Rootstock Breeding

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A. Summary

Because most of today's commercially successful almond rootstocks are interspecies hybrids, our breeding plan is to identify the specific Prunus species and hybrid combinations conferring required resistance (nematodes, salinity, diseases, etc.) and then identify individuals within those species that when used as crossing parents will also confer regionally required traits (anchorage, tolerance to waterlogged soil, boron, etc.). At the same time, we have developed the required strategies to generate large numbers of complex interspecies hybrids, identify the most promising selections within these populations and then propagate multiple copies of the most promising individuals for replicated resistance and productivity testing. Over 200 inter-species hybrids are currently undergoing cooperator evaluation, with 117 additional hybrids being propagated for 2020 planting. Ongoing genetic studies of over 1000 segregating seedlings generated by the breeding program are examining trait expression and heritability, with an additional 350-400 seedling to be planted and evaluated in 2020. DNA from over 600 individuals representing these populations is being analyzed for possible molecular markers for desired resistance and to further clarify the nature of genetic/genomic interaction in these complex intraspecies hybrids. A number of advanced selections continue to look promising after multiyear, multi-site evaluations.

B. Objectives

Develop a series of multifunctional rootstocks combining the range of required traits for continued production in the different California growing regions as well as expanded production into newer regions and climates.

C. Annual Results and Discussion

Because most successful almond rootstocks are interspecies hybrids, our goal is to identify Prunus species and hybrid combinations conferring required resistance (nematodes, salinity, diseases, etc.) and then identify individuals within those species that when used as crossing parents will also confer regionally required traits (anchorage, tolerance to waterlogged soil, boron, etc.). We are developing concurrent strategies to generate large numbers of complex interspecies hybrids, identify the most promising selections within these populations and then propagate multiple copies of the most promising individuals for replicated resistance and productivity testing.

Over 200 inter-species hybrids are currently undergoing cooperator evaluation, with an 117 additional hybrids being propagated for 2020 planting. Concurrently, ongoing genetic studies of over 1000 segregating seedlings from the breeding program are examining trait expression and heritability, with an additional 350-400 seedling to be planted in 2020. DNA from over 600 individuals from these populations is being analyzed for possible molecular markers for desired resistance and to further clarify the nature of genetic/genomic interaction in these complex intraspecies hybrids. A summary of traits being analyzed, research collaborator assisting with the analysis, and the quantity and quality of material being analyzed is summarized in Appendix A. Progress for individual traits is presented below, including a description of selections currently showing the most promise.

Nematode resistance. Hybrids between almond and peach species currently show the most potential as sources of nematode resistance (as well as salinity tolerance and vigor) with the following selections showing particular promise in early screening by Dr. Westphal in San Joaquin Valley root knot, ring, and dagger nematode infested sites:

5,17-186. Developed from a complex interspecies cross involving peach and its wild relative P. davidiana, this selection shows promise for root knot and possibly ring nematode resistance. Based on early promise, an additional 100 progeny from the original parents were generated and tested in 2018-19 for architecture and general vigor. Twelve of the most promising hybrids are currently being propagated to allow replications of 10 to 20 trees of each item, for more accurate statistical assessment.

K6733**.** Resulting from a cross between almond and nematode resistant peach, this selection as well as the related selection ***Ca40A-17*** are notable for good vigor and general adaptability to different soil types.

A8-7-310. This selection resulted from the hybridization between peach and almond followed by backcrossing to almond. It is unique in that while the backcrossing ensures that its genetic composition is mostly almond, it has retained the vigor and broad-soil adaptability of an interspecies hybrid in addition to displaying promising levels of nematode resistance. We are currently evaluating over a dozen of these unique introgression lines as their more balanced genomes may facilitate further genetic and genomic manipulations (such as three-way species hybrids).

Salinity tolerance. Early studies have supported almond by peach hybrids as promising sources of resistance. The ***K6733*** hybrid and the ***Horest*** series of almond progeny continues to show promise based on previous work by Pat Brown and others. Based on these earlier findings, a genetically diverse series of 30 interspecies hybrids is being propagated to allow replicated testing for salinity tolerance at Dr. Sandhu's facilities in Southern California.

Root asphyxia from saturated soils. My lab has used very rudimentary studies involving soil saturation of containerized plants to identify the plums, particularly P. cerasifera and P. americana and their hybrids, including UCD selections ***PG,13-3, PG,12-3,*** and ***STU,2-32***) as having potential resistance. In addition the ***SolanoGold*** almond germplasm also shows exceptional tolerance for an almond, and we been using it as a parent in plum by almond and peach by almond hybridization. These selections, as well as 14 related species and species hybrids, are being propagated for replicated testing for **Phytophthora** resistance by Greg Browne at his facility at the Kearney Agricultural Center in Parlier.

