
Integrated Conventional and Genomic Approaches to Almond Rootstock Development

Project No.: 15-HORT16-Aradhya/Ledbetter

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Objectives:

1. 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.
2. Disease testing of rootstocks to produce high quality disease phenotype data.
3. Intensify genotype-by-sequencing (GBS) based high density genotyping of rootstocks and perform association analysis to develop efficient marker assisted selection strategies.

Interpretive Summary:

Rootstocks with resistance/tolerance to soil borne pests and pathogens and drought are critical for realizing the genetic potential of scion cultivars and for sustainable production of almonds. The soil borne pathogens of almond: *Phytophthora* (PHY) rots; *Agrobacterium* induced crown gall (CG); lesion and rootknot nematodes (NEM) major limiting factors in orchard/nursery productivity and orchard longevity. While widely used fumigant in almond nurseries, methyl bromide is rapidly being phased out. Finding sustainable and environmentally sound alternatives to methyl bromide and other fumigants is a top priority for the Almond Board of California. Rootstocks with resistance to soil-borne pathogens will significantly reduce our dependence on soil fumigation for optimal productivity. Host plant resistance is the most durable and sustainable form of protection against soil borne pests and pathogens.

The ongoing long-term rootstock breeding project is focused on enlarging the taxonomic and genetic diversity of *Prunus* hybrids by involving potential donor species and genotypes in the hybrid production. We will continue to develop molecular and genomic tools to improve selection efficiency and rapid development of rootstocks. We will intensify disease evaluation and molecular characterization to decipher the genetic basis of disease resistance for development of effective marker/genomic assisted selection strategies. Development and deployment of genetic markers will facilitate 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.

Availability of diverse germplasm, high throughput marker systems for high density genotyping, efficient disease testing schemes are the key to the success of this project. Single Nucleotide Polymorphisms (SNPs) are the marker of choice for high density genotyping. This research during the past four years has focused on: (1) screening and identifying reliable sources of durable combined resistance to these soil borne diseases; (2) hybridizing potential donor species with peach and almond genotypes to produce novel rootstock genotypes; (3) SNP genotyping and disease testing; and (4) identifying markers associated with soil borne diseases to develop and validate effective marker assisted selection strategies.

Research highlights:

1. Identified marker(s) associated with an important soil borne crown gall (CG) disease.
2. Assembled a wide range of novel interspecific *Prunus* hybrids involving species that are potential donors of disease resistance and hitherto not produced.
3. Identified a number of candidate rootstocks that show high levels of resistance/tolerance to CG: P2-4 ('Nemared' x *P. argentea*); P4-25 ('Nemared' x *P. fenzliana*); P4-10 ('Nemared' x *P. fezliana*); 197-113 (*P. persica* x *P. tangutica*); 197-199 (*P. persica* x *P. davidiana*); 197-217 (*P. persica* x *P. kuramica*), and PHY: P4-1 (*P. dulcis* x *P. kuramica*); P2-4 ('Nemared' x *P. aregentea*); L1-2 (*P. cerasifera* half-sib) in initial disease evaluation trials.

Materials and Methods:

Production and disease testing on novel rootstocks

In addition to 65 hybrids produced during the past four years (**Table 1**), 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*) (**Figure 1**). These hybrids were embryo rescued and clonally propagated to produce plants for disease evaluation. We are planning to evaluate them for resistance to soil borne diseases beginning fall of 2016.

Develop and identify SNPs linked to genes mediating disease resistance

We will be building on the ongoing efforts of SNP discovery by following GBS approach. GBS is cost-effective for both SNP discovery and genotyping simultaneously (Elshire et al., 2011). 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.

