Project Title: Integrated Conventional and Genomic Approaches to Almond Rootstock Development (**Project Number: 18- HORT16 - Aradhya/Westphal)**

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Summary Response to Reviewers' comments

Breeding

The goal of our project is to develop well adapted and graft compatible rootstocks with durable resistance to soil borne diseases such as phytophthora crown and root rots, crown gall, and root-knot, lesion, and ring nematodes, all of which impact sustainable almond production in California. Initially we felt our success was dependent on our ability to generate and screen a large population of genetically diverse interspecific hybrids. Our ongoing program, although extensive, the number of hybrids that actually enter our disease evaluation pipeline drops off rapidly as they go through the stages of developments such as embryo rescue, micropropagation, seed germination and clonal propagation in cases of cross compatible crosses (Figure 1). However, the reviewers suggested our proposal contained an over emphasis on breeding versus testing international/available rootstocks. We understand the committee's concern over the generation of additional new hybrids. In response to this concern, we will tighten our breeding efforts and focus primarily on exploiting peach/almond/plum crosses. The reason for this lies in the fact that, to date, we have found the greatest level of both nematode and crown gall resistance in hybrids containing this genetic background. In response to reviewers, we will now include additional commercial rootstocks along with the industry standards such as 'Nemaguard', 'Lovell', 'Hansen 536' 'Krymsk 86' and "Marianna' that are currently being used in the disease resistance evaluations. These commercially available rootstocks will be passed through our disease resistance-screening pipeline and will serve as a baseline against which we compare all experimental genotypes evaluated in our system (i.e. both greenhouse and field-testing). (Roger Duncan has agreed to aid us in the selection of the commercially available rootstocks which will enter our screens)

Genomics

Our ultimate goal, in the application of genomic tools, is to develop reliable marker-based selection strategies to identify rootstock hybrids, in the seedling and sapling stages, genotypes that possess resistance/tolerance to the soil borne diseases. This form of juvenile selection permits rapid and cost-effective large-scale screening of hybrids as compared to expensive and time-consuming disease resistance evaluations of much more mature trees.

We have taken a low depth sequencing approach to identify single nucleotide markers and to genotype a large number of hybrids and parental genotypes/species. A thorough validation of associated markers in diverse interspecific genetic backgrounds or species-specific populations is required to effectively link genetic loci that mediate disease resistance to the markers. We have performed an initial association analysis and are planning to perform an additional analysis this year on a different set of hybrids and parental genotypes. In addition, we also will use genomic approaches to tap into polygenic/durable resistance. These efforts will require a substantial portion of the budget requested for the breeding/genomics portion of this project over the next three years of the project.

The deliverable from this portion of the project is what provides the foundation for future rootstock breeding efforts; i.e. genetic markers linked to key disease resistant phenotypes which can be used by rootstock breeders.

Progress-to-date

Over the last few years, this project has produced a larger number of genetically diverse interspecific disease resistant hybrids that are in various stages of advanced propagation and additional resistance evaluation to confirm our initial observations. (18 advanced disease resistant genotypes listed in Table 1). These advanced genotypes are being evaluated at Sierra Gold Nursery for such horticultural traits as propagation ease and graft compatibility. This now puts us in the excellent position of having nearly identified rootstocks worthy of being examined under grower field conditions. Roger Duncan, the UC Farm Advisor, Modesto also has agreed to facilitate the coordination of field trials of selected rootstocks on problematic pathogen/pest infested soils. We welcome all suggestions on this objective as we move forward with our field trails to examine “elite” selections from our program.

As mentioned above, we will more tightly focus our development of novel genetically diverse hybrids while intensifying our disease resistance screening of the wide range of commercially viable rootstocks and the novel genotypes produces in this project. As a result, we anticipate the identification of commercially viable hybrids with durable and broad spectrum of resistance to soil borne diseases.

Table 1. *Prunus* hybrids* showing resistance to soil borne diseases.

Rootstock	Parentage	RK	RL	CG	PHY
197-113	*P. persica x P. tangutica*		*	*	*
97-190	*P. persica x P. dulcis*		*		
197-199	*P. persica x P. davidiana*	*		*	*
197-200	*P. persica x P. davidiana*	*			
197-206	*P. persica x P. kensuensis*	*	*		
197-209	*P. persica x P. kuramica*		*		
197-214	*P. persica x P. bucharica*		*	*	
197-217	*P. persica x P. kuramica*			*	
197-6	*P. persica x P aregentea*	*			*
197-95	*P. persica x P. tangutica*	*		*	*
198-18	*Nemaguard x P. kensuensis*	*			
L1-2	*P. cerasifera*				*

P2-1	*Nemared x P. argentea*		*	*	
P2-2	*Nemared x P. argentea*	*			
P2-4	*Nemared x P. argentea*	*		*	
P4-1	*Nemared x P. fenzliana*			*	*
P4-10	*Nemared x P. fenzliana*	*	*	*	
P4-25	*Nemared x P. fenzliana*	*	*	*	*

RK = root knot; RL = root lesion; CG = crown gall; PHY = Phytophthora

*We have clonal copies of the selected hybrids listed above ready for an advanced field trail at the Armstrong Plant Pathology Field Station. The trail will be conducted by inculcating soils with all the three soil borne pathogens (PHY/CG/NEMs) to create optimum disease pressure for effective screening to confirm their field performance. Sierra Gold Nurseries has collected cuttings of these hybrids from our field collection of rootstock hybrids at Armstrong for propagation and grafting with scions (Nonpareil and Monterey) for field testing efforts.

We have unveiled tentative time lines with milestones for different objectives in the main proposal. Here, we have elaborated on these and provided the possible deliverables (Table 2). As suggested, objective-wise budget estimates are provided in the Table 3.

