Application of Molecular Markers for Avocado Improvement

Continuing Project: Year 4 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.

Microsatellites are among the most powerful genetic markers available to date. As co-dominant markers they can be used to infer parentage and track pollen movement within an avocado grove. The great interest in this short-term application is reflected in our collaborative efforts.

Objectives

In order to achieve MAS, we continue to move forward on several fronts to:
(1) increase the number of microsatellite markers (to at least 100),
(2) manage an experimental population of trees that will be used to assess associations between microsatellite markers and valuable traits, and
(3) 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.
Additionally this past year we were involved in several collaborative efforts that require microsatellite markers for the assessment of parentage and pollination studies (Mary Lu Arpaia, Carol Lovatt (via graduate student Lauren Garner), and John Menge).

(1) Collaborative Efforts

(a) In collaboration with Dr. John Menge we ran some 80 progeny of variety Thomas against three loci in order to address outcrossing rates prevailing in the breeding block at UC Riverside. Additional varieties have been added to the analysis after we detected outcrossing events with pollen from an unknown source. The final analysis revealed appreciable outcrossing within the breeding block.
(b) In collaboration with Mary Lu Arpaia we screened and analyzed 25 microsatellite markers against 10 varieties that will be included in a large yield trial of variety Hass interplanted with various pollinizer varieties. The role of microsatellite markers is to infer (1) levels of outcrossing within the grove, (2) assess the relationship between yields and outcrossing, and (3) study the effects of distance from the pollen source. The screens suggest that, under favorable circumstances, about six markers would be sufficient to uniquely differentiate pollen donors. However, in the event of the most common marker(s) being inherited at each locus it may only be feasible to narrow down the pool of possible pollen sources.

(c) We also assisted Lauren Garner, a graduate student of Carol Lovatt, with her study of outcrossing versus selfing in the progeny of Hass. Her work focuses on aspects of pollen germination and fruit set in selfed and outcrossed fruit.
As part of the screens for our collaborative ventures we included several additional varieties that represent the three botanical varieties of avocado (Mexican, Guatemalan, West Indian). These data have been analyzed and will be submitted for publication later this year. The results complement those derived from similar studies using VNTR and RFLP markers.

(2) Experimental Populations
We have set up a replicated experimental population consisting of progeny of variety Gwen. Yield and growth related measurements collected from these trees will enable us to identify quantitative trait loci and their associations with microsatellite markers.

One of our two replicate populations resides at South Coast Field and Experimental Station (SCFS) in Irvine (405 trees) and the other at Agricultural Operations (AgOps), Riverside (129 trees), with a residual 81 trees remaining in the lath house on campus until they reach adequate size for planting out. Planting operations were split between October/November 2001 and April 2002 (SCFS), and March 2002 (Riverside). Baseline data on plant girth and height were collected last November for the first 280 trees growing at SCFS, and again this summer at both locations.

(3) Linkage Relationships
The segregation of bands in selfed progeny provides insight into the distribution of markers on the 12 avocado chromosomes, leading to the establishment of a linkage map for avocado. To address linkage relationships we have started to run microsatellite markers against 105 Zutano progeny trees growing at AgOps. Preliminary results from some 30 trees (Table 1) suggest that most are selfed progeny of Zutano (genotype aa, ab, or bb) and very few arose from outcrossing. Although outcrossed progeny can be used in linkage assessments the analysis is simpler when progeny are segregating selfs. It is noteworthy that Zutano is heterozygous at approximately 84% of loci screened so far. A high percentage of heterozygous loci will accelerate the process of linkage assessment.

Table 1. Segregation and outcrossing in the progeny of Zutano at two microsatellite loci.

GENOTYPE	AVO102	AVD006
aa	6	1
ab	19	20
bb	6	6
outcrossed	2 (#70, 107)	4 (#38, 70, 94, 107)
TOTAL	33	31

(4) Development of Microsatellite Markers
Due to the competing activities detailed above we have not progressed in the development of microsatellite markers. However, some 300 sequences obtained previously have been edited and screened and an estimated 150 sequences are suitable for primer design.

