Application of Molecular Markers to Avocado Improvement

Continuing Project: Year 4 of 5

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Benefits to the Industry

The breeding of Avocado is poised to move from a traditional approach based on the *ad hoc* selection of open pollinated progeny from adapted cultivars to a molecular marker-guided methodology. 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 correlating closely with desirable traits. The second advantage is that the degree of genetic determination of commercially valuable traits is estimated using the statistical genetic models of quantitative genetics and this information provides a precise prediction of the likely response to selection. Hence the investigator can focus on those traits where progress will be rapid. Putting these two approaches together, the presence of a marker predicts the transmission of the major genes determining a valuable trait, eliminating the need to grow the seedling to maturity. If the marker is present, the seedling is destined to exhibit the trait upon reaching maturity. Therefore, only desirable seedlings will be raised to maturity, with enormous savings in husbandry practices and acreage.

Objectives

This project has five objectives: (1) to develop a large number of microsatellite or simple sequence repeat (SSR) marker loci; (2) to map the avocado genome with at least 100 SSR loci; (3) to establish an experimental population for quantitative genetic analysis; (4) to analyze marker-trait associations in this experimental population as a means of identifying major quantitative trait loci (QTLs) of potential commercial value; and (5) to initiate a program of marker assisted selection to accelerate the genetic improvement of commercial avocado.

Results During the Past Six-Month Period

Experimental Populations:
Our experimental populations at Agricultural Operations, UC Riverside (UCR Ag Ops), and South Coast Research and Extension Center (SCFS), Irvine, are doing well, with a minimal rate of tree loss. We have recently altered our data collection methodology by recording digital photographs of all experimental trees together with a reference scale. This library of photographic information will allow us to return to each tree at each sample point to measure new phenotypic attributes in the future. As of this writing, we have three sets of data on tree height and stem girth on all trees.

We have also scored several vegetative characters, and we have gathered data on flowering phenology. A number of the trees at SCFS set fruit in their second year in the field and one additional trait that we are now recording is precocity in fruit production.

Molecular Markers:
We now have 127 good SSR markers and we have begun to apply these to segregating progeny for genetic mapping. We have decided to suspend additional SSR development until we can complete mapping the set of SSRs already developed. We report some characteristics of the 127 markers in the Appendix below.

Schedule for the Coming Six Months
During the next six months we will concentrate on gathering data on SSR linkage relationships in the progeny of Zutano, Hass and Gwen. The data will be collected primarily using the GeneScan software of our ABI Automated Sequencer, followed by statistical analysis using MapMaker. We hope to have the mapping of these populations with the subset of markers heterozygous in each parent completed by Spring of 2004. We will also continue the extraction of DNA from our Gwen progeny to augment existing stocks that are running low. We will continue to gather measurement data from the experimental populations at SCFS and UCR Ag Ops. We plan to initiate some quantitative genetic analyses of the phenotypic data on early growth rate during the next six months. This should provide a useful indication of the degree of genetic determination of an important trait.

Publications

Ashworth, V. E. T. M. and M. T. Clegg. 2003. Microsatellite markers in avocado (*Persea Americana* Mill.). II. Genealogical relationships among cultivated avocado genotypes. (*J. Heredity*, in press).

Ashworth, V. E. T. M. and M. T. Clegg. 2003. Microsatellite markers in avocado (*Persea Americana* Mill.). I. Developing dinucleotide and trinucleotide markers. *Sciencia Horticultae* (in press).

Personnel

There have been several major personnel changes over the last few months. Dr. Vanessa Ashworth has been the lead person on this project for the past four years. Vanessa has done an outstanding job of developing SSR loci and of establishing our experimental populations of Gwen seedlings for quantitative genetic analysis. At the end of June 2003, Vanessa left the project of a one-year period to accompany her husband to Ireland where he is on sabbatical. We plan to have Vanessa return to the project in August of 2004. Paul Robinson worked 20% time on this project assisting in the maintenance of the experimental populations at SCFS and UCR Ag Ops. Paul also assisted in taking measurement data on tree growth parameters and on flowering phenology. Paul played a crucial role in the initial phases of establishing the experimental populations. Paul Robinson has decided to leave Riverside effective October 2003 and his services will no longer be available to this project. We have replaced Paul and Vanessa with Haofeng Chen, a PhD student who has just advanced to candidacy. This decision also accords with the reduced budget expected for the 2004-05 project year. Haofeng will complete

