performances are presented in Table 1. The distributions of the offspring performances for each trait are presented in Fig. 1.

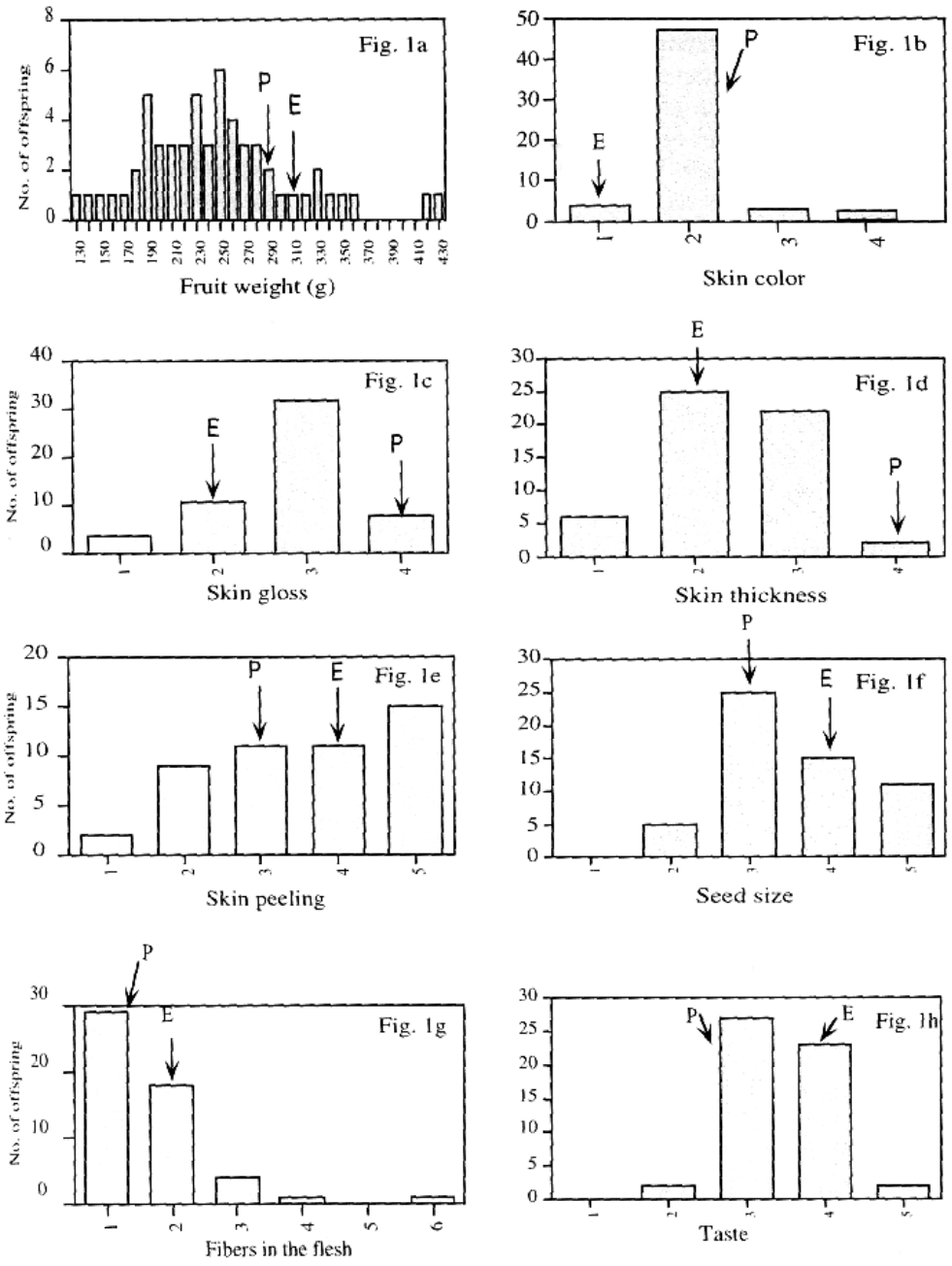

Fig. 1. (a-h) Distribution of the 'Pinkerton' x 'Ettinger' offspring according to their performance in eight avocado fruit traits. The performance of the parents is indicated by arrows.