Results and Discussion:

Novel rootstocks produced and disease evaluation

The first round of disease evaluation of novel rootstocks generated in this project has yielded encouraging results with wide variation in response to CG and *Phytophthora* screening. A number of candidate rootstocks that show high levels of resistance/tolerance to CG: P2-4 ('Nemared' x *P. argentea*); P4-25 ('Nemared' x *P. fezziana*); P4-10 ('Nemared' x *P. fezziana*); 197-113 (*P. persica* x *P. tangutica*); 197-199 (*P. persica* x *P. davidiana*); 197-217 (*P. persica* x *P. kuramica*), and PHY: P4-1 (*P. dulcis* x *P. kuramica*); P2-4 ('Nemared' x *P. argentea*); L1-2 (*P. ceracifera* half-sib) in initial disease evaluation trials will be grafted with scions and planted for evaluation of graft compatibility. We do not expect any serious graft compatibility issues among these as all the rootstocks were derived from peach-almond genetic background. A second round of CG testing with 44 hybrids is still ongoing and will be concluded in late fall, 2016. A *Phytophthora* field evaluation trial with same 44 hybrids is slated for planting in fall, 2016.

SNP discovery and genotyping of Prunus rootstocks

Previously, 190 rootstock genotypes consisting of commercial/UC/FPS/novel rootstocks produced within the project have been genotyped by following GBS method at the Institute for Genomic Diversity (IGD) at Cornell University. While disease evaluation data is being collected on the 65 new hybrids, we will be SNP genotyping them by GBS approach during current project cycle to perform association analysis.

Diseases screening and association analyses

For the previous association analysis, categorical disease screening data for CG was provided by Kluepfel lab (unpublished) at USDA-ARS. Quantitative disease evaluation data for PHY was obtained from Browne lab (unpublished), and root knot and root lesion nematode quantitative data were gleaned from online sources originating from the McKenry lab at Kearney Ag Center, Parlier.

The mixed linear model (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 or quantitative loci (QTLs) modulating CG resistance occurs in four different linkage groups. The marker with highly significant association is found located on chromosome 8 based on the peach reference genome used in this study for SNP discovery.

The analysis using the general linear model (GLM), 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 gene family appear to have a role in pathogen defense/susceptibility (van Damme et al. 2008).

In continuation of the association analyses reported above, we will be assembling disease evaluation and SNP genotype data for the new set of hybrids produced in this project to perform association analysis. The marker discovery process will continue as and when the disease data for new hybrids become available.

Research Effort Recent Publications:

Velasco, D., Aradhya, M. K. and Ross-Ibarra, J. 2016. Evolutionary genomics of peach and almond domestication. (submitted to G3:Genes/Genomics/Genetics)

References Cited:

- Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS one*, 6(5), e19379.
- van Damme M, Huibers RP, Elberse J, Van den Ackerveken G. 2008. Arabidopsis RMR6 encodes a putative 2OG-Fe(II) oxygenase that is defense-associated but required for susceptibility to downy mildew. *The Plant Journal*. 54:785-793.

Table 1. *Prunus* interspecific hybrids produced between 2010-2015

Year	Code	Mother Tree	Mother tree	Pollen Parent	Pollen Parent
		Accession	Species	Accession	Species
2010	DR10-P019.3	Pom WEO F5 3-5	<i>P. dulcis</i>	DPRU 0194	<i>P. argentea</i>
		Pom WEO F5 3-5	<i>P. dulcis</i>	Pomology	<i>P. fenzliana</i>
		Pom WEO F5 3-5	<i>P. dulcis</i>	DPRU 582	<i>P. kansuensis</i>
		Pom WEO F5 3-5	<i>P. dulcis</i>	DPRU 1467.x	<i>P. kuramica</i>
		Pom WEO F5 3-5	<i>P. dulcis</i>	DPRU 2327.x	<i>P. tangutica</i>
		Pom WEO PG 7-16	<i>P. hybrid/P. persica</i>	DPRU 194	<i>P. argentea</i>
		Pom WEO PG 7-16	<i>P. hybrid/P. persica</i>	DPRU 1871.1	<i>P. bucharica</i>
	DR10-P014.1	Pom WEO PG 7-16	<i>P. hybrid/P. persica</i>	Pomology	<i>P. fenzliana</i>
		Pom WEO PG 7-16	<i>P. hybrid/P. persica</i>	DPRU 582	<i>P. kansuensis</i>
		Pom WEO PG 7-16	<i>P. hybrid/P. persica</i>	DPRU 1467.x	<i>P. kuramica</i>
		Pom WEO PG 7-16	<i>P. hybrid/P. persica</i>	DPRU 2329.21	<i>P. pedunculata</i>
		Pom WEO PG 7-16	<i>P. hybrid/P. persica</i>	DPRU 2316.5	<i>P. tomentosa</i>
		Pom WEO PG 7-16	<i>P. hybrid/P. persica</i>	DPRU 196	<i>P. webbii</i>