his PhD thesis research on QTL mapping and quantitative genetics in avocado. He is an excellent student with outstanding laboratory and statistical skills and I am confident that Haofeng's contributions will be substantial and will allow us to keep the project moving forward in Vanessa's absence. Haofeng has taken over the collection of measurement data from Paul Robinson and he is beginning to map microsatellite markers. Finally we have had an excellent undergraduate student work in the lab this summer on the preparation of avocado DNAs from the Gwen seedling populations and from other materials. These DNAs provide a valuable resource for our continued mapping work.

Appendix

We report the current list of 127 SSR markers available to the project in this appendix. The data also include information on repeat motif, annealing temperature and genotype in Gwen, Hass and Zutano.

Marker Locus	Annealing Temp.	Motif	Fragment Size	Bands in Gwen	Bands in Hass	Bands in Zutano
AUCR.008b	56	CT22	285	2	2	2
AUCR.011	59	TC11..TC19	383	2	2	2
AUCR.017	58	TC9AC9	359	2	2	1
AUCR.020	63	AC5AG22	320			
AUCR.046	64	GA12GAA5	351	2	2	2
AUCR.050	60	TC18	323	2	2	2
AUCR.051	59	AG12	ca. 397	1	1	2
AUCR.053	61	CT6..TC11	247	2	2	2
AUCR.089	56	GA9..GA14	217	2	2	1
AUCR.103	62	CT5TT(CT)16	ca. 180	1	2	1
AUCR.107	57	CT15	219	2	2	2
AUCR.114	59	AG21	211	1	2	2
AUCR.130	64	TC16	265		1	
AUCR.159	62	GA20	274			
AUCR.160	68	GA20	306	1	1	2
AUCR.168	61	GA17	167		2	2
AUCR.181	58	GA16	244	1	2	1
AUCR.183	61	AGT5AGA(AG)17	303	2	1	2
AUCR.202	57	GA15	253	1	2	1
AUCR.213	54	TC16	102	2	2	1
AUCR.220	58	GA17.GAn	253	2	1	2
AUCR.230	58	AG4.AG12	269	2	2	1
AUCR.232	52	AG22	259	2	1	2
AUCR.233	60	GA16	257	2	1	2
AUCR.236	58	AG17	255	1	1	2
AUCR.237	51	AG15	147	1	1	1
AUCR.244	56	TG8AG17	283	1	2	2
AUCR.250	59	GA13	149	2	2	2

AUCR.252	57	TC21AC7	244	2	2	1
AUCR.257	54	AG18	201	2	2	2
AUCR.271	52	AC9.AC8	134			
AUCR.273	65	AG20	271			
AUCR.392	64	CT16CA7	378			
AUCR.395	67	AG21	191			
AUCR.403	57	CT24	331		1	
AUCR.405	61	CT18CA12	161		2	
AUCR.406	55	GA6GT(GA)6	287		1	2
AUCR.409	56	AG25	390			1
AUCR.418	57	GT12GA13	379		1	2
AUCR.446	50	AC21	210			1
AUCR.452	64	AG14	195		1	
AUCR.466	60	GGA2.GGA6	367			
AVD.002	66	CT15CA13	327	2	2	1
AVD.003II	62	TC19	192	1	2	2
AVD.006	56	TC9AC19	314	2	2	2
AVD.010	61	TG5TG8GA10	263	2	2	1
AVD.013	62	AG7..GA3.TCT 4	216	2	2	2
AVD.015	60	GT26	258	2	2	2
AVD.017	64	TC18..AC8	211	1	2	2
AVD.018	68	GA20	224	1	1	1
AVD.022	65	TC13	228	2	2	2
AVD.026	55	AG9	170	2	2	1
AVD.028	65	AG18	187	2	2	2
AVD.029	57	AC14	225	2	2	2
AVD.032	63	GAA2GA19	182	2	2	1
AVD.033	58	TG13AG21	200	1	2	2
AVD.036	62	CA3GA15…CT 8..AA…GA12	121	1	2	2
AVD.037II	59	TC18 AC13	286	1	2	1
AVD.041	61	AG14	127	2	2	1
AVD.042	65	AG17	153	2	2	2
AVD.044	66	CT15	314	1	1	2
AVD.045	62	*TTC10 TTG6 GA9	288	1	2	1
AVD.046	60	TCC5	239	1	1	2
AVD.047II	59	AG7 GA13	177	2	2	2
AVD.050	59	GA26	196	2	2	2
AVD.052	59	GA10GA8GA2 5	322	2	2	1
AVD.053	65	AG15	223	1	1	1
AVD.065	67	TC7	141	2	2	1
AVD.067	63	TC19	277	1	1	2
AVD.068	55	TC19 TACA2	233	2	2	1

