Application of Molecular Markers to Avocado Improvement

Ongoing Project

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Benefits to the Industry

Employing a molecular marker-guided methodology can accelerate the breeding of Avocado. One advantage of the molecular approach is that it eliminates the step of growing seedlings to mature trees: instead, seedlings are screened for the presence of markers correlated with desirable traits. The benefits to the industry are thus (1) a method that greatly accelerates the genetic improvement of cultivated avocado, (2) a deeper understanding of the genetic determination of complex traits of commercial value, and (3) an abundant source of molecular markers for a wide variety of applications in avocado improvement.

Objectives

The objectives of this project are to develop and apply microsatellite markers to the improvement and management of avocado. The most serious obstacle to avocado genetic improvement is the long life history of the plant. Any innovations that accelerate the breeding cycle or that improve breeding efficiency are especially valuable. Marker assisted selection of valuable traits that are genetically determined is a powerful means to accelerate the improvement of long-lived crop species. An essential prerequisite to marker-assisted selection is, of necessity, an analysis of the *degree of genetic determination* (H^2) for variation in valuable traits. Trait variation that is largely caused by environmental variation will not respond to selection because the genetic basis of the variation is small relative to environmental influences. Thus a successful program must begin by determining the heritability of trait variation and it must then associate markers with traits of high heritability. To achieve these goals we established well-characterized experimental populations in 2001 for Quantitative Trait Locus (QTL) analysis to provide resources for marker-assisted selection of valuable traits. These populations are now in their third year and are yielding valuable data on heritability for a number of traits. The second element of our program is to use standard genetic analyses to map microsatellite markers in association with valuable traits. As noted above, markers can be analyzed in seed or early seedling stages as predictors of the transmission of the valuable trait. Transmission of the marker is thus used as a screen to select particular seedlings for evaluation at the tree stage, thus saving greatly in time, land and labor resources and achieving a much higher intensity of selection.

The markers developed in this project are also ideal for the analysis of pollination. The transmission of a marker gene to a seed progeny can be used to retrospectively identify the pollen parent. This allows a precise analysis of the efficacy of particular pollination strategies in achieving optimal outcrossing, an issue of considerable interest to the industry.

Results

Microsatellite markers

We have developed a total of 127 microsatellite markers following the methods outlined in Ashworth et al., 2004. This provides a good resource for the genetic mapping of the Gwen progeny population, but our preliminary screening suggests that only about 60% of markers are heterozygous in Gwen. Since we can only map heterozygous loci we will continue to increase our inventory of microsatellite markers until we have at least 200 in order to assure a reasonable number of markers on each chromosome of avocado. Analyses reported in Ashworth et al., 2004 indicate that dinucleotide markers exhibit a higher level of allelic diversity than found at trinucleotide markers, at the same time dinucleotide markers are more likely to present gel patterns that are difficult to interpret. Table 1 below reports a characterization of a subset of microsatellite markers.

**Table 1. Avocado microsatellite markers**
The table gives the name of each marker, the mobility range on the ABI 377 gels (size range), the number of alleles observed in Hass, the percent heterozygosity in Hass, and the sequential size of allele intervals measured in nucleotides.

