Linking Candidate Genes to Biochemical Phenotypes in Avocado

July 8, 2008

I. Goal of project: to implement a program of marker-assisted selection (MAS) for nutritional traits in avocado

II. Funded jointly by CAC and UC Discovery Program: three-year project was initially funded by UC Discovery on February 1, 2008

III. Elements of project

   A. Measuring nutritional phenotypes
   B. Determining the heritability of nutritional phenotypes
      Gwen progeny population
   C. Cloning genes related to nutritional metabolism
   D. Identifying SNPs in candidate genes
   E. Implementing marker-assisted selection on Gwen progeny population
   F. Other related work

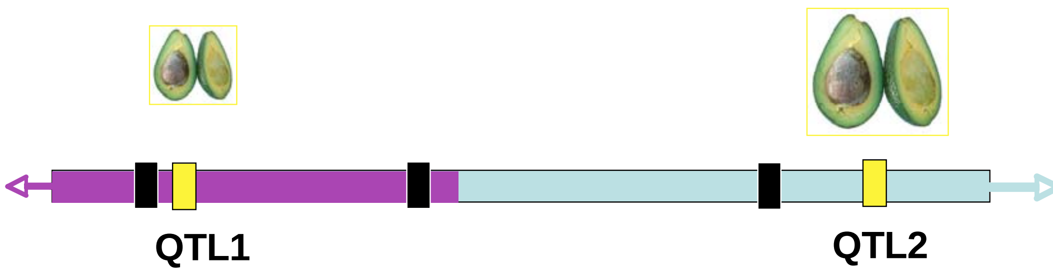
Rationale and Progress

Research suggests that consumption of avocado has a beneficial effect on human health by virtue of an array of antioxidants, vitamins, lutein, and the cholesterol-lowering and anticarcinogenic properties of β-sitosterol. Improvements in nutrition are "**value added**" traits that can be patented and sold at a premium. The development of new value added cultivars is important for the success of the California avocado industry.

- Measure these and related compounds in avocado fruit using thin layer chromatography (TLC) – *nutritional phenotype* **– initial validation of methods in progress**
- Determine the magnitude of genetic control of variation among trees in these compounds
    - Quantitative genetic analyses of Gwen progeny population – **Methods validated and populations in place**
        - Initial focus on Gwen x Fuerte progeny
- Clone genes in biosynthetic pathways that determine these compounds
    - Construct cDNA library - **Done**
    - Validate cDNA library – **In process**
    - Recover genes of interest (candidate genes) from cDNA library – **Will be the focus over the next six months**
    - Identify SNPs in candidate genes – **Will commence in year 2.**
- Employ SNPs for marker assisted selection (MAS) within Gwen progeny population – **Will initiate process at end of year 3.**
- **Personnel changes** – Vanessa Ashworth has taken a year's leave to accompany her husband on a sabbatical in Germany. Dr. Livia Tommasini joined the project as a post-doctoral scholar in June 2008 and Dr. Carlos Calderon is expected to join the project as a post-doctoral scholar in late August 2008.

Quantitative trait locus (QTL) analysis

Detects associations between magnitude of measured trait and a specific genetic marker (SSR allele)



= SSR marker

Segments of DNA inherited as a unit
SSR2 still detects QTL1

Table 1. Avocado genes to be sequenced. Sequences available in GenBank (non-chloroplast) as of late 2006 are in boldface

