Development of Genomic tools for Almond Rootstock Improvement

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OVERVIEW

PROBLEMS AND SIGNIFICANCE

Development of improved almond rootstocks possessing field resistance/tolerance to soil borne pests and pathogens is a top priority for the California Almond Industry. Although some widely used rootstocks, such as 'Nemaguard', peach, peach-almond hybrids, and other complex species hybrids, resist infestation of root knot nematodes (*Meloidogyne* spp.) they are susceptible to other soil borne pests and diseases such as *Phytophthora* (crown and root rot), crown gall (*Agrobacterium tumefaciens*), *Armillaria* (oak root fungus) and lesion and ring nematodes (*Pratylenchus vulnus* and *Criconemella xenoplax*, respectively). Development and testing of diverse interspecific hybrids utilizing wild *Prunus* spp. possessing resistance to soil borne diseases is by far the best approach. However, for far too many species the information on disease-pest reactions is not available rendering selection of species for hybridization difficult.

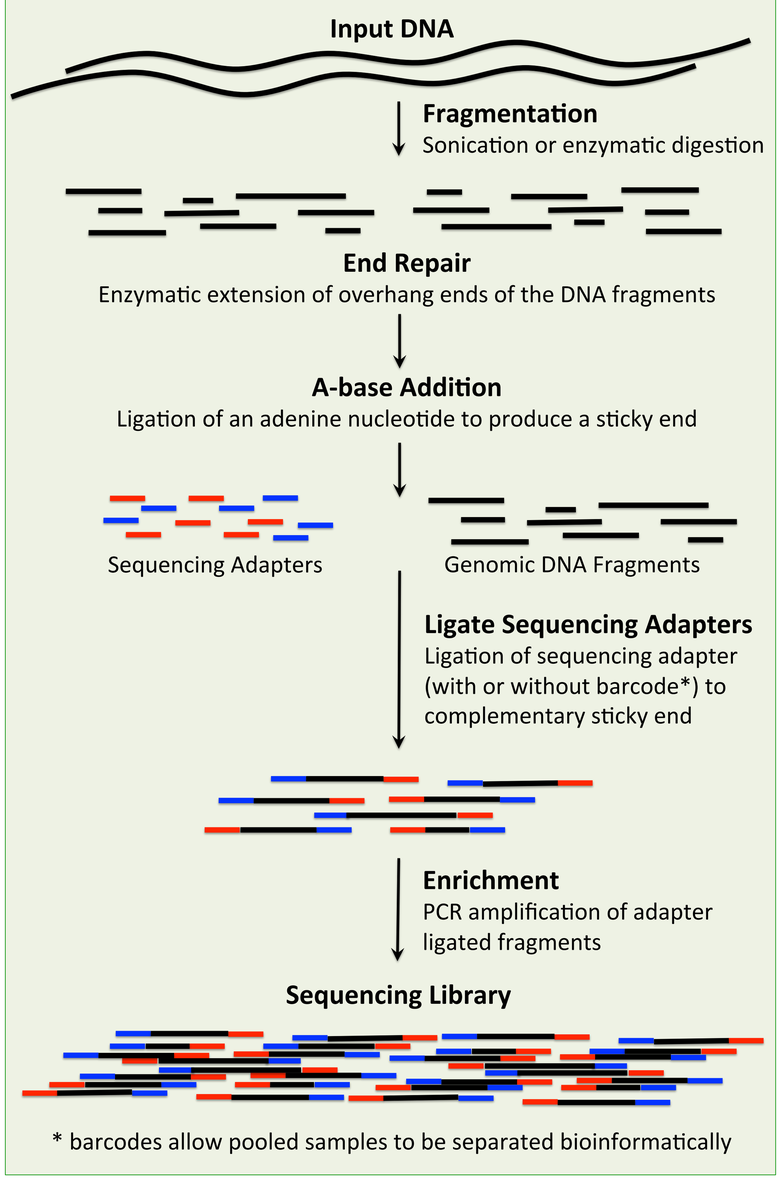
This project is focused on identifying genomic variants known as Single Nucleotide Polymorphisms (SNPs) associated with resistance to soil borne diseases and pests such as crown gall, crown and root rot, oak root fungus, root knot nematodes, ring and lesion nematodes, and replant disorder. SNPs are distributed abundantly throughout plant and animal genomes and are coming into use as standard high-throughput genotyping markers. Due to their abundant nature, they can be used for fine scale mapping of resistance gene locations and genetic association analyses targeting resistance to diseases.