Locus	Allele Size Range	Alleles of Hass	Heterozygosity [%]	Sequential Allele Size Intervals [nucleotides]
AVT005b	186–201	186, 190	64.9	2 1 1 1 1 2 3 4
AVT020gat	152–179	161, 164	48.6	3 6 3 15
AVT021	130–139	130, 136	59.5	3 3 3
AVT038	175–202	187, 190	56.7	1 2 3 4 2 6
AVT106	303–315	309	35.1	3 3 6
AVT143	202–259	211	54.1	3 3 3 36 9 3
AVT158	261–272	267, 272	27.8	3 1 1 1 1 1 1 2
AVT191	167–173	170	40.5	3 3
AVT226	293–325	298, 302	62.2	3 2 1 1 2 2 4 17
AVT372	170–182	170, 182	64.9	3 6 3
AVT386	219–233	229	62.2	1 1 2 1 5 2 2
AVT436	137–155	152, 155	67.6	3 3 3 1 2 3 3
AVT448	163–202	192	43.2	15 3 1 2 6 2 1 6 3
AVT517	217–233	229	48.6	3 3 2 1 3 3 1
AVD001	218–264	224, 226	86.5	4 2 2 2 6 4 2 2 2 2 2 2 12
AVD002	301–341	327, 341	60.0	12 1 1 3 2 2 2 2 1 3 5 2 2 1 1
AVD003II	179–217	192, 211	73.0	2 11 3 1 1 1 1 1 1 2 2 2 2 6
AVD006	309–365	314, 351	75.7	3 2 1 3 9 1 2 1 5 2 2 5 2 1 1 1 1 3 9 2
AVD013	197–250	216, 242	78.4	18 1 1 1 2 1 1 8 7 4 1 1 5 2
AVD015	215–284	258, 260	34.4	9 1 4 6 3 14 2 4 2 22 2
AVD017	208–272	211, 254	69.4	1 1 1 5 2 10 9 3 4 10 11 7
AVD018	202–240	224	74.3	12 2 2 2 2 2 2 2 4 2 6
AVD022	216–256	222, 228	88.2	2 4 2 2 2 6 8 2 1 3 2 3 3
AVO102	141–202	153, 200	86.5	8 2 2 4 3 2 2 4 2 20 2 4 4 2
AUCR418	355–403	379, 381	80.6	2 4 2 2 8 6 1 1 2 2 4 2 10 2

Experimental populations

We established experimental populations at Agricultural Operations, UC Riverside (AgOps), and South Coast Research and Extension Center (SCREC), Irvine, about three and a half years ago. These populations derive from open-pollinated Gwen seedlings clonally replicated onto Duke 7 rootstock. Four replicates of each of 400 seedling genotypes were originally produced. These were planted in a randomized block design at AgOps and at SCREC with two replicates at each location. This design allows us to estimate the phenotypic, environmental, and by subtraction the genetic variance, within genotypes both within and between locations. These estimates provide a measure of the environmental (versus genetic) determination of a trait and they provide a measure of the influence of location in determining phenotypic variability. Estimating a location effect is very important because it tells the breeder whether a desirable phenotype will perform well over different environments. It also tells the breeder how predictable a desirable trait is over environments. Secondly, this design allows us to estimate the degree of genetic determination (denoted H^2 or heritability) of a desirable trait. It is clearly important to ask this question, because traits with low H^2 values will not respond efficiently to artificial selection. The ultimate goal is to associate microsatellite markers with desirable traits that have high H^2 values.
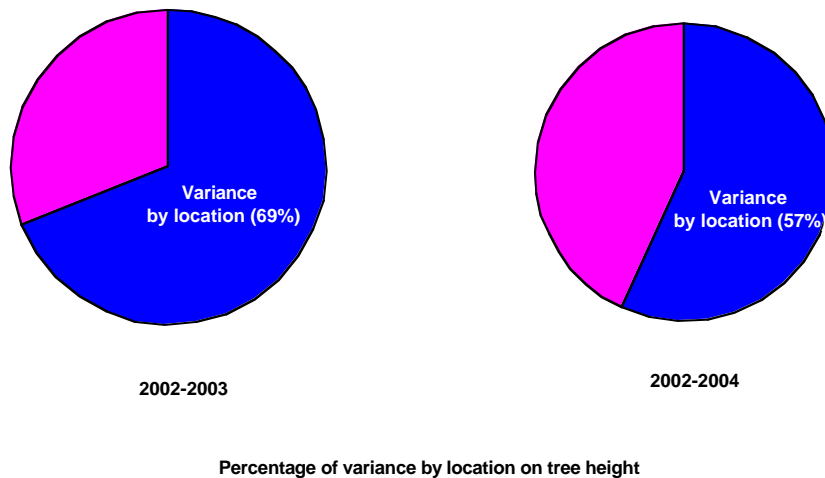
During the past year we have focused our efforts on the collection of phenotypic measurement data from the experimental populations at SCREC and at AgOps. This focus has been essential because the trees will not wait and the measurement data must be collected at several points during each year to accurately characterize tree development. The collection of precise measurement data is both essential and very time consuming.
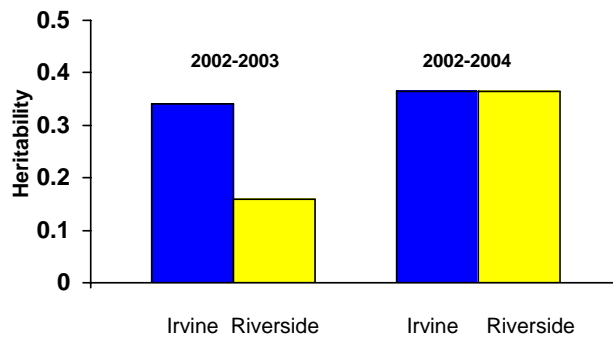
The Heritability of Canopy Diameter over time (Random model)

