Accelerated Assessment of Almond Variety Candidates

Project No.: **HORT51-Gradziel**

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A. Summary

The California almond industry is in a time of major transition. Recognizing this challenge, the Almond Board of California has established bold goals for improved production efficiencies including reductions in orchard dust, water and agrochemical inputs. While the goals and timelines have been established, the strategies to achieve these ambitious goals have not. As has been demonstrated in previous major transitions in farming methods, complementary genetic options have been essential to every success. A consequence of our long-term efforts to incorporate diverse germplasm from European and Asian almonds as well as related almond and peach species is that the UCD almond breeding program currently has more promising almond genetic diversity than any other collection in the world, including at the otherwise very impressive nearby USDA/ARS Germplasm Repository. The UCD breeding program has incorporated much of this new and diverse germplasm into California adapted selections meeting commercial requirements for nut quality and tree productivity while possessing novel characteristics such as self-fruitfulness, improved resistance to diseases, pests and environmental stresses such as drought and salinity, as well as novel tree architectures and bearing habits. The incorporation of novel traits inherently runs the risk of also incorporating undesirable traits, some of which may not be readily detectable. To rogue-out selections with undesirable traits, a series of regional trials have been developed to allow accelerated assessment of promising breeding candidates prior to larger scale Regional Variety Trial testing. In 2020/21, five new regional accelerated assessment trials were added for evaluating over 50 new UCD and international selections, totaling over 3000 trees.

B. Objectives

The objective is the creation of interim assessment plots to rapidly identify defects and deficiencies in new UCD variety candidates as well as new varieties from Spain and Australia prior to advancement to the more intensive and long-term RVT evaluations. The management flexibility present in such inherently high-turnover evaluation trials would also facilitate pioneering research targeting more efficient orchard production systems.The ultimate goal is to increase the speed and efficiency of current research targeting improved varieties and improved orchard practices for California.

Goals: 2020

1. Development multi-location sites for the interim assessment of promising UCD and international breeding selections.
   a) Establish plots at Arbuckle and Wolfskill, CA and pursue locations in the San Joaquin Valley.
   b) Collaborate with geneticist in Australia, Spain, France, Italy and Israel to identify, characterize and, if desirable, acquire for California testing the most promising new international varieties and germplasm.
2. Utilized the management flexibility and high turn-over rates of these interim plots to facilitate the assessment of different breeding lines under different orchard configurations of planting density, tree size, architectures and bearing habits.
   a) Promote and support field coupled with computer-modeling research to optimize orchard configurations for more efficient field management including off-ground harvest.

C. Annual Results and Discussion

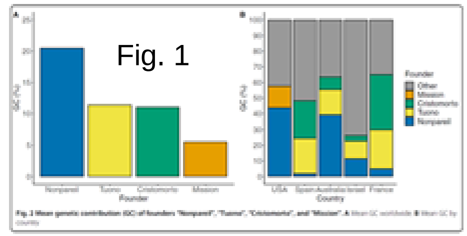
1a) **Establish plots at Arbuckle and Wolfskill, CA and pursue locations in the San Joaquin Valley.** The UCD Accelerated Evaluation block was planted at the Nickel Soils Lab in Arbuckle California on November 19, 2020. UCD selections planted included 15 new self-fruitful selections from divergent genetic backgrounds (Table 1) with Nonpareil as a standard as well as 4 UCD cultivar/rootstock experimentals. Also included were the Australian and Spanish cultivars discussed below in section 1b. UCD selections were planted on Krymsk86 in a 3 x 3 block which had previously allowed an assessment of degree of self-compatibility & self-pollinatation for the adjacent Nickels-Self-Fruitful-Evaluation Plot planted in 2008. In 2020/21, we have repurposed the Central Leader/Medium High Density block at the Wolfskill Experimental Station (WEO) in Winters California which was planted in 2018 as an unpruned, central-leader tree architecture study. With the completion of that study (see final 2020 report by Bruce Lampinen), we have continued to evaluate the 10 UCD selections for overall production and quality and more specifically, for effect of different bearing-habits and tree architectures to final yield and light-use efficiency. A very large UC selection evaluation study was also established in 2020 in Bakersfield, California to evaluate the Spanish style very high density/catch frame harvest management system (see summary Table 2). This is a long-term study involving over UCD 2500 trees representing 35 genetically and architecturally diverse UCD almond selections. Trees have also been propagated for a smaller spring, 2021 Kern County planting to test early flowering UCD selections as possible pollinizers and/or replacements for the high market-value Sonora variety. Nursery propagations have also been completed for spring 2021 plantings of 24 UCD selections in Davis California as part of an advanced selection disease evaluation trial and 30 UCD selections in WEO, Winters, California for evaluation of self-fruitfulness in advance breeding selections from different genetic sources. Propagations are also underway for a small-scale (10-20 trees per selection) grower test-planting of 15 UCD advanced breeding selections in the northern San Joaquin. Information on eight earlier planted UCD breeding selection regional trials is also summarized in Table 2.