Armillaria. Test planting in a naturally-infected field at the Wolfskill Experimental Orchards has identified plums, particularly P. cerasifera, as promising sources of resistance. In addition, almond backcrosses involving P. webbii have shown some promise considering the general vulnerability for almond. Molecular markers for oak root fungus as well as nematode resistance are currently being pursued with collaborators in Spain and Clemson as part of an AFRI proposal currently being developed for submission in early 2020. In 2019, we generated over 50 progeny from a difficult oak root fungus-resistant P. cerasifera to nematode-resistant peach cross that are currently being tissue-cultured and evaluated at Clemson. Three seed were similarly recovered from an even more difficult cross between cherry and P. cerasifera and these yet to be verified hybrids are currently being tested by collaborators at Michigan State.

Crown gall. A naturally-infected screening plot at the UC Davis orchards has so far managed to kill all of our candidate hybrids. Based on Dan Kluepfel's report that P. tangutica may be a source of resistance, we are currently testing a peach by P. tangutica hybrid (***6-1-107***) as well as 40 F2 seedlings of this hybrid to evaluate possible resistance, including inheritance. So far, the tangutica seedlings show no disease but they are still approximately 6 feet from the epicenter of the hottest-spot of crown gall activity and even nearby susceptible controls have yet to show symptoms. The tangutica seedlings are segregating for strong susceptibility to what appears to be Botryosphearia, however. We are currently germinating a peach by tangutica, F2 seedling population for 2020 controlled inoculations/evaluation by the Kluepfel lab.

Botryophaeria, Oxyporus and other wood rot diseases. Univ. of Florida collaborators have identified potential sources of resistance to Botryophaeria and possibly other heartwood-rot fungi among 40 diverse species and species hybrids provided in 2014. In 2019, an additional 210 F2 seed were generated at UCD for further resistance studies with particular emphasis towards validating a potential marker previously identified at U of F for Botryophaeria.

Somatic embryogenesis as a pathway for the single-cell plant-regeneration required for plant transformation. New genetic technologies, particular CRISPR and related methods have revolutionized our ability to modify/engineer major genes involved in plant development and disease resistance. While Prunus species, including almond are easily transformed by Agrobacterium (this occurs naturally in crown gall disease), routine transformation and plant regeneration for Prunus species (either to facilitate research or to develop improved commercial rootstocks) has not been possible because of our inability to regenerate whole plants from single cells. Based on their previous success in developing single cell- derived somatic embryos in Walnut, the Dandekar lab has recently succeeded developing similar protocols for almond-peach hybrids. The screening and protocol development process required the dissection of very young embryos from interspecific almond by peach hybrids. In 2018 we provided over 200 almond by peach hybrids for the original successful protocol development and in 2019 we provided an additional 117 hybrid seed to validate and further develop these protocols. This protocol opens the door to the use of precise genetic engineering options to complement future rootstock development and is the basis of a SCRI proposal being prepared for submission in early 2020.

D. Outreach Activities

15-Jan	PAG Conference	Almond phytomediomics	(~30 participants)
5-Jun	NIFA workshop	Breeding for food safety	(~50 participants)
21-Jan	Washington Post Interview	Climate change	(~30 participants)
8-Apr	Nursery/grower visit	Issues in new nursery varieties	(~16 participants)
14-May	Nursery Visits	Almond Bud-Failure	(~14 participants)
21-May	Farm Advisor Tour	Self-Fruitfulness in Almond	(~35 participants)
24-Jul	ABC Almond breed.workshop	UCD almond breeding	(~ 30 participants)
30-Sep	Almond Short Course	Flower develop. and pollination	(~800 participants)
1-Oct	Western Nut Grower	Article on Kester variety	(~4000 readers)
13-Nov	ABC Crackout	UCD samples	(~ 40 participants)
10-Dec	ABC Annual Conference	poster presentation	(~40 participants)
16-Dec	UCD Plant Breeders Conf.	Unique genomics for clone breed	(~90 participants)
3-Feb	UCD seminar	Breed. for Food Safety and Almond	(~30 participants)
5-Feb	American Society of Agronomy	Almond breeding challenges	(~80 anticipated)
7-Feb	ABC Rootstock workshop	UCD almond breeding	(~30 anticipated)

E. Materials and Methods

Genetic material. A diverse germplasm, including heirloom varieties, and related Prunus species and inter-species hybrids and introgression lines has been developed at the UCD almond breeding program as detailed in 2017 and 2018 annual reports.

Hybridizations, introgression and general breeding methods. Breeding strategies, including standard and modified intra-and interspecific hybridization methods as well as marker assisted breeding are routinely employed as detailed in 2017 and 2018 annual reports.

Nematode resistance. See methods in Andreas Westphal's annual report. Test plantings were also made with nursery cooperators in known root knot, ring and dagger nematode hotspots.