		DPRU 2160 A	<i>P. persica</i>	DPRU 582	<i>P. kansuensis</i>
		DPRU 2160 A	<i>P. persica</i>	DPRU 2329.21	<i>P. pedunculata</i>
		DPRU 2261 B	<i>P. persica</i>	DPRU 1871.1	<i>P. bucharica</i>
		DPRU 2261 B	<i>P. persica</i>	DPRU 581	<i>P. davidiana</i>
		DPRU 2261 B	<i>P. persica</i>	Pomology	<i>P. fenzliana</i>
		DPRU 2261 B	<i>P. persica</i>	DPRU 2327.x	<i>P. tangutica</i>
		DPRU 2261 B	<i>P. persica</i>	DPRU 2312.2	<i>P. triloba</i>
		DPRU 2261 B	<i>P. persica</i>	DPRU 196	<i>P. webbii</i>
		DPRU 2267 A	<i>P. persica</i>	DPRU 1060 & 1065	<i>P. hybrid</i>
	DR10-OT16.06	DPRU 2327.16	<i>P. tangutica</i>	Unknown	<i>Open pollinated</i>
	DR10-OT17.08	DPRU 2327.17	<i>P. tangutica</i>	Unknown	<i>Open pollinated</i>
2011	DR-11R	DPRU 0194	<i>P. argentea</i>	Unknown	<i>Open pollinated</i>
	DR-11CH	DPRU 1511	<i>P. cerasifera</i>	FPS	<i>P. hybrid</i>
	DR-11C1	DPRU 1511	<i>P. cerasifera</i>	OPS	
	DR-11DR	Pom WEO F5 3-5	<i>P. dulcis</i>	DPRU 194	<i>P. argentea</i>
	DR-11DK	Pom WEO F5 3-5	<i>P. dulcis</i>	DPRU 1467.x	<i>P. kuramica</i>
	DR-11DT	Pom WEO F5 3-5	<i>P. dulcis</i>	DPRU 2327.x	<i>P. tangutica</i>
	DR-11DV	Pom WEO F5 3-5	<i>P. dulcis</i>	DPRU 581	<i>P. davidiana</i>
	DR-11NC	Pom WEO PG 7-3	<i>P. hybrid/P. persica</i>	DPRU 1511	<i>P. cerasifera</i>
	DR-11NT	Pom WEO PG 7-3	<i>P. hybrid/P. persica</i>	DPRU 2327.x	<i>P. tangutica</i>
	DR-11NF	Pom WEO PG 7-3	<i>P. hybrid/P. persica</i>	Pomology	<i>P. fenzliana</i>
	DR-11NR	Pom WEO PG 7-3	<i>P. hybrid/P. persica</i>	DPRU 194	<i>P. argentea</i>
	DR-11W1	DPRU 0197 A/B	<i>P. webbii</i>	Unknown	<i>Open pollinated</i>
	DR-11W2	DPRU 0198	<i>P. webbii</i>	Unknown	<i>Open pollinated</i>
2012	DR-12PB	DPRU 1576. C	<i>P. persica</i>	Kearney	<i>P. arabica</i>
	DR-12PF	DPRU 1602. C	<i>P. persica</i>	Pomology	<i>P. fenzliana</i>
	DR-12PF	DPRU 2151. A	<i>P. persica</i>	Pomology	<i>P. fenzliana</i>
	DR-12PD1	DPRU 2151. B	<i>P. persica</i>	DPRU 2578.2 A	<i>P. dulcis</i>
	DR-12PH1	DPRU 2466.12 A	<i>P. persica</i>	DPRU 2915.15	<i>P. hybrid</i>
	DR-12PV	DPRU 2466.17 A	<i>P. persica</i>	DPRU 2493.X	<i>P. davidiana</i>
	DR-12PU2	DPRU 2499.1 A	<i>P. persica</i>	DPRU 0192.3	<i>P. bucharica</i>
	DR-12PK2	DPRU 2499.4 A	<i>P. persica</i>	DPRU 1467.9	<i>P. kuramica</i>
	DR-12PU1	DPRU 2499.5 A	<i>P. persica</i>	DPRU 1871.1	<i>P. bucharica</i>
	DR-12PH2	DPRU 2499.6 A	<i>P. persica</i>	FPS	<i>plum hybrid</i>
	DR-12PD2	DPRU 2544. A	<i>P. persica</i>	DPRU 2578.2 A	<i>P. dulcis</i>
	DR-12PR	DPRU 2546.3 A	<i>P. persica</i>	DPRU 0194	<i>P. argentea</i>
	DR-12PH4	DPRU 2629. B	<i>P. persica</i>	DPRU 1063	<i>plumcot</i>
	DR-12PN1	DPRU 2631. A	<i>P. persica</i>	DPRU 2316.12	<i>P. tomentosa</i>
	DR-12PS	DPRU 2631. B	<i>P. persica</i>	DPRU 582	<i>P. kansuensis</i>
	DR-12PH3	DPRU 2651.1 A	<i>P. persica</i>	DPRU 1063	<i>plumcot</i>