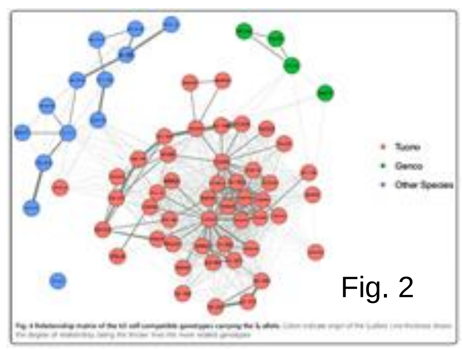
Table 1. Characteristics of advanced UCD self-fruitful selections planted at the Nickels Accelerated Assessment plot in November, 2020.

Item	Self-compatibility (SC)	Self-Compat. Source	Self-pollination	Kernel type	Shell-seal	Bloom time	Notes
UCD-20-1	Partial	Peach	Full	Sonora	Moderate	-3	Sonora type tree,Very low chill item to address climate change
UCD-20-2	Full	Peach	Full	Nonpareil	High	1	Upright and productive tree
UCD-20-3	Full	Peach	Full	Carmel	High	0	Upright tree, novel self-compatibility allele
UCD-20-4	Full	Peach	Full	Winters	Good	0	Upright tree, novel self-compatibility allele
UCD-20-5	Full	Peach	Full	Monterey	High	2	Compact tree with waterspouts suppression to facilitate hedging
UCD-20-6	Full	P. webbii	Full	Nonpareil	Good	-2	Nonpareil type tree, Good kernel quality and yield
UCD-20-7	Full	P. webbii	Full	Nonpareil	Good	-2	Nonpareil type tree, Good kernel quality and yield
UCD-20-8	Full	P. webbii	Full	Nonpareil	Good	0	Nonpareil type tree, Good kernel quality and yield
UCD-20-9	Full	P. webbii	Full	Carmel	High	1	Tolerance to drought
UCD-20-10	Full	P. webbii	Full	Sonora	High	1	Nonpareil type tree, Good kernel quality and yield
UCD-20-11	Partial	Exotic	Partial	Nonpareil	High	2	Vigorous and spreading tree,High yield consistent
UCD-20-12	Partial	Peach	Partial	Nonpareil	High	2	Compact tree with waterspouts suppression to facilitate hedging
UCD-20-13	Full	P. webbii	Full	Carmel	Moderate	1	Butte type tree, Good kernel quality and yield
UCD-20-14	Full	Mutation	Full	Sonora	Good	3	Upright and productive tree
UCD-20-15	Full	Mutation	Full	Monterey	Moderate	3	Pillar architecture to facilitate catch-frame harvest
Nonpareil	No	None	Partial	Nonpareil	Moderate	0	Evaluation Standard

1b) Collaborate with geneticist in Australia, Spain, France, the Middle East and Central Asia to identify and acquire for California testing, the most promising new varieties and germplasm. Working with California nurseries and UCD Foundation Plant Services (FPS), trees of the Australian varieties *Maxima, Capella, Rhea* and *Carina* as well as two recent Spanish varieties have been included in the Nickels Accelerated Evaluation plot. Trees have also been propagated for inclusion in the small-scale northern San Joaquin grower trials discussed above, with additional trees planted at the WEO self-fertility testing trial. Concurrent with this international transfer of breeding germplasm, we have worked with breeding colleagues in Spain, France, Italy, Isreal and Australia to characterize current breeding germplasm and the breeding implications from the observation that most new varieties released in the last 2 decades appear to be derived from the self-fruitful Italian heirloom varieties Tuono (which is genetically equivalent to the Spanish variety Guara) and Genco. Because Tuono was the recurrent breeding source for self-fruitfulness in European and

