Integrated Conventional and Genomic Approaches to Almond Rootstock Development

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BACKGROUND

Improved rootstocks resistant to soil borne pests and diseases, and drought are critical to realize the full genetic potential of scion cultivars and for sustainable production of almond. With restrictions on use of fumigants and to minimize environmental impact, the industry reliance on rootstocks with field resistance to soil borne pathogens is increasing. Host plant resistance is the most durable and sustainable form of protection against pests and pathogens. In this ongoing rootstock breeding project we will enlarge the taxonomic and genetic diversity of *Prunus* hybrids by involving potential donor species and genotypes in hybridization.

Development and deployment of high throughput molecular markers will facilitate rapid selection of resistant rootstock genotypes at juvenile stages without pathogen challenge and grow out. These advantages cannot be overstated when considering selection of a commercially viable woody perennial tree rootstock. Although peach seedlings, peach x almond hybrids, and other complex hybrids are currently used in commercial production or being tested, many important wild species of almond and other *Prunus* species with great potential as donors of resistance have not been exploited. We will continue to develop molecular tools to improve selection efficiency and rapid development of rootstocks. We will intensify disease evaluation for soil borne diseases such as phytophthora root rot, crown gall, and nematodes and molecular characterization to decipher the genetic basis of disease resistance for development of effective marker/genomic assisted selection strategies.

Availability of diverse germplasm, high density genotyping, and efficient and foolproof disease testing schemes are critical to the success of rootstock breeding project. Single Nucleotide Polymorphism (SNP) is the marker of choice for high density genotyping and we have successfully employed genotyping-by-sequencing (GBS; Elshire et al., 2011) approach to discover SNPs and genotype breeding populations.

The research during the past three years has focused on:

(1) screening and identifying reliable sources of durable combined resistance to soil borne diseases.
(2) hybridizing potential donor species with peach and almond genotypes to produce novel rootstock genotypes.
(3) SNP genotyping and disease testing.
(4) identifying markers associated with soil borne diseases to develop and validate effective marker assisted selection strategies.

OBJECTIVES

- Produce interspecific hybrids involving *Prunus* spp. that are potential donors of disease resistance to enlarge diversity among hybrids to improve selection response and genetic gains.
- Intensify GBS based high density genotyping of rootstocks and perform association analysis to develop efficient marker assisted selection strategies.
- Disease testing of commercial and experimental rootstocks to produce high quality disease phenotype data.

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PLANS AND PROCEDURES

- **Produce and evaluate diverse interspecific hybrids for tolerance to soil borne diseases (CG, PHY, NEM)**

In addition to 57 hybrids produced during the past four years, during spring, 2015, we produced a number of diverse hybrids: myrobalan plum (*P. cerasifera*) x wild peach (*P. mira*), almond (*P. dulcis*) x Japanese plum (*P. salicina*), peach (*P. persica*) x Japanese plum (*P. salicina*), and peach (*P. persica*) x Nanking cherry (*P. tomentosa*) (Fig. 1). These hybrids were embryo rescued and are in shoot multiplication stage at Sierra Gold Nursery. We are planning to evaluate them for resistance to soil borne diseases during late spring of 2016.

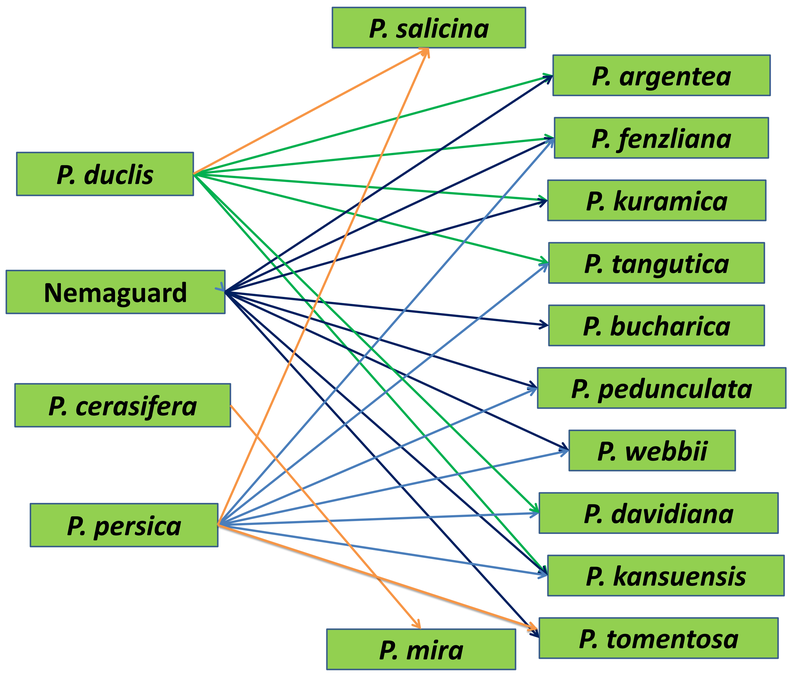

Fig. 1. Interspecific hybrid combination produced in the project. Sixty-one cross combinations with a total of >5700 clonal plants were produced for replicated disease testing trials.