The fruit weight of the parents is higher than the offspring average ('Pinkerton', 290 g; 'Ettinger', 310 g; Progeny, average 245 g). Progeny distribution is normal except for two offspring having high fruit weight (420 and 430 g). Most offspring have green skin color (rank 2), and a low degree of skin gloss (rank 3). The skin thickness of most of the offspring is thin (rank 2) to medium (rank 3). The skin peeling of the offspring is normally distributed with an average of 3.5. No offspring having small seed size (0% to 5%) were found and most of the offspring have medium (11 % to 20%) seed size. The flesh of most offspring do not contain fibers (rank I) as detected in 'Pinkerton'. Eighteen offspring have a few colorless fibers (similar to 'Ettinger', rank 2) and the rest have medium to high

levels of fibers in the flesh. Most of the offspring have poor (as 'Ettinger') to good (as 'Pinkerton') flesh taste.

ONE-WAY ANALYSIS OF VARIANCE. Sixty offspring of the cross 'Pinkerton' X 'Ettinger' were analyzed for possible associations between 90 DNA markers (50 SSRs, 23 DFP bands, and 17 RAPD markers) and eight avocado fruit traits. To avoid false positive results, a statistical significance threshold (P value) of ≤0.01 was used. The significant associations are presented in Table 2 and the mean trait values for each marker genotype are presented in Table 3. An association was found between skin gloss and three SSR markers, one DFP band and one RAPD band (the last two markers are located on the same linkage group). A highly significant association *(P*= 0.0014) was found between the SSR markers AVAG11 and AVA08 and skin gloss. One SSR marker was found to be associated with skin thickness and three SSR markers were found to be associated with skin peeling. The seed size trait was found to be associated with one SSR marker *(P* = 0.0006). The 'fibers in the flesh' trait was found to be associated with the genotype of three SSR markers which are located on linkage group 3. The significance level was very high for the marker AVA04 *(P* = 0.00001). Offspring having genotype 4 of this marker have a high level of fibers in the flesh (a mean of 3.8, Table 3). The flesh taste trait was found to be associated with one SSR and one DFP marker for which 'Ettinger' is heterozygote.

Table 2. One-way analysis of variance of avocado fruit traits.

Trait	Marker	Marker location[z]	Polymorphism source	*P*
Skin gloss	AVAG11		Ettinger	0.0014
	AVAG13		Ettinger	0.01
	AVA08		Ettinger	0.0014
	DFP03	Group 6	Pinkerton	0.005
	RAPD10[y]	Group 6	Pinkerton	0.004
Skin thickness	AVAG14		Ettinger	0.009
Skin peeling	AVAG01		Combined	0.006
	AVAC01[y]		Ettinger	0.003
	AVMIX06		Ettinger	0.005
Seed size	AVAG24	Group 8	Combined	0.0006
Fibers in the flesh	AVAG07	Group 3	Combined	0.007
	AVA04[x]	Group 3	Combined	0.00001
	AVA13	Group 3	Combined	0.01
Taste	AVMIX04	Group 5	Ettinger	0.01
	DFP05	Group 1	Ettinger	0.008

[z]Based on the avocado linkage map (Sharon et al., 1997). When not mentioned, marker's location is unknown.
[y]Deviated from Mendelian expectations ($P < 0.01$). Markers that deviate from Mendelian expectations were used for association detection in other studies as well (Uzunova et al., 1995).
[x]Deviated from Mendelian expectations ($0.01 < P < 0.05$).

The heterozygosity level of each offspring was analyzed in relation to each trait. No significant correlation values were found between heterozygosity level and the progeny performance in any of the eight traits.

INTERVAL MAPPING ANALYSIS. The location of loci controlling fruit traits was determined by interval mapping. The analysis was done for each linkage group separately (Table 4). In two cases, high log of odds (LOD) score levels (>2.0) were identified. A locus controlling skin gloss was found to be associated with linkage group 6, which is composed of two markers, for which 'Pinkerton' is heterozygote. A nonlinear decrease of the LOD score values was detected as a function of the distance between the supposed locus controlling skin gloss and the RAPD10 marker. The effect of this association explains 25% of the trait phenotypic variance.

Table 3. Trait mean for each associated marker genotype.