AVD.074	58	CT18	156	2	2	
AVD.080	65	TC8 AC12	169	2	2	2
AVD.082	57	AT5 GT14	131	2	2	1
AVD.088	66	AG18	195	1	2	1
AVD.089	64	GT13 GA20	266	2	1	2
AVD.092	61	GA13	214	1	2	1
AVD.098	59	CT18	278	2	2	2
AVD.103	58	CT20	195	1	2	2
AVD.104	58	CG4TG15AG2 2	223	2	2	2
AVD.105	66	TG16	293	2	2	1
AVD.107	61	TG15 AG8	186	2	2	2
AVD.112	59	GT5 AG21	287	2	2	1
AVD.115	56	AG16	274	1	2	2
AVD.116	59	GA5..AG23 GAs..	273	2	2	2
AVD.117	60	GA22	244	1	2	2
AVD.120	57	AnTn (AG14..T6)2x	200	2	2	2
AVD.122	58	TG28	321	2	2	2
AVD.127	68	GA16 GAn	180	2	2	2
AVO.102	58	GA12	153	2	2	2
AVO.109	59	TC22	153		2	
AVO.129b	59	TC20A(CA)9	205	1	2	1
AVO.207	57	GAA8..CT20	246		1	
AVO.303	54	..AAG5ATG9	216		1	
AVO.317	64	CAT7	138		2	1
AVT.001b	67	TGA8.	322	2	2	1
AVT.005b	62	CAT5	186	2	2	1
AVT.020gat	54	GAT9	164	2	2	2
AVT.021	65	ATC8	136	2	2	2
AVT.034	62	TCA5	229	2	2	1
AVT.038	56	TCA8	190	2	2	2
AVT.080	65	CAT6	163	1	2	1
AVT.106	68	TCA6	309	1	1	2
AVT.114	63	GAT6ATG4	298	2	1	2
AVT.133	64	ATG6,7	302	2	2	2
AVT.143	66	GAA8GAT6	211	1	1	2
AVT.144	66	TGA8	308		1	2
AVT.158	62	GAT7	267	1	2	1
AVT.191	69	ATG7TGG4	170	2	1	2
AVT.197	65	GAT5	297		2	1
AVT.219	58-65	GAT5GAT5	252		1	1
AVT.226	60	TCA6..CTT4	298	2	2	2
AVT.288	58-65	TGA7	356		2	2
AVT.295	61	TGA8	286		2	2

AVT.303	56	ATC8	233		2	1
AVT.306	58-65	TGA5	133		1	1
AVT.346	64	AC9AG14	238		2	2
AVT.364	56	TGA10	192		2	1
AVT.367	58	TGA6	233		1	1
AVT.372	58	TGA10	182	2	2	2
AVT.376	56-57	GA22	252	2	2	2
AVT.381	59-61	ATC7.ATC4	283	1	1	2
AVT.386	60	TGA8	229	1	1	2
AVT.417	67	CAT10	198	2	2	1
AVT.436	56	ATC9	152	2	2	2
AVT.448	60	GAT8	192	1	1	2
AVT.517	59	GAT6	229	1	1	2
AVT.574	58	TCA8	325	1	1	2