Biochemical Pathway or Gene Function Category	**Enzyme or Gene**
ABA Biosynthesis Pathways	1. **9-*cis*-epoxycarotenoid dioxygenase (NCED)**
	2. **Carotenoid cleavage dioxygenase (CCD)**
Amino Acid Biosynthesis Pathways	3. **Arginine decarboxylase**
	4. **Serine/threonine kinase**
Anthocyanin Biosynthesis & Phenylpropanoid Pathways	5. Anthocyanin synthase
	6. Chalcone isomerase
	7. **Chalcone synthase (CHS)**
	8. Dihydroflavonol reductase
	9. **Flavonol 3-hydroxylase (F3H)**
	10. Flavone synthase
	11. Flavonol synthase
	12. **Phenylalanine Ammonia Lyase (PAL)**
Carotenoid Pathway	13. ζ-carotene desaturase
	14. β-carotene hydroxylase
	15. Lycopene β-cyclase
	16. Phytoene desaturase
	17. Phytoene synthase
Cell Wall Hydrolyzing Enzymes	18. **Cellulase**
	19. **Endochitinase**
	20. **Polygalacturonase**
Fatty Acid Synthesis	21. **Fatty acid desaturase**
	22. **Fatty acid elongase**
	23. **Lipoxygenase (LOX)**

	24. **Stearoyl-acyl-carrier-protein**
Fruit Ripening Genes	25. **1-aminocyclopropane-1-carboxylate synthase (ACS)** 26. **Ethylene response sensor (ERS)** 27. **Ripening-related protein (pAVOe3) ACC oxidase ethylene forming enzyme**
Isoprenoid & β-sitosterol Biosynthesis Pathways	28. C-14 reductase 29. C-28 methyltransferase 30. C-8,7 sterol isomerase 31. Delta-7-sterol-C5 desaturase 32. Farnesyl pyrophosphate synthase 33. Geranyl diphosphate synthase 34. Sterol C-24 reductase 35. Sterol delta-7 reductase
Sugar Synthesis or Metabolism	36. **Beta-*D*-galactosidase (converts glucose to galactose)** 37. **Fructose-bisphosphate aldolase**
Vitamin C	38. GDP-mannose pyrophosphorylase 39. GDP-mannose-3,5-epimerase 40. L-galactono-1,4-lactone dehydrogenase 41. L-galactose dehydrogenase 42. Mannose-6-phosphate isomerase 43. Phosphoglucose isomerase 44. Phosphomannomutase
Vitamin E	45. Gamma-tocopherol methyltransferease 46. Homogentisate geranylgeranyl transferase (HGGT) 47. Homogentisic phytyltransferase (HPT) 48. Tocopherol cyclase

Miscellaneous Genes	49. **Cytochrome P-450**
	50. **Glutathione *S*-transferase**
	51. **Metallothionein-like protein**
	52. **Mitogen activated protein kinase (MAP kinase)**
	53. **Putative seed imbibition protein**

Quantitative genetics

- Progeny of ‘Gwen’ tree grafted onto ‘Duke 7 ’rootstock in 1999-2000
  - 204 genotypes, each has four replicates
  - Two locations (Irvine , Riverside)
  - Randomized block design
  - Planted in 2001
- Initial quantitative genetic analyses on growth rate and related traits (Chen et al., 2007)

Table 2. Growth rate traits

	Height	Canopy Diameter	Stem Girth	Bloom Abundance	Fruit Precocity
Broad Sense Heritability	34.31%	29.7%	28.45%	32.3%	23.44%

Table 3. Mean effect of different pollen donors

	Height (cm/mo)	Canopy Diameter (cm/mo)	T r u n k Diameter (cm/mo)	Flower Abundance	Fruit Setting
Bacon	5.931(A,B	6.045(B)	0.226(A	1.965(A	1.410(B)
Fuerte	5.002(C	6.482(A	0.213(A	1.418(C)	1.385(B)
Zutano	5.774(B	5.241(C)	0.197(B)	1.846(A	1.614(A
Mixed dono	6.289(A	6.484(A	0.223(A	1.604(B)	1.446(B)

*A,B and C mean significant difference (p=0.05)

Table 4. Correlation among traits

	Height		Stem Girth	Flowe Abundance	Fruit Setting
Height	1	0.681	0.661	0.096	0.524
Canopy diameter		1	0.66478	0.08945	0.488
St em Girth			1	0.081	0.411
Flower Abundance				1	0.179
Fruit Precocity					1