Identification of Almond Varieties and Lineages using Molecular Markers

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Project Leader: Jerry Dangl
Foundation Plant Services
UC Davis
One Shields Ave.
Davis, CA 95616
(530) 752-7540
e-mail: gsdangl@ucdavis.edu

Project Cooperators and Personnel:
Joe Connell, UCCE – Butte County
Thomas Gradziel, Plant Science, UC Davis
Judy Yang, Foundation Plant Services, UC Davis

Objectives:

1. Develop a specific set of molecular markers effective in unequivocally distinguishing among both old and new California almond cultivars and accessions.
2. Utilize these markers to identify/characterize old heritage varieties (including clones still in public and private collections whose identity is uncertain), current varieties and potential future varieties.
3. Use the genetic marker composition of these evaluated almonds to establish their probable lineage including relationships to major commercial varieties.
4. Evaluate potential positive (yield advantage, disease resistance, etc.) and negative (graft incompatibility, bud-failure and disease susceptibility, etc.) association with different lineages as an aid to predicting future opportunities/problems, and to assist breeding efforts.

Interpretive Summary:

In 1924 Milo Wood of the USDA published a description of almonds in California in which he described over 164 different varieties being grown on approximately 90,000 acres. Two of those varieties, Nonpareil and Mission (then known as Texas) continue to be grown today. The molecular marker identification (genetic fingerprinting) methods developed in this research conclusively demonstrate that virtually all currently important commercial varieties grown in California are

progeny of Nonpareil by Mission crosses, thus documenting a tremendous loss of genetic variability over the last 100 years. Leaf samples of over 30 of the turn-of-the-century heirloom almond varieties described by Dr. Wood were collected for molecular analysis. Results highlight a tremendous genetic variability within this early germplasm. However, of the currently important almond varieties only Padre, Sonora, and Winters (all UC - Davis bred varieties) benefited from the incorporation of some of this diverse early germplasm. Previous molecular marker studies indicated that the Peerless variety may have substituted for Mission in the parentage of certain current varieties, thus increasing current genetic variability (and decreasing genetic vulnerability to disease, Noninfectious Bud-failure, etc.). However, the more effective molecular markers developed in this study show that this was not the case. Molecular analysis of advanced UC - Davis breeding selections now in grower trials demonstrate an extensive increase in genetic variability resulting from recent UC - Davis breeding efforts to incorporate new germplasm with improved disease/pest resistance and particularly self-compatibility and self-fruitfulness. Field levels of self-fruitfulness of UC - Davis selections were also verified through genetic fingerprinting of resulting progeny. Genetic fingerprinting using these markers also proved effective in identifying miss-labeled varieties as well as showing trueness-to-genotype of some 'off-type' selections (i.e. despite abnormal tree appearance/performance, the selections were shown to be the expected variety rather than a propagation error). These molecular markers, because of their accuracy and unambiguity, are also proving very useful in our ongoing efforts to determine probable parentage of elite breeding material as well as determining the genetic improvement efficiency of different crossing combinations.

Materials and Methods:

Details can be found in Dangl et al. 2009 (Recent Publications). In brief, leaves were collected from 180 almond trees, including old heritage varieties, current varieties and potential future varieties. The trees sampled are summarized in **Tables 1 and 2**. The leaves were placed between blotting paper in sealed, labeled envelopes and rapidly dried at room temperature using chemical desiccants. Genomic DNA was extracted from approximately 20 mg of the dried leaf tissue using a commercial extraction kit (DNeasy® 96 Plant Kit, Qiagen, Valencia, CA).

Nine Simple Sequence Repeat (SSR) DNA markers were selected for their ability to distinguish among both old and new California almond cultivars and accessions. Specific marker fragments were amplified by Polymerase Chain Reaction (PCR) using standard protocols. The amplified marker fragments were characterized by size in base-pairs using capillary electrophoresis on a Genetic Analyzer (ABI Prism 3100, Applied Biosystems). The resulting data were organized into spreadsheets and formatted to facilitate detailed analysis including use as a reference database for almond DNA profiles.

Results and Discussion:

Clear reproducible DNA profiles at 9 SSR markers were generated for 180 samples of almond varieties, heirloom varieties, almond breeding lines and advanced selections. These profiles were compared to one another and compared to an existing database of DNA profiles of many commercially important almond varieties (Dangl et al. 2009, **Table 3**). It is highly probable that any two samples with matching, identical, DNA profiles using these 9 markers are linked by vegetative cuttings to the original seedling tree. However, there is the possibility that the match is a random event. On the other hand, non-matching profiles absolutely eliminate the possibility that the trees are linked by cuttings to the same original seedling. A negative result (non-matching profiles) is conclusive; a positive match has to be discussed in terms of probabilities.