Salinity tolerance. See methods in Patrick Brown's 2017 annual report and D. Sandhu 2020 proposal.

Root asphyxia from saturated soils. My lab has used very rudimentary studies involving soil saturation where containerized plants would be submerged until Nonpareil seedlings (used as susceptible controls) began to show moderate damage. At this point, containers were drained and plants rated for damaged. Selections showing promise from this initial study will be more accurately evaluated by the Phytophthora and asphyxia screening facilities of Greg Browne at KAC, Parlier. See Browne proposal for methods.

Armillaria. **Crown gall**. Test plantings were made at known disease hotspots in Winters and Davis, respectively. Controlled laboratory inoculations/evaluation is also occurring at the Gasic lab at Clemson University for Armillaria and (in spring 2020) at the Kluepfel lab at UC Davis for crown gall.

Botryophaeria. Oxyporus and wood-rot diseases. See protocols developed by Bob Johnson in his 2017 annual report.

Somatic embryogenesis . An essential component of inducing somatic embryogenesis was determining the critical time of early embryo development were individual cells would retain regenerability. To determine the proper time for successful cell culture a large number of hybrid embryos had to be sacrificed for dissection and testing.

F. Publications that emerged from this work

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

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

Liu, Ting-Hang, Mohammad A. Yaghmour, Miin-Huey Lee, Thomas M. Gradziel, Johan Leveau, and Richard M. Bostock. 2019. A roGFP2-based bacterial bioreporter for redox sensing of plant surfaces. Phytopathology September 4, 2019. https://doi.org/10.1094/PHYTO-07-19-0237-R

Gradziel, T. M., and. B. Lampinen. 2019. 'Kester' Almond: A Pollenizer for the Late 'Nonpareil' Bloom with High Yield and Kernel Quality. HORTSCIENCE 54(n):1–2. 2019. Https://doi.org/10.21273/HORTSCI14398-19

Gradziel, T. M. 2019. 'Kester' Almond: A Pollenizer for the Late 'Nonpareil' Bloom with High Yield and Kernel Quality. Western nut grower, Fall, 2019.

Appendix A. A summary of traits under evaluation, research collaborator doing the evaluation, and the quantity and quality of material analyzed for 2019-2020.

Trait	Cooperator	Material under evaluation	Species evaluated	New material being propagated for 2020 evaluation
Root lesion Ring, and Root-knot nematode	Andreas Westphal	25 clones	a, dv, m, p, t, w	20 additional accessions including 12 new hybrids from promising parents
Salinity tolerance	Brown & Sandhu	12 clones	d, a, , f, m, p, t, w	30 diverse species hybrids
Replant decline	Various nurseries	20 clones & ~1000 seed	a, dv, m, p, s, t,	[Testing not yet completed]
Asphyxia	In-house	~100 seed	d, p	11 new populations of almond-peach-plum hybrids
Phytophthora	Greg Browne	3 clones	pl	20 additional species hybrids
Botryophaeria resistance	Jose Chaparro (UF)	40 clones, 100 seedlings	a, b, f, m, pd, p,	120 additional Selfed (F2) seedlings from resistant parent
Oxyporus and other wood rot diseases	Rizzo/Johnson	15 clones	d, a, , f, m, p, t, w	[Testing not yet completed]
Armillaria	In-house	~200 seedlings	d, p	48 new plum by peach hybrids; 3 plum by cherry hybrids
Crown gall	Dan Kluepfel	~200 seedlings, ~400 seed	p x t (F2, F3)	>200 seedlings field planted, ~100 seedlings -greenhouse
Compatibility with Nonpareil & Replant	Various nurseries	50 clones	a, b, dv, m, p, pl	[Testing not yet completed]
Effect on scion architecture	Fowler Nursery Wonderful Farms	7 clones	a, dv,	[Testing not yet completed]
Heat Tolerance	Mathew Gilbert	15 clones	a, f, m, p, w	Evaluations completed, reported in 2018-19 annual report
Dryland culture	Andrew Langford	Almond seedlings	d	[Testing not yet completed]
General architecture for high density plantings	Thorp, Wirthensohn, Lampinen	20 clones and ~400 seedlings	d, , f, m, p, w	12 almond species clones
Embryogenesis, transformation	Abhaya Dandekar	~200 developing seed; 6 clones	d, p, dv	120 additional hybrids based on 2019 successful embryogenesis

Almond {P.dulcis} (d), Peach {P.persica} (p), P.argentea (ar), P.fenzliana (f), P.mira (m), P.webbii (w), P.bucharica (b), P.pedunculata (pd), Plum spp. (pl), P.tangutica (t), P.triloba (tr), P.davidiana (dv), P.scoparia (s).