

INTRODUCTION

Almond (*Prunus dulcis* (Mill.) D. A. Webb; Rosaceae) is a temperate, deciduous, insect-pollinated tree native to southwest Asia. Commercial US almond production is limited to the California Central Valley, and accounts for 80% of world almond production (USDA-FAS, 2009). Almonds are the highest value agricultural export (Matthews et al., 2010) and third highest valued agricultural product (USDA-NASS, 2008) in California with an annual value of \$2 billion.

Soil borne pests and pathogens considerably reduce almond yields in California. Countering this problem requires developing rootstocks with durable resistance to pests and diseases. Disease evaluation and molecular characterization of available rootstocks, new interspecific hybrids, and wild *Prunus* germplasm are critical to identify novel sources of durable resistance and develop marker-assisted selection (MAS) methods.

Genetic markers permit rapid, accurate, and large scale screening of germplasm and breeding populations to select desired gene combinations that confer durable resistance. MAS permits genetically sound juvenile selection of resistant plants at seedling and sapling stages, without challenging them with pests and pathogens, and is therefore much more cost and time efficient as compared to trait-based selection. An effectively MAS strategy will allow for the rapid development of rootstocks with field resistance.

Effective MAS strategies require: (1) high throughput molecular marker systems to perform rapid and cost effective fine scale genotyping of large populations; (2) identification of markers that co-segregate with the resistance traits; and (3) reliable disease screening techniques to assay large populations.

SOLUTIONS

Molecular characterization combined with extensive disease and pest resistance evaluation of rootstocks currently available and the ones in evaluation trials, interspecific hybrids, and germplasm accessions

- Discover single nucleotide polymorphism (SNP) markers for high-throughput genotyping
- Identify novel sources of resistance in *Prunus* germplasm collections and interspecific hybrids
- Link SNP markers with disease and pest resistance through genetic and association mapping
- Develop marker-assisted breeding tools for large-scale juvenile selection of resistance to pests and diseases