Australian varieties it inevitably results in inbreeding if not rectified by the simultaneous inclusion of a more diverse breeding germplasm. Utilizing both both breeding records and molecular analysis, recently published results (see publication 1) confirm extensive inbreeding particularly for Spanish and Australian varieties. Figure 1 shows the outsized influence of just 4 cultivars Nonpareil, Mission, Tuono and Cristomorto to new cultivars for California (USA), Spain, Australia, Israel and France. The very narrow genetic base is of particular concern for California since the varieties Nonpareil and Mission are widely planted with most other California varieties being chance seedling selections which turn out to be mainly progeny of Nonpareil by Mission. (The main exceptions are purposely bred varieties from UCD such as Sonora, Padre, Winters, Sweetheart, and Kester, which have a much more diverse parentage). The extent of inbreeding reported would explain the reduced productivity observed in many of the newer Spanish varieties. Because almond evolved as a self-sterile species requiring outcrossing for any seed-set, it was able to rapidly diversify the genetics of individual trees as well as the regional populations and this more expanded genetic “toolkit” allowed more successful adaptation over a wider range of environments in central Asia and Europe. This diverse genetics would also lead to the accumulation in both the population and individual tree of a much higher number of semi-lethal alleles of genes. Normally, the expression of these deleterious forms is masked by the companion allele from the other parent. But with inbreeding these deleterious alleles become concentrated and expressed, leading to reduced plant vigor and so productivity. Publication-1 further analyzes relatedness and so level of inbreeding for newer varieties from these regions. Figure 2 shows the relatedness of 65 self-compatible selections from these countries. Since virtually all European and Australian self-fruitful varieties use the Italian variety Tuono (and to a lesser extent the related Italian variety Genco) as the source of self-compatibility, the resultant inbreeding becomes even more pronounced. The only exceptions are listed in blue (as *other species* sources) with all items being UCD self-fruitful selections as well as Zaiger-bred *Independence* (derived from peach). [As shown by the breeding relatedness lines, we have also incorporated Tuono and the related variety Supernova into our breeding germplasm. The conclusion of this study is that, of all the regional breeding programs, only California has made the necessary effort to avoid inbreeding depression through the early incorporation diverse sources of self-compatibility (and concurrently novel sources of resistance/tolerance to important diseases, pests and environmental threats). Consequently, while the Australian and Spanish self-fruitful varieties may make valuable contributions to US commercial production (though unlikely given their very hard shells and lower crack out) their potential value to enrich UCD breeding program appears negligible. [For more detailed analysis, see the 2020 annual report for Almond Variety Development].





2. Utilized the accelerated assessment plots to facilitate preliminary evaluation of tree performance under different orchard configurations, architectures and bearing habits. To be advanced to regional testing, UCD breeding selections must first demonstrate good nut and kernel quality, general adaptation to California environments and evidence for high productivity (see Table 1 and publication-2). The genetically diverse UCD sources for self-compatibility, ranging from cultivated almond and peach to wild peach and almond species, have also contributed extensive variability for tree architecture, size and growth and bearing habit. The resulting diversity, not only allows the selection of the most promising tree types for different production regions, climates and management styles, but also provides insight concerning the specific needs of new farming methods such as ultra-high density plantings and catch-frame harvest even when the trials may eventually prove unsustainable. In addition, these diverse tree architectures and bearing habits provide opportunities to develop accurate computer- models of almond production, that can then be used to test various "what if" orchard management strategies more rapidly and comprehensively to facilitate field-based trials. Towards this model-development goal, these diverse test plots are being made available to all interested researchers (such as Dr. Brian Bailey, who is currently working to develop an almond production model) as well as engineers involved with harvest mechanization.

D. Outreach Activities

Rather than "boots on the ground" the goal for the COVID19 impacted 2020 season was "plants in the ground". Table 2 summarizes recent regional evaluation plot development as a complement to earlier UCD plots located throughout California production regions.

Table 2. Evaluation plot	Location	Year planted	Number of UC selections	Total Number of trees
Sonora pollinizer/replacements	Kern County-3 locations	2021	3	30
Disease Resistance Testing	Davis, CA	2021	24	48
Self-fertility Testing	Winters, CA	2021	30	60
Accelerated Evaluation	Arbuckle, CA	2020	20	180
High-density catch frame	Bakersfield, CA	2020	35	2600
Central Leader/High Density	Winters, CA	2018	10	120
Minimal Training/High Density	Fowler, CA	2018	6	30
Regional Variety Trials	Butte, Stanislaus, Madera Co.	2014	14	1260
High Temperature Tolerances	Arizona	2013	7	140
Billings RVT	Delano, CA	2009-11	44	466
New Columbia/Wonderful	Firebaugh, CA	2009	12	768
Nickels Self Fruitful's	Arbuckle, CA	2008	10	150
NBF Foundation Source Testing	Wasco, CA	2000	10	600

E. Materials and methods

Materials are summarized in Table 1 and 2. Standard nursery practices were used for all propagations and plantings. Tree and crop assessments use the same evaluation criteria as used in the RVT's as summarized in annual reports and publication-2. Methods for molecular and pedigree analysis of the extent of inbreeding in breeding programs worldwide are described in publication-1.

