Year-End Progress Report

IMPLEMENTING MARKER-ASSISTED SELECTION FOR BIOCHEMICAL PHENOTYPES IN AVOCADO

Harley Smith
UC Riverside

Mary Lu Arpaia
UC Riverside

Team Members from UC Riverside: Vanessa Ashworth and Livia Tommasini

---

Traditional breeding approaches are used for the development of new avocado cultivars. In these approaches, fruit/seed are selected from cultivars with favorable traits including fruit quality. After germination, it takes 4 to10 years for the selected trees to reach a reproductive competent state and an additional 5 years to characterize each cultivar for fruit production and quality. One of the problems with the traditional breeding approach is a majority of the trees are discarded after characterization of fruit production due to unfavorable phenotypes. Therefore, traditional breeding approaches are time consuming, labor intensive and costly. The goal of this project is to implement marker-assisted selection (Collard and Mackill 2008; Tester and Langridge 2010) to identify avocado varieties with improved nutritional traits at the seedling stage. Previous research had identified molecular and biochemical markers that predict high carotenoid, beta-sitosterol and vitamin E content. The goal of this grant is to select for genotypes enriched in these nutrients in order to develop new cultivars that can be marketed for their health-related benefits.

During February and March, we evaluated fruit load in eight Gwen progeny genotypes (103, 112, 125, 138, 148, 162, 178, and 196) exhibiting elevated nutrient levels for one or more of these markers. Approximately 200 fruits from each genotype were harvested. These fruits were allowed to ripen and their seeds were excised, washed, and transported for professional germination at Farm ACW in Fallbrook, CA.

The traits of interest in the genotypes chosen are as follows:
- high carotenoid content: 103, 138, 178
- high beta-sitosterol content: 162, 178
- high vitamin E content: 112, 125, 196, 148
- desirable selection indices (sj values): 178 and 112
- multiple desirable properties: (sj value, carotenoids, and beta-sitosterol): 178

Approximately, 95% of the seeds germinated. Leaf tissue was collected from approximately 1600 seedlings and stored for DNA extraction. Because we had to extract DNA from a large number of samples (leaf tissue), a semi-high-throughput DNA

extraction assay was developed. This procedure allows for the isolation of DNA from 96 samples at a time. Since September, we have isolated DNA from over 1200 seedlings (82% of the total), with overall DNA concentrations averaging 31.6 ng/ul (Table I).

Box	Average DNA concentration	Sample number
1	20.15	96
2	44.05	96
3	**21.62**	96
4	21.52	96
5	19.45	96
6	34.30	96
7	35.13	96
8	22.91	96
9	17.35	96
10	38.61	96
11	50.36	96
12	In progress	(96)
13	41.2	96
14	In progress	(96)
15	44.2	96
16	In progress	(80)
TOTAL	31.2	1248 (of 1520)

Table I: Average concentration (ng/ul) of DNA extracted from seedling leaf tissue germinated from 8 progeny genotypes from the Gwen open-pollinated population.

Lastly, we have optimized the TaqMan PCR-based assay to identify molecular markers (SNPs) that predict the nutritive phenotypes (Figure 1).

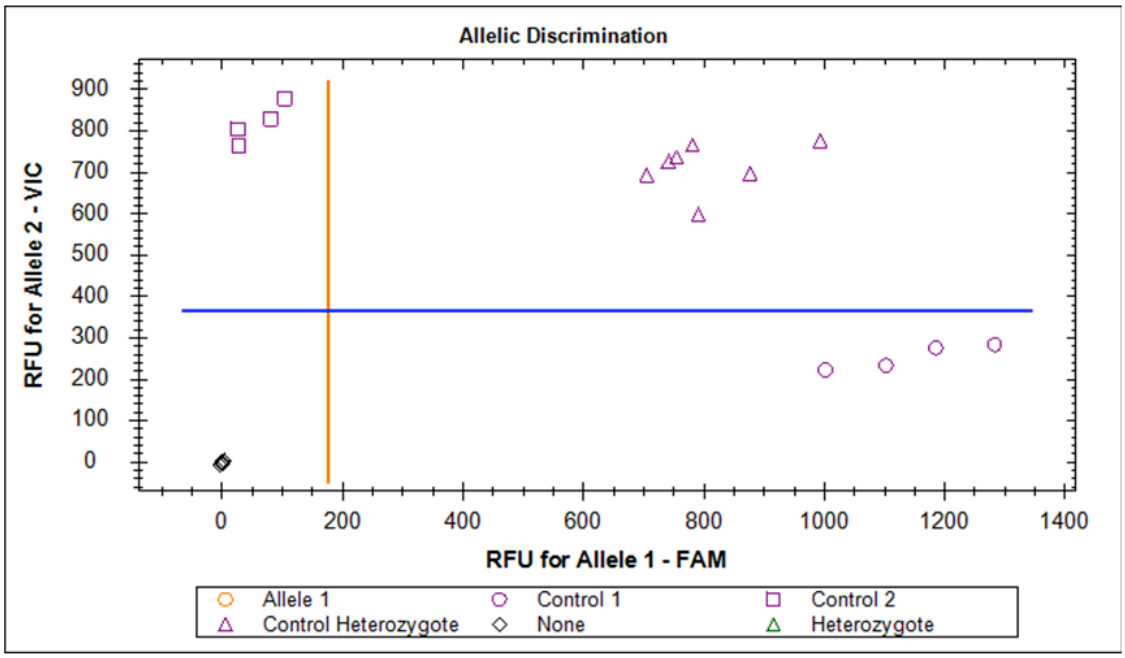

Figure 1: DNA concentration for each sample was measured using a Nanodrop instrument and successively diluted to 1-20 ng for the optimal template concentration for TaqMan SNP genotyping assay. This assay was performed with the putative lutein (LUT5) gene that had been previously cloned in the Gwen open pollinated mapping population. The PCR conditions were optimized on a BIO-RAD CFX96 real-time PCR instrument at the UCR core genomics facility. Results show that three control genotypes (control 1 = homozygous for allele a; heterozygous (ab), and control 2 = homozygous for allele b) can be successfully discriminated using this assay.

SELECTED REFERENCES:

Collard, B. C. Y. and Mackill D. J. (2008) Marker-assisted selection: an approach for precision plant breeding in the twenty-first centure. Phil. Trans. R. Soc. B 363: 557-572.

Tester, M. and Langridge P. (2010). Breeding technologies to increase crop production in a changing world. *Science* 327: 818–822.