
Interspecific Breeding Germplasm for Rootstock Research and Development

Project No.: 12-HORT10-Gradziel

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Objectives:

- A.** Catalog individual interspecific breeding populations currently available from the almond and peach scion breeding programs. Compile available breeding information including initial parental source, detailed pedigrees, and field evaluation notes for dissemination to interested public and private researchers.
- B.** Propagate promising selections for distribution to interested researchers. In consultation with researchers, develop seed populations of 100+ individuals for the most promising breeding lines for potential use in subsequent trait/molecular marker segregation proposals/projects.
- C.** Transfer promising core germplasm to the USDA Prunus Germplasm Repository for long-term maintenance and public-domain access.

Interpretive Summary:

Interspecific germplasm sources within the UCD almond and peach breeding program which show value for use in rootstock genetic improvement have been identified. Detailed pedigree relationships as well as parent and limited progeny molecular and phenotypic information has been transferred to the RosBreed web-site (<http://www.rosbreed.org/>) to allow end-user analysis. Germplasm represented by these populations demonstrate extensive diversity both molecularly and phenotypically and include a wide range of species including *Prunus tangutica*, *P. persica*, *P. davidiana*, *P. mira*, *P. argentea*, *P. scoparia*, *P. dulcis*, and *P. webbii*. Field evaluation of the germplasm has identified unique and potentially useful characteristics in parents of some of the lineages, including modification of tree architecture by *P. scoparia* hybrid rootstocks, a high level of drought tolerance in a peach by *P. argentea* hybrid, and a pronounced invigoration of scion growth in several advanced interspecies introgression lines. The most promising selections have been propagated, either clonally or by seed propagation, for distribution to USDA germplasm repository and interested researchers. An ongoing challenge is germplasm dispersal since research momentum in rootstock and disease screening has stalled due, in part, to insufficient program funding. As a consequence, several

progeny populations will be maintained as ungerminated seed and limited sample-size plantings the more promising breeding lines are being planned for 2014 in order to better assess final rootstock breeding value.

Introduction:

Changes in almond cropping and cultural management patterns have led to the need for a new generation of compatible rootstocks. Responding to this need, a number of public and private efforts have been initiated to research, develop and test new rootstocks and interstocks for almond and related stone fruit.

Germplasm derived from interspecies hybrids is often pursued to attain the largest possible range of vigor and desirable horticultural traits. However, the acquisition of such exotic germplasm is often difficult and time-consuming. As part of the long-term almond and peach scion development programs at UCD, breeding lines have been developed combining almond, peach as well as related *Prunus* species including *P. argentea*, *P. bucharica*, *P. davidiana*, *P. fenzliana*, *P. domestica*, *P. mira*, *P. orthosepala*, *P. scoparia*, *P. tangutica* and *P. webbii* (Figure 1). Although early selections within this germplasm have demonstrated evidence for desirable rootstock/interstock traits such as drought and disease resistance, dramatic cutbacks in UCD field support have resulted in some of this germplasm being already discarded, with much of the remainder slated for removal within the next 2 years. This project would make propagations of remaining germplasm available to interested researchers and would develop suitably large (100-200 individuals) seedling populations from targeted interspecific genotypes prior to parent tree removal.

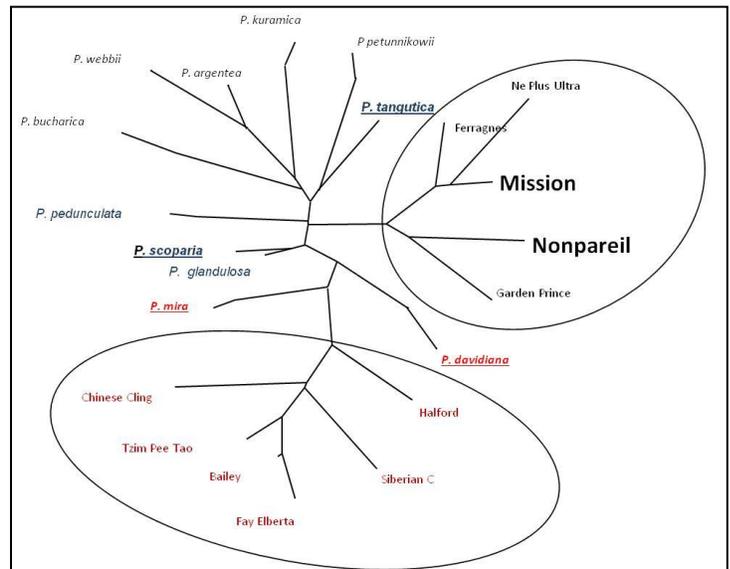


Figure 1. Taxonomic relationships among species evaluated.

Materials and Methods:

- A. Catalog currently available interspecific breeding populations within the UCD almond and peach scion breeding programs. Identify the specific parental source and detailed lineage, where known. Provide pedigree information in the publically available RosBreed PediMap software format (<http://www.rosbreed.org/>) to allow end-user visualization of pedigree relationships as well as genotypes still available at UCD. Compile all relevant phenotypes and field evaluation notes for dissemination to interested public and private researchers.
- B. Propagate promising selections by softwood cuttings or T-bud-propagations for distribution to interested researchers. Use selfed (forced-selfed for self-incompatible selections) seed for cutting-recalcitrant selections. Using controlled crosses, generate seed populations of 100-200 individuals for end-user identified breeding selections for use in trait/molecular marker segregation proposals/projects.