Several DNA profiles from almond varieties in **Table 1** matched those in the reference database. New samples of Mission, Nonpareil, Sonora and Sweetheart all exactly matched their respective reference profiles. These matches suggest, with a very high degree of probability, that these trees have been faithfully propagated by cuttings back to the original seedling tree. The probabilities that these matches happened at random are over one in a billion. One mislabeled tree of Solano was found to be Peerless and the DNA profile one misidentified Peerless tree did not match any from the database nor within this study, leaving its identity unknown.

This methodology of DNA fingerprinting cannot distinguish between mutations, bud-sports, within a variety though such mutations may be very significant. The data set contains, for example, several mutations of Nonpareil; all have identical DNA profiles. Though being able to distinguish bud-sports would be a useful tool, the ability to match a bud-sport back to the original variety is very useful, as well. One of the samples analyzed came from a young commercial orchard of Sweetheart; some trees appeared to be off type, perhaps due to incorrect identification of the nursery stock. The DNA profile of the atypical Sweetheart exactly matched the Sweetheart reference profile. The result suggests the tree may be a mutation, or the problem may be nutrition or disease related, not an error at the nursery. This result demonstrates the very significant real world applicability of this technology.

There were 27 new unique profiles from previously untested almond variety noted in Table 1. These profiles more than double the number of varieties in the DNA profile reference database. For some varieties two trees were sampled: Arboleta, Kutsch, Lewelling, Nonpareil and Tuono. For each of these varieties the two trees had identical profiles. Testing multiple trees of a variety and finding identical profiles adds confidence to the validity of a reference profile especially when the trees visually appear true to type.

The majority of trees analyzed in this study are breeder's selections or from various breeding lines, including several species hybrid populations. These 130 trees resulted in roughly 100 unique DNA profiles. Obviously there were trees within this group with matching profiles, though none matched any of the reference profiles. Trees within these breeding lines should all be selected seedlings. The matching profiles therefore are not the result of vegetative propagation; they must in fact be random matches.

Limited genetic variability, as would be seen in a population of siblings of the same cross, greatly increases the chance that the DNA profiles of two different seedlings will be identical. The genetic variability, reflected in the DNA profiles, is even more limited if a parent is a self or when testing a backcross population. One selection from the Winters x 179 population actually shares the same profile with the parent Winters, documenting a level of field self-fruitfulness in this variety. For one of the peach x almond backcross lines three of the four samples tested had matching profiles. In very specific cases we have seen identical profiles from distinct seedlings. Such situations could be resolve if necessary by analyzing the parents, by making and analyzing test crosses or by employing additional markers. We have developed the necessary markers and protocols for such cases. The system does unambiguously distinguish between typical almond varieties.

The unambiguous molecular marker identification (genetic fingerprinting) methods developed in this research conclusively demonstrate that virtually all currently important commercial varieties grown in California are progeny of Nonpareil by Mission crosses. Leaf samples of over 30 of the turn-of-the-century heirloom almond varieties were collected for molecular analysis. Results highlight a tremendous genetic variability within this early germplasm. However, of the currently important almond varieties only Padre, Sonora, and Winters (all UC - Davis bred varieties) benefited from the incorporation of some of this diverse early germplasm. Previous molecular marker studies indicated that the Peerless variety may have substituted for Mission in the parentage certain current varieties, thus increasing current genetic variability (and decreasing genetic vulnerability to disease, Noninfectious Bud-failure, etc.). However, the more effective molecular markers developed in this study show that this was not the case. Molecular analysis of advanced UC - Davis breeding selections now in grower trials demonstrate an extensive increase in genetic variability resulting from recent UC - Davis breeding efforts to incorporate new germplasm with improved disease/pest resistance and particularly self-compatibility and self-fruitfulness.

The DNA profiles generated in this study are being used to deduce or document parent/progeny relationships. Statistical analysis of the profiles can also illuminate broader relationships among the varieties: broader family groups, geographic origins and species composition. These further, in depth, analyses are in progress. The results will be published in a peer reviewed journal. The

Almond Board of California will be provided with manuscripts and final publications.

Recent Publications:

Dangl, Gerald S., Judy Yang, Thomas Gradziel, Deborah A. Golino. 2009. A practical method for almond cultivar identification and parental analysis using simple sequence repeat markers. Euphytica 168 (1): 41-48. On-line at: http://www.springerlink.com/content/n75l611429277407

Table 1. Forty nine almond trees, including current and heirlooms varieties were analyzed with nine Simple Sequence Repeat DNA markers. Profiles identical to reference profiles or identical to profiles within this study are indicated.

