Assessing the Genetic Determination of Valuable Avocado Traits Using Microsatellite (SSR) Markers and Quantitative Trait Locus (QTL) Analysis

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Avocado Improvement

This research aims to strengthen our knowledge of the genome of avocado (*Persea americana* Mill.) and to meld molecular and measurement (phenotypic) data in order to expedite the process of variety improvement. QTL analysis is designed to detect associations between the presence of a particular molecular marker and the magnitude of a measured trait. Such associations can be translated into marker-based decisions on tree selection and breeding strategies.

Trait-Marker Associations

Varietal improvement in avocado has long relied on multi-year field trials, during which large numbers of seedlings are grown to maturity and compared for desirable characteristics. Inferior trees are removed from the breeding block as their deficiencies become apparent, leaving only the most promising genotypes. However, the time, land resource, and labor costs associated with growing trees to the appropriate stage of development are considerable.

The pace of varietal improvement would accelerate substantially through the application of molecular markers that are detectable using DNA extracted from seedlings. If transmitted along with desirable traits, the markers can be used as surrogates for these traits and can be applied quickly to a large number of seedlings to enrich the initial pool of trees for traits that are of interest to the Industry.

Our research is designed to identify markers that are co-transmitted with genetic factors conferring desirable characteristics in avocado. Our objectives are (1) to link avocado traits of interest to growers with molecular markers and (2) to harness this information via marker-assisted selection. This marker-guided method of variety improvement has the potential to increase selection intensity by several orders of magnitude.

Haplotyping

Another approach to the study of the avocado genome is to gather DNA sequence data. Distinct avocado genotypes can be characterized by means of differences in their DNA sequence at particular loci by comparing frequency and patterns of nucleic acid substitutions in each of the two parental sequence strands (haplotypes). When applied to a panel of wild and cultivated avocados, this comparison sheds light not only on current levels of genetic diversity but also on past domestication activity.

We (Haofeng Chen) generated haplotype data for 33 cultivated and 21 wild accessions of avocado at four gene loci. The wild genotypes formed three clusters corresponding to the three botanical races of avocado. An assignment test was then used to assess the membership of each of the 33 cultivars to these clusters (Figure 1). Minimal membership of the tested cultivars to the West Indian cluster was predictable, but the relative representation of the Mexican and Guatemalan haplotypes in several well known hybrid cultivars was surprising.

Haplotype data at the gene loci flavanone-3-hydroxylase, cellulase, chalcone synthase, and serine-threonine kinase indicated that genetic diversity in the cultivars is comparable to that present in the wild accessions and hence is not limiting for future breeding activities.

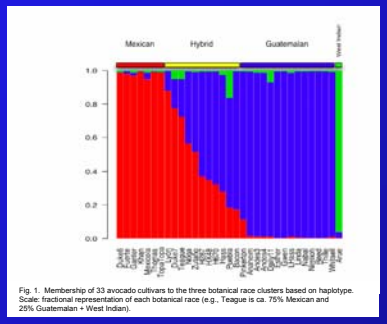

Fig. 1. Membership of 33 avocado cultivars to the three botanical race clusters based on haplotype. Scale: fractional representation of each botanical race (e.g., Teague is ca. 75% Mexican and 25% Guatemalan + West Indian).

Table 2. Broad-sense heritability and genotype x environment interactions for three measures of growth rate (tree height, canopy diameter, stem girth), flower abundance, and fruit load per tree. These values are based on over 90,000 data points.

	Tree height	Canopy diameter	Stem girth	Flower abundance	Fruit load
Broad-sense heritability (%)	34.4	29.7	28.5	32.3	23.4
G x E interaction (%)	NS	NS	NS	23.9	17.6

Table 3. Mean effects of pollen donor on growth rate (tree height, canopy diameter, stem girth; all in centimeters per month), flower abundance, and fruit load per tree.