The project has thus far assembled 67,194 peach and almond SNPs from various online databases and they will be subjected to validation soon. A set of ~150 interspecifc hybrids from ~15 different cross combinations are being micropropagated for disease evaluation. This project is also part of a larger project focused on all aspects of rootstock breeding for almond and walnut crops funded by a USDA Specialty Crop Block Grant.

OBJECTIVES

1. Develop a set of molecular markers linked to disease-pest resistance so that they can be incorporated in rootstock breeding programs
   - Assemble then validate *in silico* discovered single nucleotide polymorphisms (SNPs) of peach and almond from public databases
   - Discover SNPs based on espressed sequence tags (ESTs) of cDNA developed from a set of diverse peach, almond, and wild species
2. Produce and test a number of diverse *Prunus* interspecific hybrids involving useful species with potential to possess disease resistance attributes
   - Molecular characterization of peach and almond wild relatives
   - Production and testing of interspecific hybrids to identify novel sources of resistance to soil borne diseases

Library Construction for Illumina Sequencing



* barcodes allow pooled samples to be separated bioinformatically

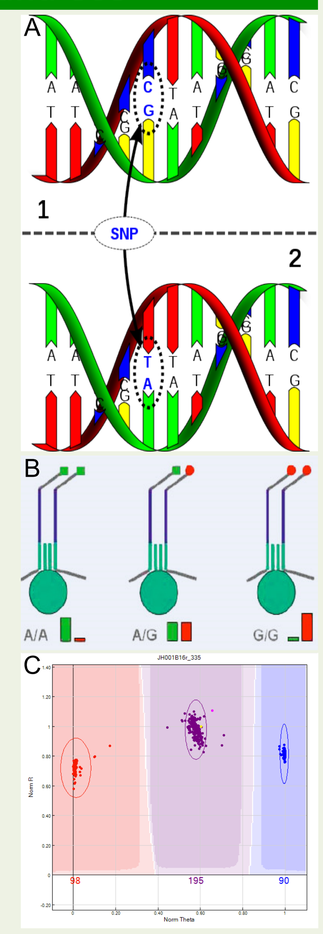
Table 1. Assembled *In silico* SNPs public databases

Resource	Crop Source	Number
ESTree	Peach and Almond	17 291
GDR	Peach	40 794
NCBI	Almond	109
IRSC (at GDR)	Peach	9 000
	Total	***67 194***

Abbreviations: GDR, Genome Database for Rosaceae; IRSC, International Rosaceae SNP Consortium; NCBI, National Center for Biotechnology Information

SNP Discovery and Use

Single nucleotide polymorphisms (SNPs) are DNA sequence variations of single base mutations. They are found throughout the genomes of higher organisms, including plants, and permit fine scale mapping. They are the only marker system to allow fast, efficient, large-scale genotyping of individuals using high-throughput platforms to evaluate from 3,000 to 1,000,000 SNPs simultaneously. Discovery of SNP markers associated with resistance to pests and diseases will enable the evaluation of available genetic resources leading to identification of multiple sources of resistance for use in disease resistant rootstock development. Resistance-associated SNP markers have the potential to enable pyramiding resistance in single genotypes. SNPs also facilitate genetic association analyses targeting disease resistance utilizing historic recombination found in germplasm collections and natural populations consisting of genotypes of unknown or mixed ancestry that represent a common gene pool.



SNPs and SNP genotyping. **A.** Illustration of a SNP (C/G to T/A) by Dave Hall. **B.** Schematic of SNP detection on a high throughput genotyping platform. **C.** Example of genotyping results for 383 individuals at a single SNP locus.