Trait	Marker	Genotype[z]	Trait mean	SD
Skin gloss	AVAG11	1	2.5	0.12
		2	3.3	0.17
	AVAG13	1	3.1	0.16
		2	2.5	0.14
	AVA08	1	3.2	0.16
		2	2.4	0.14
	DFP03	1	3	0.12
		2	2.3	0.19
	RAPD10	1	3	0.12
		2	2.3	0.23
Skin thickness	AVAG14	1	2.8	0.17
		2	2.2	0.13
Skin peeling	AVAG01	1	3.5	0.58
		2	4.3	0.36
		3	2.2	0.52
		4	3	0.44
	AVAC01	1	4.5	0.34
		2	3.2	0.21
	AVMIX06	1	2.7	0.36
		2	4.1	0.26
Seed size	AVAG24	1	3	0.20
		2	4.1	0.17
		3	3.6	0.21
Fibers in the flesh	AVAG07	1	2.8	0.28
		2	1.5	0.24
		3	1.4	0.29
		4	1.4	0.19
	AVA04	1	1.4	0.17
		2	1.2	0.24
		3	1.7	0.21
		4	3.8	0.34
	AVA13	1	2.7	0.39
		2	1.6	0.18
		3	1.5	0.22
Taste	AVMIX04	1	3.4	0.12
		2	3.9	0.14
	DFP05	1	3.3	0.14
		2	3.8	0.11

[z]Based on the avocado linkage map (Sharon et al., 1997). Ordered from the shortest alleles combination (1) to the largest (4).

Table 4. Linkage between loci controlling fruit traits and DNA markers loci based on interval mapping.

Trait	Linkage group	LOD score	Detected map[z]	Effect (SDs)	Variance explained[y] (%)
Skin gloss	6	2.9	Pinkerton	1.17	25
Fibers in the flesh	3	5.4	Combined	0.62	37.5
Taste	5	1.5	Ettinger	−0.81	16

[z]Linkage groups that are polymorphic in one parent are marked by the parent name ('Pinkerton' or 'Ettinger'). Linkage groups polymorphic in both parents are marked combined (Sharon et al., 1997).
[y]The percentage of the trait variance explained by the detected association.

A Locus Associated With Fibers In The Flesh. High LOD score levels were found between a putative locus associated with fibers in the flesh and the markers located on linkage group 3 (Table 4, Fig. 2). This linkage group is composed of four SSR markers, and the locus controlling fibers in the flesh showed the highest LOD score value of 5.4, close to AVA04. The locus effect explains 37.5% of the trait phenotypic variance.

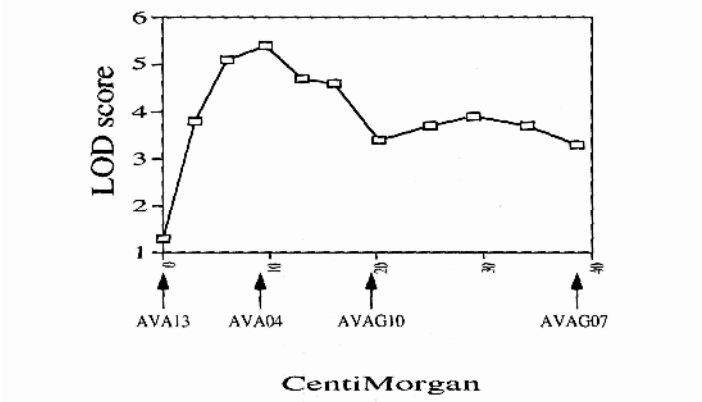

Fig. 2. The LOD score values for the location of locus controlling fibers in the flesh on linkage group 3. Marker location: 0cM = AVA13, 9.6cM = AVA04, 20.1cM = AVAG10, 38.5cM = AVAG07.

Figure 3a presents the parental phases of the marker alíeles on linkage group 3. This linkage group contains four SSR markers—both parents are heterozygotes for different alleles in two marker loci (AVA04 and AVAG07). Both parents are heterozygotes having the same alleles in the locus AVA13, while 'Pinkerton' is heterozygote and 'Ettinger' is homozygote for the locus AVAG10. Therefore, this latter marker is located on the 'Pinkerton' map only (distance of 11.6 cM from AV A04). The offspring are thus divided to various haplotype combinations while the four parental groups are the majority. Figure 3b presents a 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 controlling fibers in the flesh and the alleles of each marker on El +P1 haplotype combination is significant (except for the marker AVAG 10). This result indicates that a locus controlling fibers in the flesh is located within this linkage group close to the marker AVA04 and having high significant level *(P*=- 4.4 x 10^{-8}). Furthermore, this locus is associated with a high level of fibers in the flesh, only when a specific allelic combination is present (interaction between alleles). More specifically, only when P_1 alleles interact with E_1 alleles, the level of fibers in the flesh is significantly high.

