Application of Molecular Markers for Avocado Improvement

Continuing Project; Year 3 of 5

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Benefit to the Industry

Molecular markers serve as reference points that help locate agriculturally important traits on a genetic map. The denser the clustering of the markers, the greater is the precision with which an agronomic trait can be pinpointed. A trait and a tightly linked molecular marker are likely to be inherited together, such that selection for the marker can replace selection for the agronomic trait. Unlike many yield-related traits that only become apparent after 3-6 years in mature avocado trees, molecular markers can be identified at the seedling stage, eliminating the necessity of maintaining large numbers of seedlings of which only very few may prove to have superior genotypes. Once a particular marker has been shown to be tightly linked to a trait of interest a pool of candidate seedlings can be pre-screened, retaining only those seedlings that possess the marker (and hence the trait). This highly efficient process is called marker-assisted selection (MAS) and makes avocado breeding both faster and more efficient.

Objectives

1. In order to achieve MAS, we are moving forward on three fronts to:
2. develop a large number of microsatellite markers (at least 100),
3. establish an experimental population of trees that will be used to assess associations between microsatellite markers and valuable traits, and
4. study the inheritance of markers to determine linkage relationships (chromosomal positions) of the traits in question and hence permit the construction of a linkage map.

We are also involved in several collaborative efforts that require microsatellite markers for the assessment of parentage (John Menge's rootstock selections) and pollination studies (Mary Lu Arpaia, Carol Lovatt (via graduate student Lauren Garner), and John Menge).

Discussion and Summary

***(1) Development of microsatellite markers*:**

To date we have sequenced 1250 clones of avocado DNA which has lead to the identification of about 50 microsatellite loci. We have sequenced both dinucleotide- and trinucleotide microsatellite regions and their comparison suggests that while dinucleotide microsatellite loci are far more abundant in the avocado genome than trinucleotide loci, many are difficult to interpret compared with the superior resolution of trinucleotide loci. Table 1 compares the two microsatellite types in terms of relative efficiency at different stages in the marker development process. Interestingly, both marker types yield similar numbers of promising loci in relation to the total number of

clones sequenced, but differ in their most labor-intensive step. Marker development will continue until at least 100 loci have been identified. Acquisition of an ABI377 automated sequencer will boost the sequencing throughput.

***(2) Experimental population*:**

In collaboration with Reuben Hofshi at ACW Groves, we have grafted four replicates each of 205 open-pollinated progeny of variety Gwen onto a uniform Duke 7 rootstock. Two replicates will be grown at South Coast Field Station and two at UC Riverside Agricultural Operations in a randomized block design. Some of the seedlings are ready for outplanting this fall, although a majority is expected to be ready next spring. At the time of planting, we shall record data on seedling height, branching, internode distance along the main stem, and root vigor to establish baseline data, and progress will be monitored at 6-month intervals. Yield-related traits and flowering will be measured when the trees have reached maturity.

(3) Linkage relationships:

Although our experimental population consists of the open-pollinated progeny of variety Gwen we predict, on the basis of previous results, that most of the progeny will have originated by selfing. Selfed progeny and co-dominant markers (such as microsatellite markers) provide insight into the distribution of markers and any tightly linked traits on the 12 avocado chromosomes, thereby closing the final .gap in the establishment of a genetic map for avocado.

Collaborative efforts:

Microsatellite markers are central to the assessment of parentage and pollination patterns. Pollination sources and levels of outcrossing can be inferred where the maternal parent is known and potential pollen donors are short listed (see examples in Figs. 1 and 2). A preliminary step is to screen the relevant genotypes (avocado varieties) for a range of microsatellite loci to determine which will differentiate best between the genotypes. Ideally, a single locus is sufficient to distinguish selfing from outcrossing or to uniquely identify the pollen donor. Typically, several loci are necessary to narrow down the pool of pollen donors and to rule out or confirm outcrossing versus selfing. When the candidate genotypes are closely related, e.g. have a common maternal parent the task of distinguishing between them becomes more difficult and usually involves a large number of loci.

Table 1. Comparison of trinucleotide (AVT) and dinucleotide (AVD) loci. The AVD data is preliminary.