- **Develop and identify single nucleotide markers (SNPs) linked to disease resistance among hybrids**

We will be building on the ongoing efforts of single nucleotide polymorphism (SNP) discovery by following genotyping by sequencing approach. Genotyping-by-sequencing is cost-effective for both SNP discovery and genotyping simultaneously. GBS permits high density genotyping and flexibility for genetic and association mapping. The SNP genotype data in combination with disease screening data will permit us to analyze for the association of markers with disease resistance loci. Association analyses will be performed with a mixed-model integrating the marker-inferred population structure at an estimated number of subpopulations (Q-matrix) and the pair-wise co-ancestry.

- **Develop effective marker based selection strategy for rapid development of rootstocks.**

Validation of markers linked to resistance is the key to formulate selection strategies. Appropriate assays will be designed to genotype rootstock populations with the markers.

RESULTS AND DISCUSSION

Novel rootstocks produced within the project

The first round of disease testing of novel rootstocks generated in the project has yielded encouraging results with wide variation in response to CG and Phytophthora screening. The three rootstock genotypes showing resistance to Phytophthora were planted in Wolfskill Experimental Orchards in Winters after budding them with 'Nonpareil' for further evaluation. These experimental hybrids, which have undergone one round of disease testing along with others from various disease testing trials (190 genotypes) from Kluepfel and Browne lab were included in the association analysis. A second round of CG testing with 31 genotypes is still ongoing and will be concluded in late Spring, 2016.

The GBS data from the IGD contained 221,115,528 sequencing reads of which 203,429,249 could be filtered by barcode producing 18,596,235 unique Tags. Resulting SNPs ranged from 164,742 to 909,352, depending on the calling method and filtering parameters. The genotype data in combination with disease phenotype data generated by collaborating plant pathologists was utilized for association analysis. The multidimentional scaling (MDS) analysis performed on the SNP data for 190 genotypes indicated simple genetic structure, which suggests the least influence of genetic structure on the genotype-disease phenotype

Association Analyses

The results of association analyses performed using two different methods, mixed linear model as implemented in the TASSEL, which along with genetic structure considers coefficient of coancestry of genotypes in computing association are considered more robust than the general linear model implemented in the PLINK, which uses only genetic structure as covariate to compute associations. While these analyses are still preliminary, at this time we have identified significant association of markers with CG, but the analyses failed to come up with any markers for either root knot or lesion nematode infestations. We are further filtering the data set using a number of filtering criteria to eliminate leaky data among the SNP loci identified in the GBS analysis. We expect association of SNPs with other diseases (RNK/RLN) once the data is cleaned up.

The MLM results indicated several SNPs across the genome with significant association ($p<0.05$) with CG with R^2 values ranging from 0.09 to 0.11, which is considered significant for traits such as disease resistance with complex inheritance patterns with generally low heritability. It appears that the genetic loci or quantitative loci (QTLs) modulating CG resistance occurs in four different linkage groups. The marker with highly significant association is found on chromosome 8 with respect to the peach reference genome used in this study for SNP discovery and genotyping. The probability values indicate potential for association, but the low marker R^2 (marker-trait linkage) suggests the markers are only weakly associated with the phenotype.

The analysis using the GLM in PLINK, however, showed a significant association of a SNP with CG located on chromosome 1 at the end of an Exon for a putative iron oxidoreductase enzyme gene in *Arabidopsis thaliana*, which appears to be homologous to a transcript of a putative gene in *P. persica*. Some members of this family appear to have a role in pathogen defense/susceptibility (van Damme et al. 2008).

REFERENCES

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FUTURE PLANS

- Enlarge the taxonomic and genetic diversity of interspecific hybirds to include diverse gene pools of species that are potential donors of disease resistance and drought tolerance.
- Develop effective and high throughput marker-assisted selection schemes to increase selection efficiency and trait integration for improved almond rootstocks.