	Tree height	Canopy diameter	Stem girth	Flower abundance	Fruit load
ÕbaconÕ	5.931(a,b)	6.045(b)	0.226(a)	1.965(a)	1.410(b)
ÕhuerteÕ	5.002(c)	6.482(a)	0.213(a)	1.418(c)	1.385(b)
ÕqutanoÕ	5.774(b)	5.241(c)	0.197(b)	1.846(a)	1.614(a)
Mixed	6.289(a)	6.484(a)	0.223(a)	1.604(b)	1.446(b)

Table 4. Average fruit weights [grams], with sample numbers in parentheses.

	SCREC	AgOps	Both locations
ÕbaconÕ	280.6 (48)	257.8 (41)	269.2 (89)
ÕhuerteÕ	268.3 (46)	264.4 (27)	266.4 (73)
ÕqutanoÕ	266.8 (38)	248.2 (30)	257.5 (68)
Overall	271.9 (132)	256.8 (98)	264.4 (230)

Table 5. Fruit shape (scored using IPGRI descriptors) arranged by pollen parent. Values are counts for AgOps and SCREC combined. Shape scores are: ÑzÖ spheroid; ÑöÖ obovate; ÑjÖ narrowly obovate; and ÑlÖ elavate.

	ÑzÖ	ÑöÖ	ÑöÖ	ÑlÖ	Totals
ÕbaconÕ	31	27	21	10	89
ÕhuerteÕ	26	11	20	15	72
ÕqutanoÕ	10	9	30	18	67
Total	67	47	71	43	228

Conclusions

Information on the progress of our research is presented in Tables 1–6. It highlights the need for extensive and ongoing data collection and analysis. The more exhaustive the data collected—both phenotypic and molecular—the greater the likelihood of detecting trait-marker associations and the greater the accuracy of marker-assisted selection.

Table 1. Summary of facts and figures relating to our markers and experimental trees.

1. 205 distinct genotypes of open-pollinated ÕflowerÕprogeny
2. Four clones of each genotype at SCREC (Irvine) and Agricultural Operations (AgOps, Riverside); two clones planted at each location
3. Grafted onto Duke 7 rootstock
4. Trees planted out in 2001 (SCREC) and 2002 (AgOps)
5. 398 and 285 trees (= 683 trees) at each site, respectively
6. 127 microsatellite markers
7. 364 and 161 trees (525 trees) bore fruit this year
8. 34.7% of genotypes were sired by ÕBaconÕ39.8% by ÕhuerteÕand 25.5% by ÕqutanoÕ
9. Fruit dry weights (MarchŠApril): 15.6Š43.8%, averaging 29.3 ± 5.0% DW at SCREC and 33.5 ± 5.4% at AgOps
10. Fruit weights: <100 g to 799 g, with an average of 281 g (SCREC) and 255 g (AgOps) (preliminary data)
11. Fruit load/tree: high (>100 fruit/tree) in 27.3% of trees, medium (50Š99 fruit/tree) in 38.3%, low (1Š49 fruit/tree) in 26%, and 8.5% of trees bore no fruit.

Table 6. Updated table on labor requirements for collection of data on growth rates, flowering, and fruit evaluations. Readings = number of measurements taken as part of the evaluations. Noninvasive[a] evaluations include fruit shape, weight, width, length, horizontal circumference, vertical circumference, and skin texture. Invasive[b] measurements include ripe fruit weight, 4 seed attributes, 4 skin attributes, and 4 flesh attributes.

Evaluation type	# Years or year	Readings per trait	# Fruits per tree	Tree #	Data points
Tree height	5	1	n/a	700	3,500
Canopy diameter	4	2	n/a	700	7,000
Trunk diameter	4	2	n/a	700	5,600
Flowering	3	6	n/a	700	75,600
				Subtotal:	**91,700**
Fruit[a]	2005	7	1Š15	62	6,510
Fruit[b]	2005	13	1Š3	62	2,418
				Subtotal:	**8,928**
Fruit[a]	2006	7	2Š8	525	29,400
Fruit[b]	2006	13	1Š3	525	20,475
				Subtotal:	**49,875**
				TOTAL:	**150,503**

Hours/evaluation: [a]Noninvasive: ca. 50 fruits in 3 hours = 16.7 fruits/h [3.6 min/fruit] [b]Invasive: ca. 30 fruits in 3 hours = 10 fruits/h [6 min/fruit]