Variety	Profile Match	Comments
Arboleta Almond	Arboleta	new reference
Arboleta Almond	Arboleta	new reference
Kutsch	Kutsch	new reference
Kutsch	Kutsch	new reference
Lewelling	Lewelling	new reference
Lewelling	Lewelling	new reference
Livingston	Livingston	new reference
Livingston bitter branch	Livingston	Livingston mutation
Mission	Mission	
Langeudoc	Mission	
Mission bud failure	Mission	Mission mutation
Walton	Mission	
Nonpareil	Nonpareil	
Nonpareil	Nonpareil	
Nonpareil	Nonpareil	
Nonpareil bud failure	Nonpareil	Nonpareil mutation
Softshell Unknown	Nonpareil	Historic interest, Chico, Nonpareil mutation
Tardy Nonpareil	Nonpareil	Nonpareil mutation
Vivescent	Nonpareil	Nonpareil mutation
West Steyn	Nonpareil	Nonpareil mutation
Jeffries	nothing	Putative Nonpareil mutation
Peerless, wrong	nothing	Does not match Peerless reference
Solano, wrong	Peerless	Does not match Solano reference
Goldenstate	Sonora	
Sonora	Sonora	
Sweetheart	Sweetheart	
Sweetheart, atypical	Sweetheart	Tree from a commercial orchard
Tuono	Tuono	
Tuono	Tuono	
13-1	Winters	Winters variety
2-19E	nothing	new reference
Arbuckle	nothing	new reference
Bigelow	nothing	new reference
Davey	nothing	new reference
Eureka	nothing	new reference
Ferragnes	nothing	new reference
Harriott	nothing	new reference
IXL	nothing	new reference
Jordanolo	nothing	new reference
La Marie	nothing	new reference
La Prima	nothing	new reference
LeGrand, Delta	nothing	new reference
Marcona	nothing	new reference
Milow	nothing	new reference
SansFaute	nothing	new reference
SmithIXL	nothing	new reference
SydneySpecial	nothing	new reference
Trusito	nothing	new reference
Vesta	nothing	new reference

Table 2. Summary of breeding stock and other trees tested.

Type	Selection or Population	Comments
Almond grower selection	Bee Bio Late	
Almond grower selection	Booth Almond	
Almond selection-haploid	Stokey F5C, Haploid	Stokey F5C, haploid and diploid types have IDENTICAL PROFILES
Almond selection-diploid	Stokey F5C	Stokey F5C, haploid and diploid types have IDENTICAL PROFILES
Almond species cross	D3-	5 different selections tested
Almond species parent	*Prunus fenzliana*	
Heirloom variety cross	05, 9-	3 different selections tested. One selection MATCHES WINTERS, a random match to a close relative
Heirloom variety cross	NP x 232 + 3-40 hex pop.	10 different selections tested
Heirloom variety cross	Winters x 179 (plus self)	10 different selections tested, one selection MATCHES WINTERS, a random match to a close relative
Self compatible breeding line	several populations	39 selections tested, , 2 have IDENTICAL PROFILES
Self incompatible breeding line	several populations	20 selections were tested, several sets have IDENTICAL PROFILES
Historic interest	Bidwell Almond	Trees of historic interest, Chico, variety unknown
Historic interest	Hite Trees	Trees of historic interest, Chico, variety unknown
Peach - almond breeding line	several populations	7 different selections tested
Peach - almond chimera	95, 12-130 and 97,13-89	5 different selections tested all have IDENTICAL PROFILES
Peach parent	NSW2-36	The 2 NSW2 selections have IDENTICAL PROFILES
Peach parent	NSW2-37	The 2 NSW2 selections have IDENTICAL PROFILES
Peach parent	18,8-11	peach parent
Peach parent	Dr Davis	peach parent
Peach parent	Hesse	peach parent
Peach x almond backcrosses	EL	4 different selections tested, 3 have IDENTICAL PROFILES
Peach x almond backcrosses	F10C	2 different selections tested
Peach x almond backcrosses	F8,1-42	
Peach x almond backcrosses	LeGrand OP (10-3+4)	
Species cross with *P. persica*	several populations	11 selections were tested, *P. persica* crossed with several other Purnus species

Table 3. Almond varieties comprising a reference database of DNA marker profiles.

Accession	Source[a]	Accession	Source[a]
ALDRICH	FPS, CN1	PADRE	FPS, CN1
BUTTE	FPS, CN1	PEERLESS	FPS, CN1
CARMEL	FPS, CN1	PRICE	FPS,
FRITZ	FPS, CN1	RUBY	FPS, CN1
KAPAREIL	FPS	SOLANO	CN2, WEO
KOCHI	FPS	SONORA	FPS, CN1
MISSION	FPS, CN1	SWEETHEART	FPS
MONTEREY	FPS, CN1	THOMPSON	CN2, CO2, WEO
NE PLUS ULTRA	FPS	TITAN	FPS
NONPAREIL	FPS, CN1	WINTERS	FPS
NORMAN	CO1		

[a]Abbreviations: FPS, Foundation Plant Service, U.C. Davis. CN1, Commercial Nursery 1.CN2, Commercial Nursery 2. C01, Commercial Orchard 1. CO2, Commercial Orchard 2. WEO, Wolfskill Experimental Orchard, U.C. Davis.