RESULTS

Objective 1a

A total of 67,194 SNPs from publicly available sources have been assembled (**Table 1**). Of the total, 17,291 are from peach and almond from the ESTree database, 40,794 are from peach from GDR, 9,000 peach SNP genotyping chip, and 109 are from almond from the NCBI database. The data has not been evaluated for duplicate SNPs, which can potentially reduce the total number of putative SNPs to between 40,794 and 67,194. The best way of validating the *in silico* SNPs is by verifying the presence of primer sequences in the genomic DNA sequences of species involved in hybrid production. Contig assembly of genomic DNA sequences generated from a set of diverse peach, almond, and wild *Prunus* spp. will be searched for the presence of primer sequences using readily available bioinformatic tools.

Currently the genomic libraries of selected diverse *Prunus* spp. involved in hybrid combinations are under construction and they will be barcoded and sequenced to allow for sequencing multiple individuals/species in the same sequencing reaction on the Illumina HiSeq producing approximately 15X coverage of each genome, based on an approximate genome size of 250-300 Mb. *In silico* SNP primer sequences will also be validated against these genome sequences.

Objective 1b

Root tissue derived RNA profiles of rootstock genotypes with contrasting disease reactions to various soil borne pests and pathogens will provide information regarding differentially expressed genes between susceptible and resistant rootstocks. This will facilitate identification of SNPs located within genes of interest thus providing useful SNPs linked to candidate resistance genes. These SNPs along with others identified using genomic sequences will allow us to fine scale map resistant genes. A few candidate rootstocks considered for cDNA library construction are listed in **Table 2** along with their known pest and disease evaluation scores from various sources. Sequencing of both genomic and cDNA libraries should be completed early 2012.

Objective 2

Several thousand pollinations were made in 2011 to produce interspecific hybrids involving a number of wild *Prunus* spp. with potential use in rootstock development based on available scientific literature. About 150 pollinations yielded hybrid seeds in nine cross combinations (**Table 3**). Previously, during 2010, 116 hybrid seeds were produced from twenty crosses and put into culture at the Davis repository at the end of 2010 after stratification, but few have thrived. The immature fruits resulting from 2011 crosses were harvested and delivered to California Seed & Plant Laboratory (Elverta, California) for culturing in June 2011. The immature embryos were excised from the seeds and germinated on an embryo culture medium, and surviving seedlings are currently undergoing shoot multiplication (**Figure 1**) to produce 50 clonal copies of each hybrid genotype. Once rooted, the clones will be distributed for evaluation with three major soil borne diseases and pests (crown gall, *Phytophthora*, and nematodes).

Additionally, an F2 interspecific mapping population of *P. persica* 'Andross' by *P. argentea* (DPRU 194) will be propagated early 2012. Cuttings from the initial parents and F1 were evaluated for clonal propagation by rooting in mid-2011. Only cuttings of 'Andross' and the F1 hybrid rooted in this initial test.

Table 2. Candidate rootstocks for root expression profiling

Trait	Lovell	Nemaguard	Nemared	Hansen 536	Nickels
Soil Type Adaptability	Well drained soils	Well drained, sandy-loam soils.	Well drained, sandy-loam soils.	Well drained soils, drought tolerant, calcareous soils okay.	Better adaptability than Hansen 536.
Wet Soil	Moderately susceptible	Very susceptible	Very susceptible	Very susceptible	Moderately susceptible
Anchorage	Good	Good	Good	Very good	Very good
Vigor	Moderate	High	Moderate	Very high	Very high
Root Suckers	None	None	None	None	None
Phytophthora Resistance	Low	Low	Low	Very Low	Very Low
Bacterial Canker Resistance	High	Low	Low	Unknown	Unknown
Crown Gall Resistance	Fair	Fair to Good	Fair	Fair	Fair
Root Knot Nematode Resistance	Low	High	High	High	High
Oak Root Fungus Resistance	Low	Low	Low	Low	Low