	DR-12PT	DPRU 2651.2 A	<i>P. persica</i>	DPRU 2327.x	<i>P. tangutica</i>
	DR-12PL	DPRU 2654.1 A	<i>P. persica</i>	DPRU 2464 A	<i>P. salicina</i>
	DR-12PK1	DPRU 2655.1 A	<i>P. persica</i>	DPRU 1467.9	<i>P. kuramica</i>
	DR-12PN2	DPRU 2656.2 A	<i>P. persica</i>	DPRU 2316.12	<i>P. tomentosa</i>
2015	DR15-CM1	DPRU 2982.4	<i>P. cerasifera</i>	DPRU 3112	<i>P. mira</i>
	DR15-CM2	DPRU 2982.4	<i>P. cerasifera</i>	DPRU 3112	<i>P. mira</i>
	DR15-CM3	DPRU 2982.4	<i>P. cerasifera</i>	DPRU 3112	<i>P. mira</i>
	DR15-CM4	DPRU 2982.4	<i>P. cerasifera</i>	DPRU 3112	<i>P. mira</i>
	DR15-CM5	DPRU 2982.4	<i>P. cerasifera</i>	DPRU 3112	<i>P. mira</i>
	DR15-CM6	DPRU 2982.4	<i>P. cerasifera</i>	DPRU 3112	<i>P. mira</i>
	DR15-CM7	DPRU 2982.4	<i>P. cerasifera</i>	DPRU 3112	<i>P. mira</i>
	DR15-CM8	DPRU 2982.4	<i>P. cerasifera</i>	DPRU 3112	<i>P. mira</i>
	DR15-DS1	DPRU 2962.22	<i>P. dulcis</i>	DPRU 424	<i>P. salicina</i>
	DR15-DS2	DPRU 2962.22	<i>P. dulcis</i>	DPRU 424	<i>P. salicina</i>
	DR15-DS3	DPRU 2962.22	<i>P. dulcis</i>	DPRU 2806	<i>P. salicina</i>
	DR15-DS4	DPRU 2962.22	<i>P. dulcis</i>	DPRU 2806	<i>P. salicina</i>
	DR15-DS5	DPRU 2962.22	<i>P. dulcis</i>	DPRU 2806	<i>P. salicina</i>
	DR15-DS6	DPRU 2960.8	<i>P. dulcis</i>	DPRU 2806	<i>P. salicina</i>
	DR15-DS7	DPRU 2960.8	<i>P. dulcis</i>	DPRU 2806	<i>P. salicina</i>
	DR15-DS8	DPRU 2960.8	<i>P. dulcis</i>	DPRU 2806	<i>P. salicina</i>
	DR15-DS9	DPRU 2960.8	<i>P. dulcis</i>	DPRU 2806	<i>P. salicina</i>