	AVT (complete)	**AVD (in progress)**
Clones sequenced	**492**	**233**
Duplicated clones	**9%**	**7%**
No repeats present	**69%**	**14%**
Primers designed	**11%**	**64%**
Promising loci	**28 (57%)**	**6 (of 12 loci screened)**

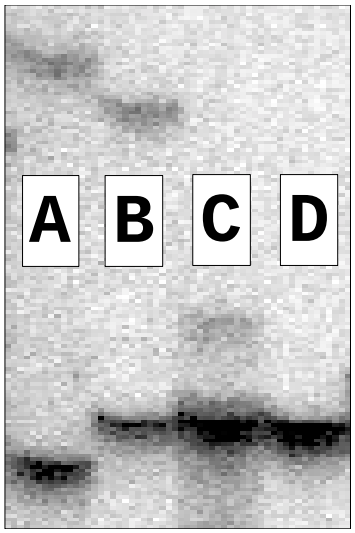

Figure 1. Excerpt of an autoradiogram of dinucleotide locus AVO102 including four genotypes that we are screening for Carol Lovatt. Lanes A through D are Hass, Bacon, Topa Topa and an unknown variety growing in the vicinity of the other three trees. Selfed progeny of Hass can be unambiguously distinguished from outcrossed progeny as the two bands of Hass differ from those present in Bacon, Topa Topa or the unknown tree.

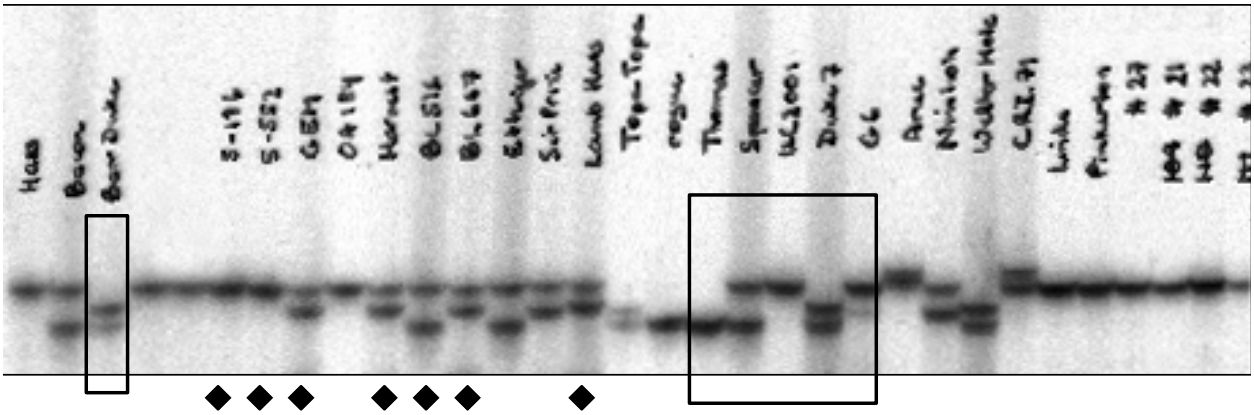

Figure 2. Autoradiogram of the trinucleotide locus AVT.386. Each lane corresponds to a single genotype. Diamonds mark varieties whose maternal parent is Gwen and are (from left to right) 5-186, 5-552, GEM, Harvest, BL516, BL667 and Lamb Hass (banding of Gwen itself is identical to the first two genotypes). This locus suggests that GEM, Harvest, BL516 and BL667 originated by outcrossing whereas 5-186 and 5-552 could have arisen by selfing. These varieties are being screened for a collaborative effort with Mary Lu Arpaia. Boxed genotypes are part of a study (collaboration with John Menge) that seeks to distinguish between selfing and outcrossing in Thomas interplanted with 5 other varieties (from left to right: Barr Duke, Thomas, Spencer, UC2001, Duke 7, G6). This locus will enable outcrossing to be confirmed in cases where Thomas was pollinated by UC2001 or G6, as these varieties have a different band to Thomas. Outcrossing can also be detected in cases where Thomas progeny inherited a band that differs from the one in Thomas, e.g. the upper band of Spencer, Barr Duke or Duke 7. Progeny exhibiting a single band identical to that of Thomas could be either selfed or outcrossed by Spencer, Barr Duke or Duke 7.