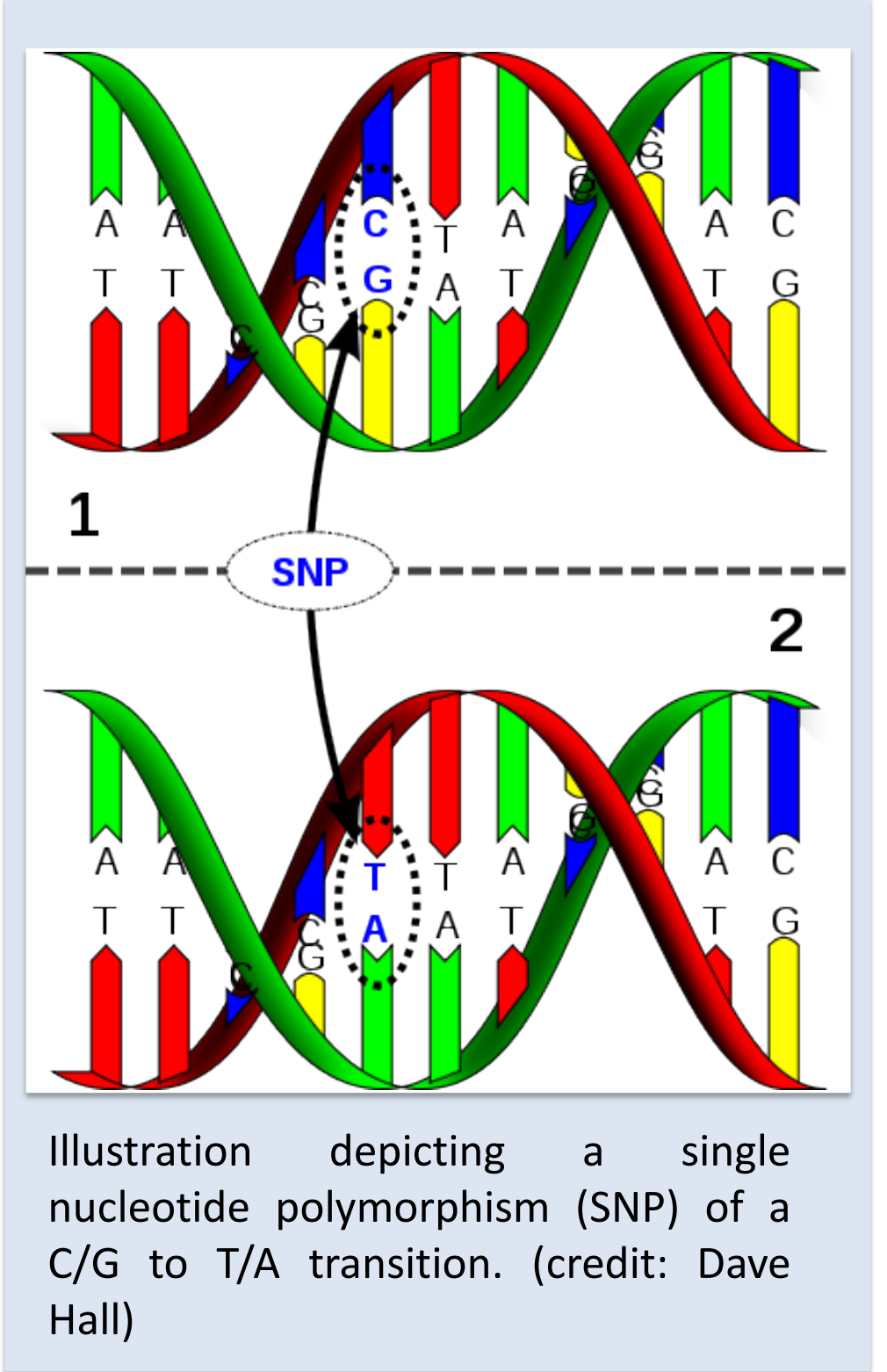
Marker-Assisted Selection

Why Marker-Assisted Selection?

- Trait-based selection is difficult, time-consuming, and resource-intensive
- Permits juvenile selection to take advantage of rare recombination events in large breeding populations
- Preferred over trait-based selection for difficult traits, especially biotic and abiotic stresses, including disease and pest resistance
- For traits with low heritability
- Allows for pyramiding resistance genes

SNPs - Molecular Marker of Choice

Single nucleotide polymorphisms (SNPs) are DNA sequence variations of single base mutations. They are the only marker system to allow fast, efficient, large-scale genotyping using high-throughput platforms to evaluate tens of thousands to hundreds of thousands of SNPs simultaneously. SNPs occur abundantly and are distributed uniformly throughout plant genomes permitting fine scale genome mapping. This enables the evaluation of all available genetic resources with varying resistance to pests and diseases. Identification of multiple resistance sources will permit gene pyramiding in interspecific hybrids. SNPs facilitate genetic association analyses targeting resistance to diseases using natural populations, such as germplasm collections consisting of genotypes of unknown or mixed ancestry that represent a common gene pool.



OBJECTIVES

1. Assemble and validate *in silico* discovered SNPs of peach and almond.
2. Discover SNPs from genomic and EST sequences from a set of diverse peach, almond and wild species resources
3. Molecular characterization of peach and almond wild relatives, and production and testing of interspecific hybrids (see Table 1) to identify novel sources of resistance to soil borne pests and diseases.

Available Resources

Germplasm

Prunus gene pools and interspecific hybrids



Almond relatives: *P. webbii* - hardiness, late bloom, self-fertility, modified growth habit and tree size; *P. pedunculata* - cold hardiness; *P. bucharica* - hardiness and late bloom; *P. argentea* - modified tree size; *P. tangutica* - hybrids easily obtained; *P. fenziiana* - putative wild progenitor, hybridize well; *P. kuramica* - vigor, low chill, tree architecture

Peach & Peach relatives: *P. persica* - vast source of genetic resources, produces fertile F1s with *P. dulcis*; *P. davidiana* - nematode resistance; *P. kansuensis* - hybrid vigor imparted to scion; *P. mira* - leaf curl resistance

Other Prunus: *P. cerasifera* - adaptation to wet, waterlogged soil, moderate resistance to crown rot, cold hardiness, possible resistance to RKN and oak root fungus; *P. tomentosa* - cold hardiness, dwarfing; *P. triloba* - cold hardiness

Interspecific Hybrids

Table 1. Interspecific crosses made in 2010.

	<i>P. dulcis</i>	<i>P. persica</i>	Nemaguard	Nemared
<i>P. argentea</i>	22	0	2	0
<i>P. bucharica</i>	0	2	1	0
<i>P. cerasifera</i>	-	0	-	0
<i>P. davidiana</i>	0	2	-	-
<i>P. fenziiana</i>	3	3	1	0
<i>P. kansuensis</i>	2	2	1	-
<i>P. kuramica</i>	5	0	1	-
<i>P. pedunculata</i>	-	2	1	0
<i>P. tangutica</i>	3	2	0	0
<i>P. tomentosa</i>	-	0	1	0
<i>P. triloba</i>	0	1	-	-
<i>P. webbii</i>	-	4	1	0
Plumcot	-	54	-	-

Additional Materials

- Nursery rootstock trial materials
- F₂ population of *P. persica* x *P. webbii* (C. Ledbetter)
- USDA National Clonal Germplasm Repository almond and peach collections, including wild relatives

Genome & EST Sequences

Publicly available peach genome sequence to use in sequence assembly and alignment, and SNP discovery



Collaboration

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

EXPECTED RESULTS

- Discover genes and QTLs conferring resistance to soil borne pests and pathogens, and linkage to SNP markers.
- Molecularly and phenotypically characterized rootstock and germplasm for resistance to soil borne pests and diseases.
- Develop effective and high throughput marker-assisted selection schemes to increase selection efficiency and trait integration in the genetic improvement of almond rootstocks.