(5) Integration of the ABI 377 Automated Sequencer
A portion of the past year was spent setting up and integrating our new high-throughput ABI377 automated sequencer into the processes of DNA sequencing and running of microsatellite markers. The equipment is capable of handling 96 samples at a time (Figure 1), which corresponds to a two-fold increase over previous methods. Due to the high cost of fluorescently labeled primers for the ABI377 we cannot use the equipment for the process of screening new loci or troubleshooting problematic loci. These latter activities continue to be done by the traditional method of acrylamide gel electrophoresis using radioactive label.

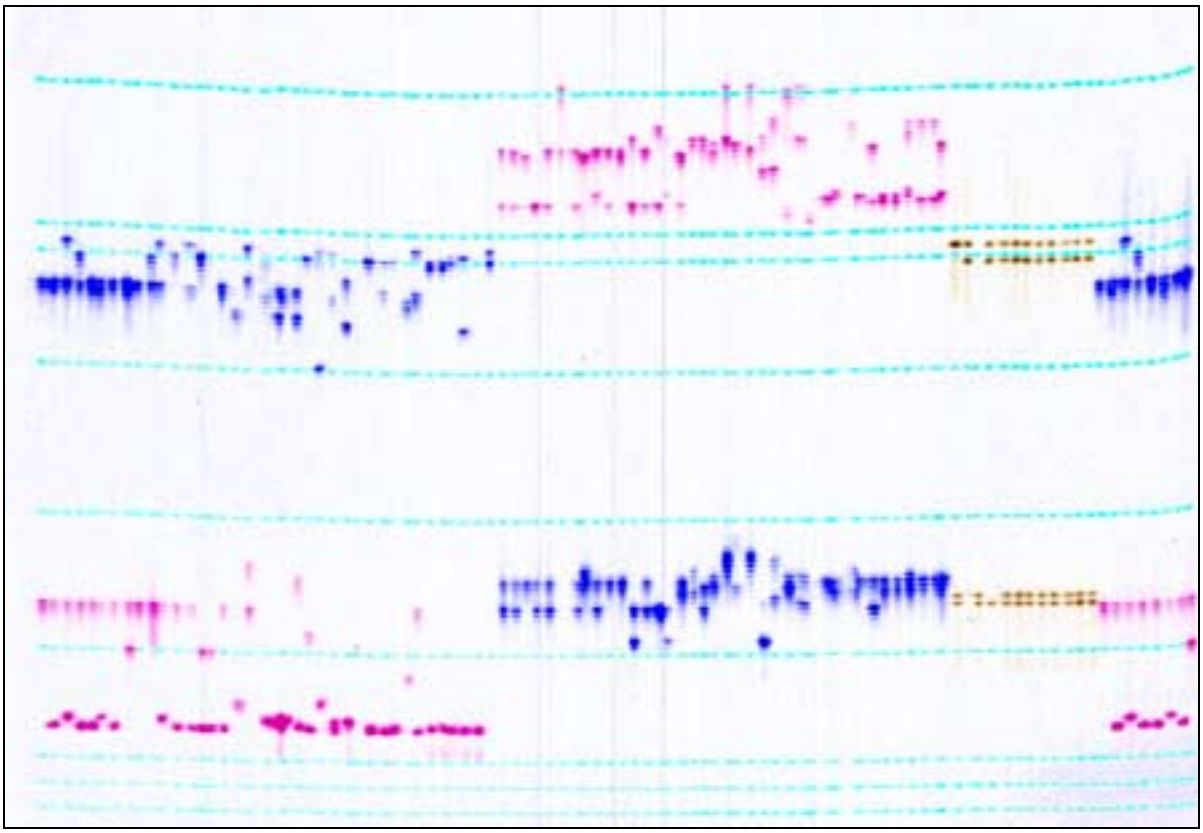

Figure 1. Electropherogram of a 96-well gel generated on an ABI377 automated sequencer. The first 76 lanes show four microsatellite loci, each locus occupying a different region on the gel and representing 38 separate genotypes. Fainter horizontal lines of bands are size standards that are used to control for minor variations in the gel matrix.