Development of Genomic Tools for Almond Rootstock Improvement

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INTRODUCTION

Almond rootstocks with resistance to soil borne and replant diseases are key to sustainable production of almond and therefore a top priority for the California Almond Industry. With restrictions on fumigant use and the need to minimize environmental impact, industry reliance on rootstocks with field resistance to soil borne pests and diseases is increasing. Though widely used rootstocks (e.g. 'Nemaguard', peach, and peach x almond hybrids) tolerate or resist root knot nematodes, they are susceptible to other soil borne pests and diseases, such as lesion and ring nematodes, crown gall, *Phytophthora* and *Armillaria*. In this project an attempt has been made to produce novel interspecific hybrids involving peach, wild almond species and diploid plums that are potential donors of resistance to soil borne diseases and in some cases drought tolerance.

Wild *Prunus* spp. are excellent sources of resistance to soil borne diseases, but data on disease-pest interactions is lacking rendering selection of species and individuals within species for use in hybridization difficult. For this reason it is critical to phenotypically evaluate and genetically characterize wild *Prunus* spp., commercially available rootstocks, and newly generated interspecific hybrids (see poster 64 for more details).

While hybrids are being screened for disease resistance, genomic tools and methods are being developed to assist in efficient selection of resistant hybrids. Genome sequences of a dozen *Prunus* spp. genotypes used in generation of the interspecific hybrids (*P. dulcis, P. persica*, *argentea, P. kansuensis, P. davidiana, P. bucharica, P. kuramica, P. arabica, P. tangutica*, and *P. fenzliana*) are currently being analyzed to identify SNPs compatible across species. A second approach to discover SNPs and concurrently genotype a set of all rootstocks currently in use or undergoing field testing along with our novel hybrids are is also underway using a new technique called genotyping-by-sequencing (GBS).

OBJECTIVES

- Develop a set of molecular markers linked to disease-pest resistance for use in almond rootstock breeding programs
- Discover single nucleotide polymorphisms (SNPs) using existing peach and almond sequences, root specific EST sequences, and genotyping by sequencing profiles.
- Genotyping of commercially available roostocks and newly produced genetically diverse interspecific hybrids

MATERIALS AND METHODS

Screening for disease resistance (ongoing)

Interspecific hybrids produced at the USDA National Clonal Germplasm Repository (NCGR) from 2010-2012 from 20 different interspecific crosses involving 14 wild *Prunus* species, of which 43 hybrids with sufficient clonal plants have under gone a year of disease testing for crown gall and *Phytophthora* crown and root rots. An additional 40 hybrid genotypes are currently undergoing multiplication via micropropagation at California Seed and Plant Laboratory (CSPL) in Elverta, California.

Disease evaluation nurseries and replicated experiments have been planted in Davis and Parlier locations by USDA-ARS Crops Pathology group (D. Kluepfel and G. Browne) for crown gall, *Phytophthora*, and replant syndrome evaluations. Fumigated and non-fumigated treatments have been imposed in the *Phytophthora* and replant disease evaluation experiments (**see poster 64**).

During 2013, 13 new interspecific hybrids were tested in replicated trials for crown gall and *Phytophthora* resistance. The results indicated that three genotypes P-4-2, P-4-10 and P-4-25, all 'Nemared' x *P. fenzliana* hybrids, are found to posses resistance to crown gall (**Figure 1**). The genotypes P-2-4 ('Nemared' x *P. argentea*), P-4-1 ('Nemared' x *P. fenzliana*) and L-1-2 (an open-pollinated *P. cerasifera*) are showing promising levels of resistance to *Phytophthora* (**Figure 2**). A second round of disease testing for these hybrids and a first round of disease testing for additional hybrid genotypes are planned for 2014.

Genomic tools – development of SNP markers (ongoing)

A diverse panel of twelve *Prunus* genotypes used in hybrid development was resequenced at BGI (Hong Kong) to an average minimum depth of 30X resulting in ~10 gigabases of data each. The nine genomes received have undergone quality evaluation (**Figure 3a-c**), read mapping to the peach reference genome (**Figure 3d-l** and **Table 1**), and initial SNP discovery (**Table 3**). The percentage of reads aligning with mapping quality 30 or above (MAPQ >=30) ranged from 50.6% (*P. arabica*) to 74.8% (*P. persica* cv 'Lovell') (**Table 1**). The percentage of reads with the poorest mapping quality (MAPQ<3) ranged from 17.7% (*P. davidiana*) to 37.4% (*P. arabica*). Comparing each genome to the peach reference generated from 0.18 to 3.1 million putative SNPs, and a co-analysis of all nine genomes produced 6.6 million putative SNPs (**Table 3**).

DNA from 190 interspecific hybrids and numerous commercial and experimental rootstocks was isolated and shipped to the Institute for Genomic Diversity at Cornell University for genotyping-by-sequencing (GBS; Elshire *et al.* 2011). This technique identifies SNPs located near restriction sites from throughout low repeat regions of the genome.