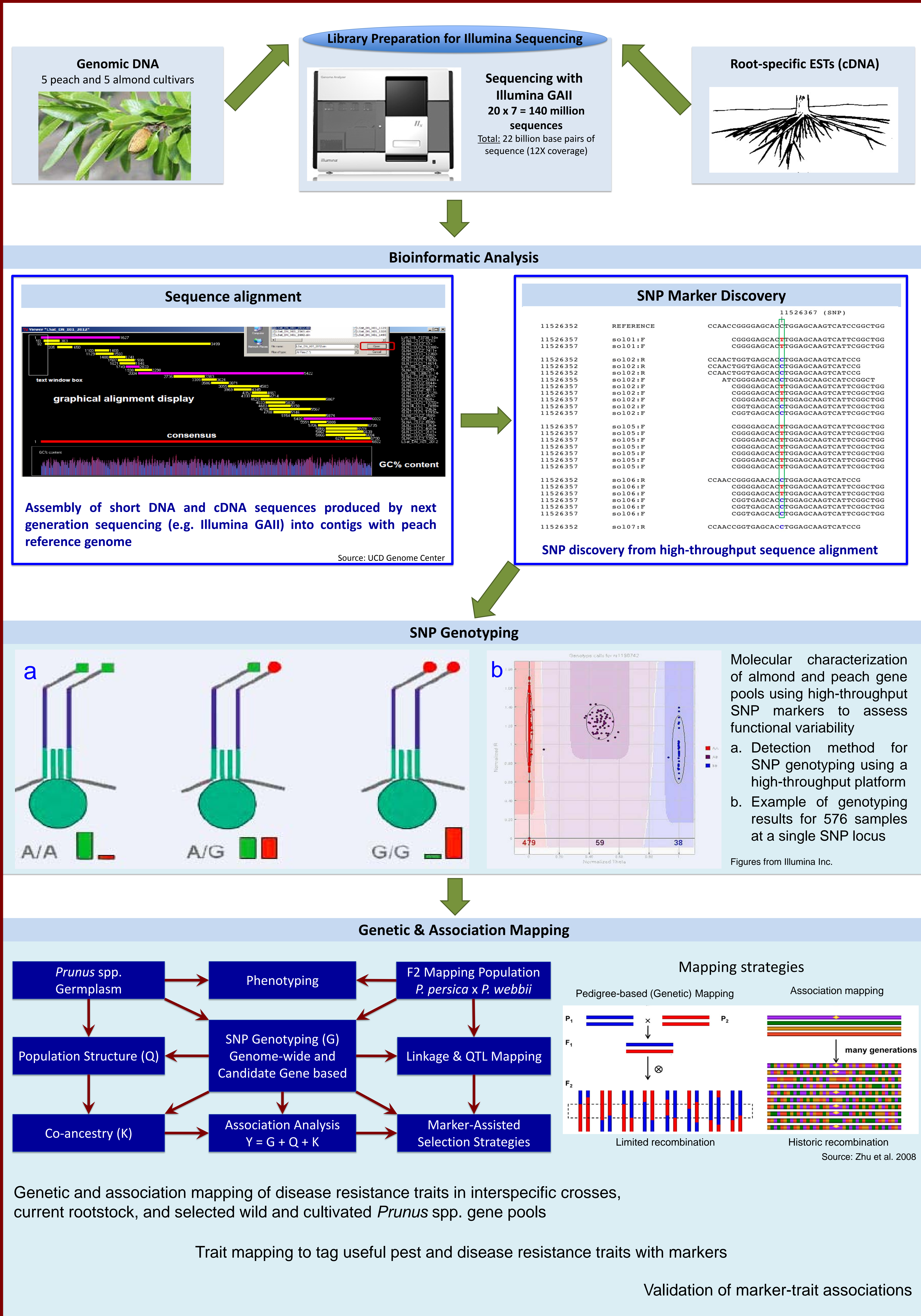
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ACKNOWLEDGEMENTS



Genomic and EST Sequencing, SNP Discovery, and Mapping



Genetic and association mapping of disease resistance traits in interspecific crosses, current rootstock, and selected wild and cultivated *Prunus* spp. gene pools

Trait mapping to tag useful pest and disease resistance traits with markers

Validation of marker-trait associations