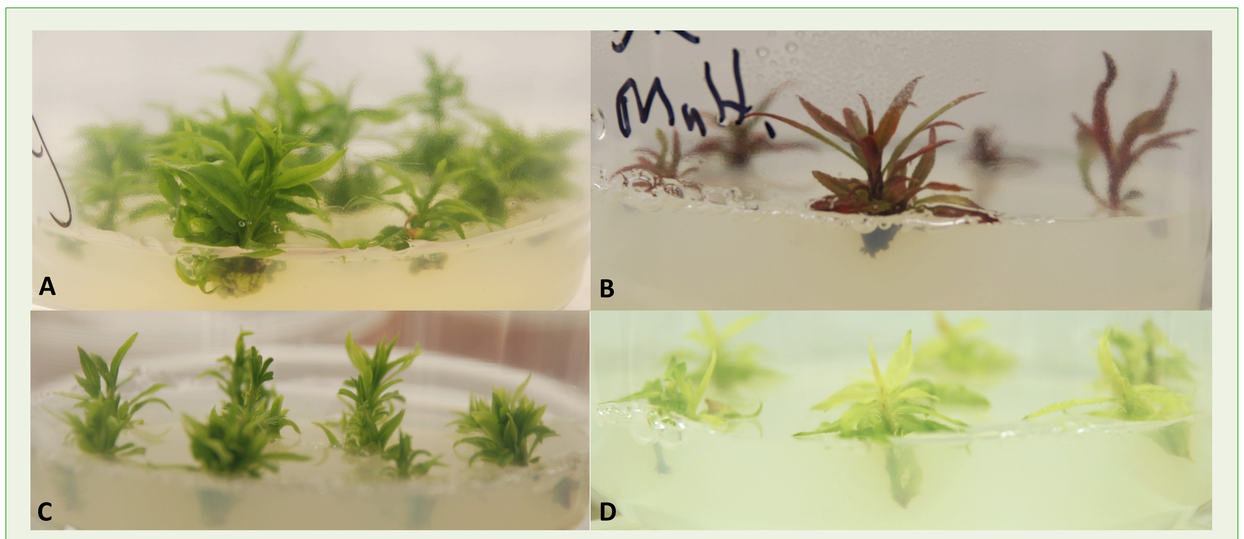


Fig. 1. Interspecific Prunus hybrids in culture. A. 'Tardy Nonpareil' x *P. tangutica*, B. 'Nemared' x *P. fenzliana*, C. 'Tardy Nonpareil' x *P. argentea*, and D. *P. cerasifera* x 'Nickels'.

Table 3. Interspecific Prunus hybrids from 2011

NCGR ID	Mother tree	Pollen source	Seeds produced	Surviving genotypes	Plants in culture
DR-11 DT	Tardy Non-Pareil (UC Davis)	*P. tangutica* DPRU 2327.x	2	2	51
DR-11 DV	Tardy Non-Pareil (UC Davis)	*P. davidiana* DPRU 581	1	1	4
DR-11 DR	Tardy Non-Pareil (UC Davis)	*P. argentea* DPRU 194	4	4	57
DR-11 DK	Tardy Non-Pareil (UC Davis)	*P. kuramica* DPRU 1467.x	9	9	171
DR-11 NT	Nemared (UC Davis)	*P. tangutica* DPRU 2327.x	23	9	37
DR-11 NR	Nemared (UC Davis)	*P. argentea* DPRU 194	23	17	57
DR-11 NC	Nemared (UC Davis)	*P. cerasifera* DPRU 1511	9	5	14
DR-11 NF	Nemared (UC Davis)	*P. fenzliana* (UC Davis)	71	32	96
DR-11 CH	*P. cerasifera* (DPRU 1511)	Nickels (peach-almond)	3	2	16
		TOTALS	***145***	***81***	***503***

FUTURE DIRECTION

- Develop SNP genotyping platform containing SNPs linked to disease and pest resistance for use in evaluating existing rootstock cultivars and germplasm for future rootstock breeding efforts, and evaluating rootstock progeny populations.
- Develop effective and high throughput marker-assisted selection schemes to increase selection efficiency and trait integration in the genetic improvement of almond rootstocks.

ACKNOWLEDGEMENTS

Performed in collaboration with CDFA-SCRI block grant: "Development of almond, stonefruit and walnut rootstocks with improved resistance to soil borne diseases". Coordinated with Greg Browne's evaluation of rootstocks for *Phytophthora* and replant disease complex. Molecular resources developed in this project will directly contribute to addressing the objectives set out in the CDFA-SCRI project.