	DR15-DS10	DPRU 2960.8	<i>P. dulcis</i>	DPRU 2806	<i>P. salicina</i>
	DR15-DS11	DPRU 2960.8	<i>P. dulcis</i>	DPRU 2806	<i>P. salicina</i>
	DR15-DS12	DPRU 2960.8	<i>P. dulcis</i>	DPRU 2806	<i>P. salicina</i>
	DR15-DS13	DPRU 2960.8	<i>P. dulcis</i>	DPRU 2806	<i>P. salicina</i>
	DR15-DS14	DPRU 2959.8	<i>P. dulcis</i>	DPRU 2806	<i>P. salicina</i>
	DR15-DS15	DPRU 2959.8	<i>P. dulcis</i>	DPRU 2806	<i>P. salicina</i>
	DR15-DS24	DPRU 2959.8	<i>P. dulcis</i>	DPRU 2806	<i>P. salicina</i>
	DR15-PS1	DPRU 3159	<i>P. persica</i>	DPRU 424	<i>P. salicina</i>
	DR15-PS2	DPRU 3159	<i>P. persica</i>	DPRU 424	<i>P. salicina</i>
	DR15-PS3	DPRU 3159	<i>P. persica</i>	DPRU 424	<i>P. salicina</i>
	DR15-PS4	DPRU 3159	<i>P. persica</i>	DPRU 424	<i>P. salicina</i>
	DR15-PS5	DPRU 3159	<i>P. persica</i>	DPRU 424	<i>P. salicina</i>
	DR15-PS6	DPRU 3159	<i>P. persica</i>	DPRU 424	<i>P. salicina</i>
	DR15-PS7	DPRU 3159	<i>P. persica</i>	DPRU 424	<i>P. salicina</i>
	DR15-PT1	DPRU 3159	<i>P. persica</i>	mixed pollen	<i>P. tomentosa</i>
	DR15-PT2	DPRU 3159	<i>P. persica</i>	mixed pollen	<i>P. tomentosa</i>
	DR15-PT3	DPRU 3159	<i>P. persica</i>	mixed pollen	<i>P. tomentosa</i>
	DR15-PSA1	DPRU 3190	<i>P. persica</i>	DPRU 424	<i>P. salicina</i>
	DR15-PSA2	DPRU 3190	<i>P. persica</i>	DPRU 424	<i>P. salicina</i>
	DR15-PSA3	DPRU 3190	<i>P. persica</i>	DPRU 424	<i>P. salicina</i>
	DR15-PSA4	DPRU 3190	<i>P. persica</i>	DPRU 424	<i>P. salicina</i>