Associate SNPs with resistance (early stages)

Using the disease screening data (phenotype) and the molecular data (genotype) developed above, we will identify SNP markers associated with resistance to soil borne disease. Once confirmed, we will use these SNPs to develop juvenile selection strategies for rapid rootstock improvement.

RESULTS

Figure 1. Results of first year crown gall screening of interspecific hybrid genotypes (l-r) P-2-11, P-2-4, P-4-25, and P-4-10. The crown gall testing shows genotype P-2-11 is clearly susceptible, whereas genotypes P-2-4, P-4-25, and P-4-10 show resistance at this testing stage.

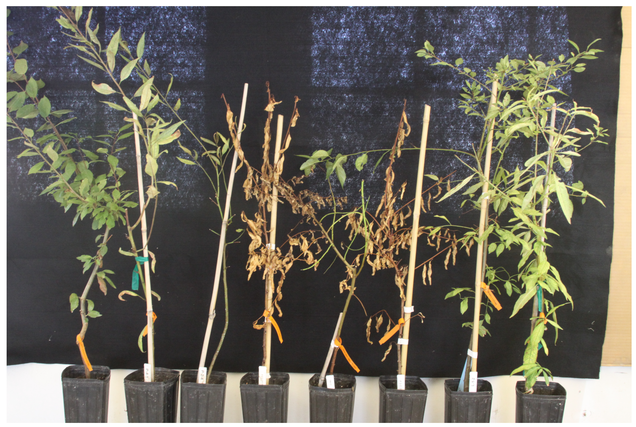

Figure 2. First year interspecific hybrid screening for *Phytophthora* crown and root rot. The *Phytophthora* screen shows (l-r) Marianna 26-24, P-4—1, P-2-4, three dead project interspecific hybrids, L-1-2, and 'Nemaguard'.

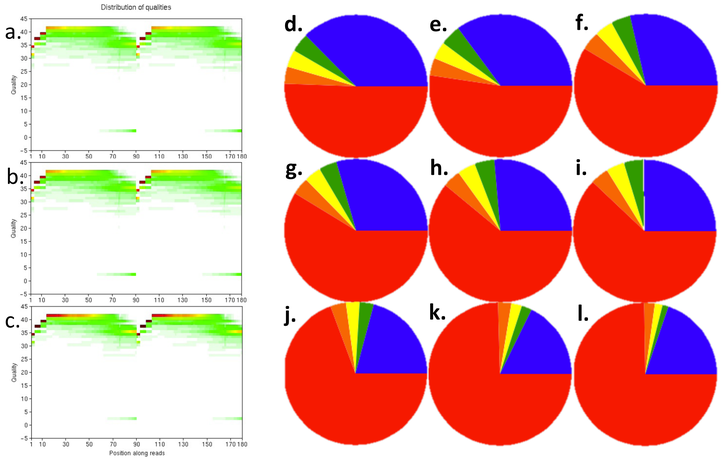

Figure 3. Left: A sample of the sequencing read quality distribution as provided by BGI. The change in the middle is due to the switch from the forward end to reverse end of the paired ends. The quality was consistent across all nine sequenced genomes as shown from the three samples above a) *P. dulcis* 'Tardy Nonpareil', b) *P. arabica*, and c) *P. cerasifera*. Right: Mapping quality of read alignments for resequenced genomes of d) *P. arabica*, e) *P. cerasifera*, f) *P. dulcis* cv. 'Tardy Nonpareil', g) *P. fenzliana*, h) *P. argentea*, i) *P. kuramica*, j) *P. tangutica*, k) *P. davidiana*, and l) *P. persica* cv. 'Lovell' in ascending order (left to right, top to bottom) of percent reads aligned to the peach reference genome with mapping quality greater than or equal to 30 (red).

DISCUSSION

Disease evaluation and genotyping of existing and novel rootstocks (ongoing)

Wild *Prunus* species are potential donors of resistance to soil borne diseases. We are screening both commercially available rootstocks and newly developed *Prunus* interspecific hybrids for disease resistance including replant disorder. The *Prunus* hybrids have broad genetic backgrounds in novel combinations, which we hypothesize contain resistance to multiple diseases. While many of the newly developed interspecific hybrids are undergoing clonal propagation several genotypes have undergone one round of disease evaluation following standard replicated statistical designs.

After one year of disease evaluations for crown gall, Phytophthora, and replant syndrome, Kluepfel and Browne have identified genotypes with potential resistance (**Figures 1 & 2**). Additional disease testing of these genotypes and first year testing of additional hybrid genotypes are planned for 2014.