Table 2. Main Goal(s), key objectives, timelines and deliverables

Objective(s)	Date to be accomplished	Milestones and deliverables associated to the objective
Objective 1	July 2020- July 2021	*Prunus* interspecific hybrids
Objective 2	July 2019-Sept2022	High-quality disease resistance data
Objective 3	July-2022	Markers associated with resistance to soil borne diseases. Development of selection strategies
Objective 4	July 2020 –July 2022-	Identify promising novel disease resistant rootstock selections. Characterize the CG, Phytophthora and nematode resistance phenotypes for the top commercially available rootstocks.

Objective 1: Rootstock breeding is a "numbers-game" and success in getting field resistance to multiple diseases depends on production and disease testing of a wide spectrum of taxonomically and genetically diverse two-, three- and possible higher order crosses involving more than three species in interspecific hybrids. This objective addresses this important goal and the deliverables are large numbers of interspecific hybrids that increase the probability of success of rootstock breeding objectives set out for this project. However, as mentioned above, we will now tighten our focus to what we anticipate will be highly productive plum X peach/almond hybrids.

Objective 2: Disease resistance screening is central to this project. Reliable disease evaluation permits selection and development of improved rootstock with combined durable resistance to soil borne diseases. The deliverables here are the highly reliable disease resistance data that will be used to develop marker associations and genomic selection strategies to accelerate development of rootstocks by avoiding expensive and time consuming disease evaluations.

Objective 3: High-density molecular markers such as single nucleotide polymorphisms (SNPs) and reliable disease evaluation data are prerequisites for developing efficient marker-assisted and genomic selection strategies. As we work with highly diverse interspecific hybrids, population structure and kinship pose serious difficulties in the computations of associations and genomic selection criteria. Deliverables here are markers (e.g. SNPs) and marker and genomic selection strategies.

Objective 4: Ultimately, evaluation of field performance of rootstock selections is critical in order to assess the value of selected rootstocks. The deliverable here are rootstock selections that emerge from field testing in problematic soils with scores greater than currently accepted rootstock standards.

Only the most promising hybrids will be increased in a commercial nursery in preparation for on-farm field trails. As mentioned above, efforts are already ongoing at Sierra Gold Nursery (as in-kind contribution) to evaluate horticultural characteristics of several of our putatively disease resistant genotypes. We will soon have answers to such questions as: Is this genotype easily propagated? Is this genotype graft-compatible? Is the root system supporting a typical almond tree? With this basic information, further selection of the more favorable rootstocks will be made. Roger Duncan has agreed to assist in the identification of field sites and facilitation of field trial establishment in cooperation with key farm advisors. We anticipate establishment of at least four locations across the almond producing areas of California. Almond production areas will be identified for rootstock trials which will contain up to ten elite clones emerging from our screening efforts. These trials will be placed in different soil types which exhibit pathological challenges. In all trials, two to three commercially available rootstocks will be used as standards against which all elite genotypes will be evaluated.

Table 3. Estimated budget for different objectives in the Almond Rootstock Project.

Project Title:	**Almond Rootstock Development**
Project Leader(s):	**Aradhya, Ledbetter, Kluepfel,Browne, Westphal**

Objective	**Year 1**	**Year 2**	**Year 3**	**Total**
Propagation of hybrids*				
Student Assistant III (2 x $12.25/hr)	$ 25,400	$ 25,600	$ 25,800	$ 76,800
Supplies	$ 8,000	$ 8,000	$ 8,000	$ 24,000
Propagation of hybrids Total	**$ 33,400**	**$ 33,600**	**$ 33,800**	**$ 100,800**
Pathology (PHY)				
Jr. Specialist, 60% time (PHY)	$ 25,260	$ 25,513	$ 25,768	$ 76,540
Jr. Specialist (PHY) Benefit @ 41.2%	$ 10,357	$ 10,460	$ 10,565	$ 31,382
Supplies	$ 3,000	$ 2,000	$ 2,000	$ 7,000
Maintenance fees	$ 2,000	$ 3,000	$ 3,000	$ 8,000
Pathology (PHY) Total	**$ 40,617**	**$ 40,973**	**$ 41,332**	**$ 122,922**
Pathology (CG)				
Salary				
Jr. Specialist 30% time (CG)	$ 21,000	$ 21,210	$ 21,450	$ 63,660
Jr. Specialist (CG) Benefit @ 41.2%	$ 8,610	$ 8,696	$ 8,795	$ 26,101
Supplies (CG)	$ 7,000	$ 6,000	$ 5,000	$ 18,000
Pathology (CG) Total	**$ 36,610**	**$ 35,906**	**$ 35,245**	**$ 107,761**
Pathology (Nematodes)				
Junior Specialist @ 75%	$ 29,625	$ 30,514	$ 31,430	$ 91,569
Junior Specialist @ 75% (Composite Benefit Rate)	$ 15,731	$ 16,878	$ 18,114	$ 50,723
Supplies	$ 2,500	$ 3,000	$ 3,000	$ 8,500
Maintenance fees	$ 10,000	$ 10,000	$ 10,000	$ 30,000
Pathology (NEM)	**$ 57,856**	**$ 60,392**	**$ 62,544**	**$ 180,792**
Travel	**$ 2,000**	**$ 2,000**	**$ 2,000**	**$ 6,000**
TOTAL FUNDING REQUESTED	**$170,483**	**$172,871**	**$ 174,921**	**$ 518,274**
*Propaga**tion** of exisiting hybrids only				

Figure 1. Pictorial depiction of how the numbers of hybrids drops off as they go through various stages of development from pollination in field though disease resistance evaluation to final selection for field testing.