F. Publications that emerged from this work

1. Felipe Pérez de los Cobos, Pedro J Martínez-García , Agustí Romero , Xavier Miarnau, Iban Eduardo , Werner Howad, Mourad Mnejja, Federico Dicenta, Rafel Socias i Company, Maria J Rubio-Cabetas, Thomas M Gradziel, Michelle Wirthensohn , Henri Duval, Doron Holland, Pere Arús , Francisco J Vargas and Ignasi Batlle. 2021. Pedigree analysis of 220 almond genotypes reveals two world mainstream breeding lines based ononly three different cultivars. Horticulture Research (2021) 8:11. https://doi.org/10.1038/s41438-020-00444-4.
2. Gina Sideli, Ted DeJong, and Sebastian Saa. 2020. Almond Variety Program; The Continuum of Variety Development, Screening, and Evaluation. ABC Special Technical Report. 54 pages.
3. Angela S. Prudencio, Raquel Sánchez-Pérez, TM Gradziel, Pedro J. Martínez-García, Federico Dicenta, Thomas M. Gradziel and Pedro Martinez Gomez.. 2020. Genomic Designing for New Climate-Resilient Almond Varieties . In: Chittaranjan Kole (Ed.) Genomic Designing of Climate-Smart Fruit Crops. ISHS Jhlpd68505c015976.
4. Gradziel, T.M. 2020. Redomesticating Almond to Meet Emerging Food Safety Needs Frontiers in Plant Science, Volume 11, 12 June 2020. 89/fpls.2020.00778. https://doi.org/10.33
5. Kourosh Vahdati, Saadat Sarikhani, Neus Aletà, Charles A. Leslie, Abhaya M. Dandekar, Mohamad Mehdi Arab, Beatriz Bielsa,, Thomas M. Gradziel, Álvaro Montesinos,, María J. Rubio-Cabetas,, Gina M. Sideli, Ümit Serdar, Burak Akyüz, Gabriele Loris Beccaro, Dario Donno, Mercè Rovira, Louise Ferguson, Mohammad Akbari, Abdollatif Sheikhi0, Mahmoud Reza Roozban, LJ Grauke, Keith, Kubenka, Warren Chatwin, Amandeep Kaur, Srijana Panta, Lu Zhang, Shawn A. Mehlenbacher, Xinwang Wang. Physiological and molecular aspects of nut crops rootstock-scion interactions: current and future. (in-press). Kourosh Vahdati, Saadat Sarikhani, Neus Aletà, Charles A. Leslie, Abhaya M. Dandekar, Mohamad Mehdi Arab, Beatriz Bielsa,, Thomas M. Gradziel, Álvaro Montesinos,, María J. Rubio-Cabetas,, Gina M. Sideli, Ümit Serdar, Burak Akyüz, Gabriele Loris Beccaro, Dario Donno, Mercè Rovira, Louise Ferguson, Mohammad Akbari, Abdollatif Sheikhi0, Mahmoud Reza Roozban, LJ Grauke, Keith, Kubenka, Warren Chatwin, Amandeep Kaur, Srijana Panta, Lu Zhang, Shawn A. Mehlenbacher, Xinwang Wang. Physiological and molecular aspects of nut crops rootstock-scion interactions: current and future. (in-press).
6. Johnson, E.P., Preece, J.E., Aradhya, M., Gradziel, T. Rooting response of Prunus wild relative semi-hardwood cuttings to indole-3-butyric acid potassium salt (KIBA). Scientia Horticulturae, Volume 263, 15 https://doi.org/10.1016/j.scienta.2019.109144.

7. Katherine M. D’Amico-Willman, Chad E. Niederhuth, Matthew R. Willman, Thomas M. Gradziel, Wilberforce Z. Ouma, Tea Meulia, and Jonathan Fresnedo-Ramírez. (in Press) DNA methylation status is associated with divergent exhibition of non-infectious bud failure, an age-related disorder, in twin almonds. The Plant Journal.
8. Gradziel, Thomas M. and Jonathan Fresnedo-Ramírez. (2019). Noninfectious Bud-failure As a Model for Studying Age Related Genetic Disorders in Long-Lived Perennial Plants. Journal of the American Pomological Society 73(4): 240-253 2019
9. Gradziel T, B. Lampinen and J.E. Preece. (2019). Propagation from Basal Epicormic Meristems Remediates an Aging-Related Disorder in Almond Clones. Horticulturae 2019, 5(2), 28; https://doi.org/10.3390/horticulturae5020028