Fig. 3A

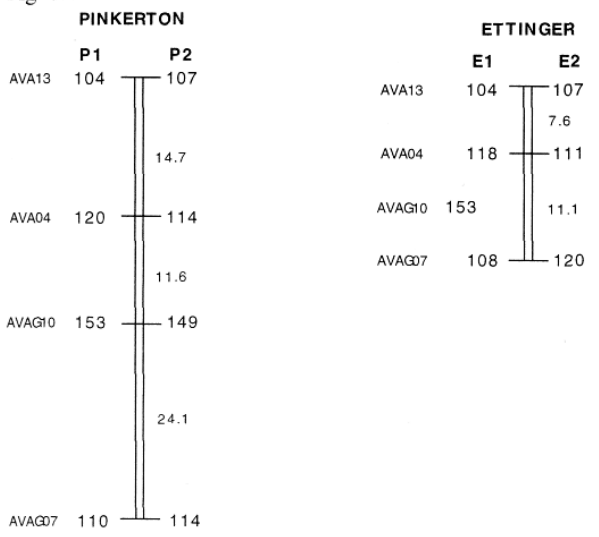

Fig. 3B

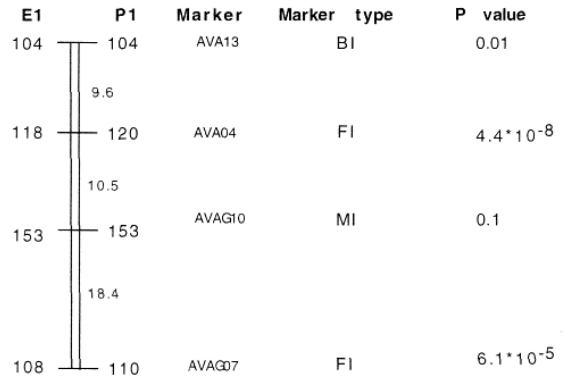

Fig. 3. (A) Parental haplotypes of linkage group 3. (B) Association between fibers in the flesh and simple sequence repeat (SSR) markers on linkage group 3. The order of the marker alleles on the linkage group for each chromosome is the haplotype and is labeled as P_1 and P_2 for 'Pinkerton' and E_1 and E_2 for 'Ettinger". Marker classification: MI (maternal informative) loci, informative for the maternal gametes only (the parental genotype is homozygous); PI (paternal informative) loci, informative for the paternal gametes only (the maternal genotype is homozygous); BI (both informative) loci, heterozygous in both parents with the same two alleles; FI (fully informative) loci, both parents are heterozygous and the number of alleles is three or four. P value = significance of contrast analysis.

Discussion

The detection of association between genetic markers and QTL's is complicated in families resulting from a cross between heterozygote parents (Knott and Haley, 1992; Leonards-Schippers et al., 1994). The main problems include error type I in which a QTL is falsely detected as linked to a marker, and error type II in which no linkage is detected even though such linkage does exist (Jansen, 1994). Error type I is frequent when association between high number of loci and high number of traits is searched. Error type II may be found when high LOD score and *P* values are used. Thus, QTL's which are distant from the marker or having a small effect on the trait would not be detected. In the present study, 90 markers and eight traits (total of 720 pair-wise comparisons), were analyzed. A significance value of 6.9×10^{-5} (0.05/720) should be used to avoid error type I. This value should be corrected due to the existence of 12 linkage groups having 34 linked markers (544 pair-wise comparisons) resulting in a significant value of 9.2×10^{-4} (0.05/544). However, this high *P* value can cause an error type II. In this study, association between DNA markers and fruit traits having *P* values of ≤ 0.01 were presented. Only the association between linkage group 3 and the locus controlling fibers in the flesh had a *P* value of $<9.2 \times 10^{-4}$ and therefore was further on studied.

A few factors may prevent the detection of association in this study.

1. A limited number of progeny. This obstacle reduces the statistical power of the association detection, and thus limits the number of detected associations.
2. Lack of heterozygosity in a certain parent of the marker locus and the putative QTL.