DR15-PSA5	DPRU 3190	<i>P. persica</i>	DPRU 424	<i>P. salicina</i>
DR15-PSA6	DPRU 3190	<i>P. persica</i>	DPRU 424	<i>P. salicina</i>
DR15-PSA7	DPRU 3190	<i>P. persica</i>	DPRU 424	<i>P. salicina</i>
DR15-PSA8	DPRU 3190	<i>P. persica</i>	DPRU 424	<i>P. salicina</i>
DR15-PSA9	DPRU 3190	<i>P. persica</i>	DPRU 424	<i>P. salicina</i>
DR15-PSA10	DPRU 3190	<i>P. persica</i>	DPRU 424	<i>P. salicina</i>

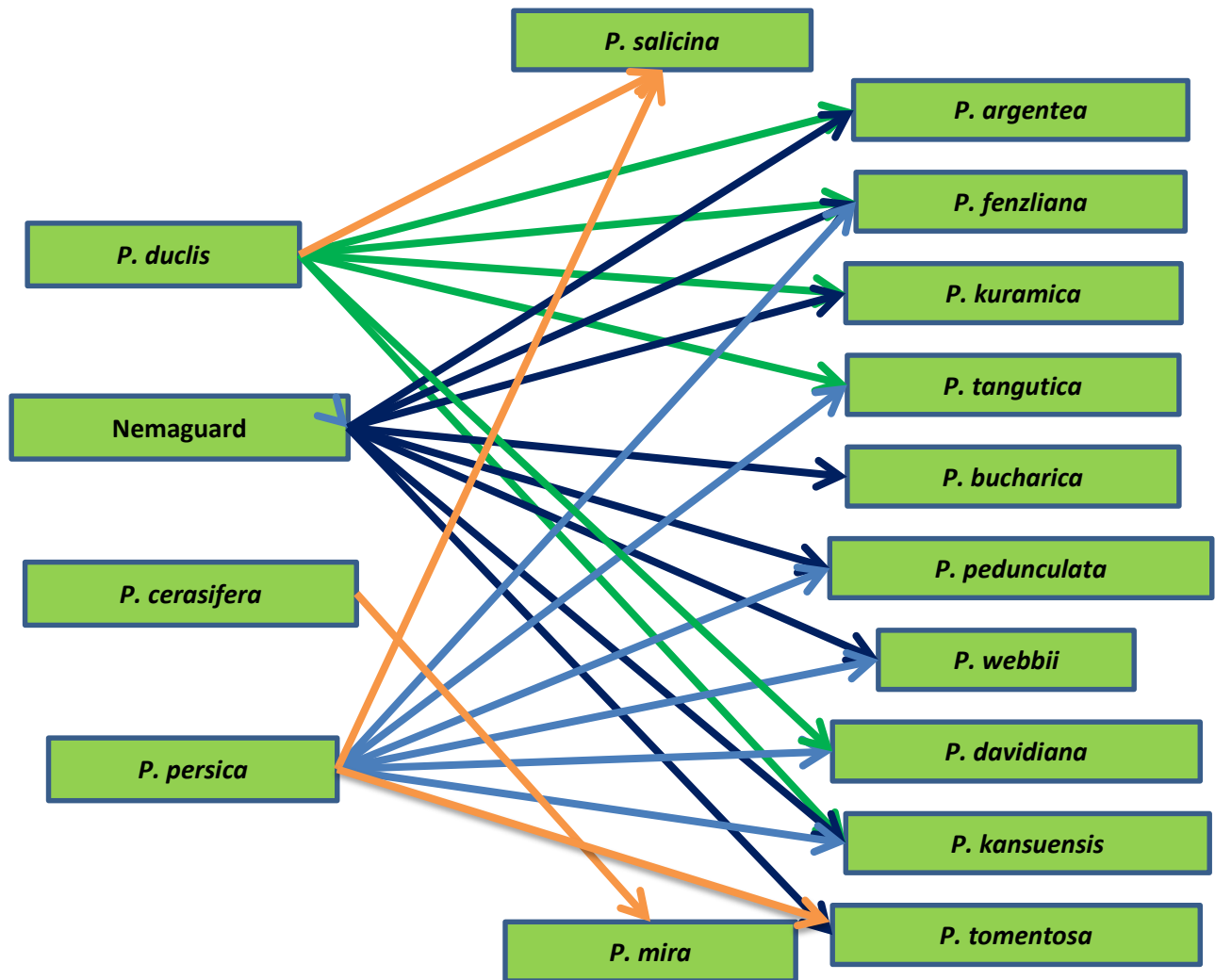


Figure 1. Interspecific hybrids produced in the project.