Molecular data development and association analyses (ongoing)

Genome resequencing SNP discovery (in process)

Nine diverse resequenced genomes (**Table 1**) have been received and are undergoing bioinformatic analysis to discover common polymorphic SNPs. A preliminary analysis identified over 3 million putative SNPs in the *P. cerasifera* sample when mapped to the peach reference genome and over 6.5 million when all nine genomes are evaluated concurrently. The 1.98 million SNPs putatively identified for *P. davidiana* (DPRU2493.7) are similar in quantity to, but slightly higher than, the 1.67 and 1.68 million SNPs discovered earlier using a publicly available resequenced *P. davidiana* genome by similar analysis (*unpub.*) pipeline and Verde *et al.* (2013), respectively. An additional three resequenced genomes, including *P. kansuensis*, (**Table 2**) were just received from BGI. A publicly available resequenced *P. kansuensis* genome previously analyzed produced 1.19 and 0.95 million SNPs from the same methods used with the publicly available resequenced *P. davidiana*.

Some reads mapping with low quality are likely contamination and the parameters for read alignment require modification so they no longer map even with low quality, which should have little impact on the total number of SNPs discovered as the discovery pipeline includes quality filtering. However, other low mapping quality reads may be due to species divergence, such as seen with *P. arabica* and *P. cerasifera*.

Rootstock genotyping (in process)

One hundred and ninety commercial, experimental, and new interspecific hybrids produced from this project are currently in the process of GBS, which will identify several thousands of SNPs from this diverse material. Combined first year disease evaluation data for the hybrid rootstocks and multiple years of data for several commercial rootstocks will allow genome-wide association and identification of markers associated with resistance to the major soil borne diseases. Additionally, the genotype data can be used for association analysis with any future disease screening of the genotyped rootstocks.

Rootstock expression profiling (pending)

In Spring 2014 we will begin gene expression profiling (RNAseq) of root tissue from resistant and susceptible commercial rootstock material in both challenged and unchallenged conditions. RNAseq will allow identification of genes that are differentially expressed in challenged and unchallenged conditions within and between genotypes. This data will provide insight into which genes may be important for resistance to soil borne disease.

Table 1. Mapping quality (MAPQ) of read alignments for resequenced genomes as reflected in Figure 2.

Species	Total Reads (milliions)	Aligned Reads MAPQ >= 30 %	MAPQ >= 30 raw	MAPQ < 3 %	MAPQ < 3 raw
P. arabica	116.8	50.6	59095368	37.4	43731873
P. cerasifera	117.5	52.3	61518653	35.1	41284005
P. dulcis	117.6	58.6	68890700	28.6	33611270
P. fenzliana	117.3	58.7	68867782	29.5	34570305
P. argentea	117.5	61.0	71680733	26.4	30999655
P. kuramica	118.1	62.0	73274743	25.3	29892844
P. tangutica	118.4	69.3	82079054	20.9	24687083
P. davidiana	117.5	74.6	87625390	17.7	20807045
P. persica	117.5	74.8	87821785	19.7	23173437

Table 2. Species with resequencing data just received from BGI.

Species	Accession	Type
P. kansuensis	DPRU 0582	peach
P. bucharica	DPRU 1871.1	almond
P. dulcis	DPRU 2578.2	almond

Table 3. Preliminary SNPs discovered from alignment with the peach reference genome from a) each resequenced individual and c) multiple resequenced individuals concurrently. The average number of SNPs by type from read alignments of individuals (b) for comparison with co-analyzed alignment. Note: the cultivar 'Lovell' is the heterozygous progenitor of the individual used as the peach reference genome sequence, which account for the low number of SNPs.

Species	Accession	Type	SNPs
P. persica	Lovell	peach	178064
P. davidiana	DPRU 2493.7	peach	1982521
P. argentea	DPRU 0194	almond	2791469
P. fenzliana	*P. fenzliana*	almond	3061028
P. arabica	*P. arabica*	almond	2982388
P. dulcis	Tardy Nonpareil	almond	2942116
P. kuramica	DPRU 1467.9	almond	2817836
P. tangutica	DPRU 2327.16	almond	2604548
P. cerasifera	DPRU 0579	plum	2948852
	Average (incl. Lovell)		2478758
	Average (not incl. Lovell)		2766345

Average by Type	# Samples	SNPs	
peach type	2	1080293	(w/ Lovell)
peach type	1	1982521	(w/o Lovell)
almond type	6	2866564	
plum type	1	2948852	

Co-analyzed	# Samples	SNPs	Non-Unique
peach type	2	2062291	98294
almond type	6	5125824	12073561
all types	9	6572887	15735935

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UC DAVIS Department of Plant Sciences

REFERENCES

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The International Peach Genome Initiative, Verde *et al.* (2013). The high-quality draft genome of peach (*Prunus persica*) identifies unique patterns of genetic diversity, domestication and genome evolution. Nature Genetics 45:487-494.

FUTURE PLANS

- Perform genome-wide association analysis combining GBS genotype and disease evaluation data to identify markers linked to resistance/tolerance.
- Develop effective and high throughput marker-assisted selection schemes to increase selection efficiency and trait integration for improved almond rootstocks.