Mapping experiments

Microsatellite profiles are being collected from each experimental tree using the GeneScan software of our ABI Automated Sequencer, followed by statistical analysis using MapMaker. We anticipate that the mapping of these populations with the subset of markers heterozygous in the Gwen parent should be completed during the summer of 2005. We must also continue the extraction of DNA from our Gwen progeny to augment existing stocks that are running low.

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2002-2003　　　　　　　　　　　　　**2002-2004**

Variance by location (69%)　　　　Variance by location (57%)

Percentage of variance by location on tree height

Figures 1A and B. The two figures above display initial values of H^2 for canopy diameter and the proportion of the variance in tree height that is accounted by the between-location component based on the first two years' worth of data.

Data analysis

The statistical methods of quantitative genetics are being employed in the analysis of the measurement data. The SAS package of statistical tools is being used in data analysis and Professor Shizhong Xu is a collaborator on the statistical analyses. We are fortunate that Professor Xu is one of the world's foremost authorities on statistical genetics.

Collection of measurement data

We have data from 2002, 2003, and 2004 for tree height, stem girth and canopy diameter; we measure the trees once a year, during the period from July to September. We have just completed the cycle of measurements for this year. Data are being collected on precocious fruit bearing to ask whether potentially valuable traits have a strong genetic component. At SCREC, about 40% of all the trees developed fruit last year, while at AgOps, about 20% of all the trees developed fruit. (It is important to note that the AgOps trees were planted almost a year behind the SCREC trees so this difference is most likely a normal developmental effect.) We will continue the measurement of fruit number again this year.

Flowering data have been collected for 2003 and 2004. Collecting flowering data is quite time consuming. We record discrete categories for bloom stage, bloom intensity, bloom open percentage, bloom drop percentage, and fruit crop intensity. Data collection begins at the end of February and ends in the middle of May. Data should be collected once a week or once every two weeks until all of the flowers drop and fruit set begins. We then collect the fruit set data. We will continue these data collection regimes through the 2004–05 year and hopefully beyond. In

addition, we are beginning to measure fruit moisture content and oil content as fruit yields become substantial. Other characteristics will also be measured, including fruit shape, skin texture and color, taste, and maturation time, and we will record important yield characteristics such as propensity for alternate bearing.

Resistance to Persea *mite*

We discovered obvious segregation at SCREC for resistance to *Persea* mite. Some trees appear to resist *Persea* mite infestations and grow well, while others appear to be susceptible. Susceptible trees turn yellow and grow poorly. We have recorded this trait and will analyze its genetic determination during the next year.

Conclusions

Results obtained during the past year show a substantial H^2 for growth traits. These results also reveal a substantial location effect on phenotypic variation for growth-related characters. The methodology is now in place to begin to measure various fruit quality-related traits. Microsatellites are an abundant source of markers in avocado, and there is ample polymorphism within and between cultivars for successful QTL analysis.

Publications

ASHWORTH, V. E. T. M., M. C. KOBAYASHI, M. DE LA CRUZ, AND M. T. CLEGG. 2004. Microsatellite markers in avocado (*Persea americana* Mill.): development of dinucleotide and trinucleotide markers. *Scientia Horticulturae* 101: 255-267.