After the detection of association between a marker and a putative QTL, its location can be determined based on the LOD score values along the linkage group. The putative QTL controlling fibers in the flesh is probably located close to the marker AVA04 (the highest LOD score value along linkage group 3). The location of the putative QTL controlling skin gloss can only be roughly located on linkage group 6. The nonlinear decrease of the LOD score value along linkage group 6 indicates that the locus controlling skin gloss is located very close to the marker RAPD10.

We used two statistical approaches: one-way analysis of variance and interval mapping (the latter can be performed only after a genetic map is generated). In most cases when association was found in the one-way analysis of variance, the association was also detected by the interval mapping except for three cases (i.e., the association between the marker AVAG24 and seed size, between AVMIX04 and taste and between DFP05 and the taste). In these cases only two to three alleles of the markers segregated in the family, and the LOD score values of the interval mapping analysis were between 0.5 and 1.5.

No association was detected between fruit weight and any of the marker loci *(P* values of 0.02 and 0.03 were detected with two markers), even though a significant difference in the mean fruit weight is observed between the offspring mean and the parents mean. Possible explanations could be as follows.

1. The trait is highly affected by the environment, and heritability is quite low (Lavi et al., 1991b).
2. Fruit weight is controlled by a large number of loci each having a small effect, and the number of typed progeny was quite limited.

3. Fruit weight is controlled by a single locus which is not close to the analyzed markers.
4. The variance in fruit weight results mainly from interaction between various loci.
5. Markers linked to genes controlling fruit weight were not tested in this population.

Similarly, Mhameed et al. (1995) reported that no linkage between DFP bands and fruit weight was found.

No association was found between skin color and any of the markers, probably due to the low variation level in this trait (Fig. 1b) and the limited number of analyzed progeny. Mhameed et al. (1995) detected such a linkage in a larger family. Each of the other traits was found to be associated with at least one marker at a significance value of $P = 0.01$ or less.

The possibility that the level of heterozygosity (percent of heterozygous loci) of the offspring is correlated with the performance in each of the traits was studied. No association was detected between the performance of the progeny in the various traits and the heterozygosity level.

The contrast analysis between the four genotypes of the marker locus AVA04, showed that a specific genotype, originated from haplotypes E_1 and P_1, has a higher level of fibers. This result can be explained by a negative dominant factor which is located on the haplotypes E_2 and P_2. Thus, the fibers level of the offspring having one of these haplotypes (E_1 or P_2) is low in comparison to the offspring with the combination of E_1 and P_1 haplotypes. Furthermore, this linkage group demonstrates the importance of using highly polymorphic markers for detecting association with fruit traits. Two out of the four markers on this linkage group have two different alleles in the parents (FI = full-informative markers), one marker has the same alleles in both parents (BI = both-informative marker) and one marker has two alleles in 'Pinkerton' and is noninformative in 'Ettinger' (MI = maternal-informative marker). The significance of the association is very high for FI markers, is medium for BI marker and no significance values can be detected using the MI markers. Using the FI markers, one can distinguish between the four marker genotypes, and perform a χ^2 test for each group against the average of the other three. On the other hand, BI markers can distinguish between three groups only and thus the significance is lower (still one can distinguish between haplotype $E_1 + P_1$ and the remaining haplotypes). Unfortunately, the marker AVAG10 has two alleles only in 'Pinkerton' and thus only two genotypes are available. Using this marker, one can not distinguish between the haplotype combinations $E_1 + P_2$ and $E_1 + P_1$ resulting in a probable masking of the association *(*$P = 0.11$).

The difference between the marker types emphasizes the importance of using highly polymorphic markers for detection of linkage with QTL's. The SSR markers, which were the majority of the mapped markers, allow better detection of association with QTL in comparison to RFLP, RAPD, or DFP markers. The latter types of markers were used to identify associations between unmapped markers and avocado traits (Mhameed et al., 1995). Although DFP markers are highly polymorphic, only the presence or absence of one allele could be scored for each marker locus, while when typing SSR markers one can use the advantage of scoring up to four alleles segregating in a locus. Furthermore, using markers with three or four alleles, one can detect association with the genotype of each locus in each offspring.

The advantage of using mapped markers over single unmapped markers (Mhameed et al., 1995) is the opportunity to apply Interval Mapping using LOD score analysis and thus to genetically map the putative QTL.

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