- C. Identify core germplasm (i.e., capturing fullest range of genetic/trait diversity or highest levels of promising traits) based on PediMap analysis and in consultation with USDA Repository scientists. Propagate by T-budding onto Lovell rootstock and transfer selections to the USDA Prunus Germplasm Repository/Davis, CA for long-term maintenance and to allow ready public access. Provide passport data and relevant trait/molecular information for inclusion into the USDA GRIN database.

Results and Discussion:

A. Catalog and phenotype.

We have identified specific interspecific germplasm sources and lineages currently available within the breeding program (**Table 1**). Detailed pedigree relationships as well as parent and limited progeny molecular and phenotypic information has been transferred to the RosBreed web-site (<http://www.rosbreed.org/>) to allow end-user analysis. Over 500 RosBreed molecular markers, which are relatively evenly distributed over the eight Prunus chromosomes, have been used to characterize each genotype. In addition, phenotypic information concerning horticultural characteristics (fruit type, bloom and maturity time, fruit and seed mass, etc.) has also been compiled with the RosBreed data. Germplasm represented by these populations demonstrate extensive diversity both molecularly and phenotypically (see **Figure 2**). Species represented within this germplasm include *Prunus tangutica*, *P persica*, *P davidiana*, *P mira*, *the argentea*, *P scoparia*, *P dulcis*, and *P webbii*.

The availability of this diverse germplasm has also allowed continued genetic and horticultural analysis. A recent analysis of the S-allele loci which control self-and cross-compatibility in Prunus have identified several new potentially useful alleles (**Figure 2**). In addition, the extended evaluation period has allowed identification of unique and potentially useful scion tree architectures and growth habits in parents of some of the progeny populations (**Figure 3**).

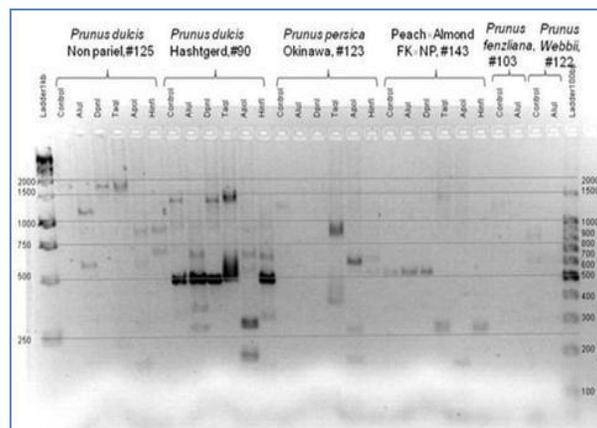


Figure 2. Novel S-alleles controlling self-and cross-compatibility in Prunus species including wild relatives as identified with Iranian and Spanish collaborators..



Figure 3. Representative range of aboveground scion tree architectures contained within the current germplasm

Table 1. UCD peach and almond species germplasm selections for which detailed molecular and phenotype information is available through the RosBreed web-site (<http://www.rosbreed.org/>).

Selection	Parent1	Parent2	Source	Number of Selections, F1 or F2s
2008_3_196	Loadel	Yumyeong	<i>P.persica</i>	22
2005_17_1	Loadel	Vilmos	<i>P. dulcis</i>	42
2005_17_255	Carson	persXdavidiana	<i>P.davidiana</i>	23
2005_17_155	Loadel	persXdavidiana	<i>P.davidiana</i>	1
2006_1_107	18_8_11	P_Tang_Mix	<i>P.tangutica</i>	1
2003_1_329	DrDavis	P.mira19	<i>P. dulcis</i>	1
P.mira#19	P.mira	P.mira	<i>P.mira</i>	1
2001_7_180	Andross	P.argentea	<i>P.argentea</i>	1
2000_2_8	Loadel	P.argentea	<i>P.argentea</i>	2
2005_20_192	91_16_154	Ogawa	<i>P. dulcis</i>	1
Hansen1	Almondseed2	Nemaguard	<i>P.davidiana</i>	1
Nickels	CP_5_33	Nemaguard	<i>P. dulcis</i>	1
2000_3_205	Andross	MissionxScoparia	<i>P.scoparia</i>	1
Carmel	Nonpareil	Mission	<i>P. dulcis</i>	1
Jordanolo	Nonpareil	Harriott	<i>P. dulcis</i>	1
Panamint	BabcockxBoston	GoldminexRioOsoGem	<i>P.persica</i>	1
2000_2_16	Loadel	F8_5_166	<i>P. dulcis</i>	1
2005_22_204	91_17_195	F8_5_159	<i>P. dulcis</i>	2
98_2_132	Pallas	F8_1_96	<i>P. dulcis</i>	1
2005_16_172	OHenry	F8_1_42	<i>P. dulcis</i>	19
99_16_131	F8_1_121	F8_1_121	<i>P. dulcis</i>	1
2005_17_5	Loadel	F10C_12_28	<i>P. dulcis</i>	1
Woltemade	Kakamas	F_Wolvamade	<i>P.persica</i>	1
Stukey_6_9BF	Nonpareil	F_Stukey69BF	<i>P. dulcis</i>	1
Stukey_6_8	Nonpareil	F_Stukey68	<i>P. dulcis</i>	1
Stukey_6_27H	Nonpareil	F_Stukey627H	<i>P. dulcis</i>	1
Stukey_6_27	Nonpareil	F_Stukey627	<i>P. dulcis</i>	1
St_John	ChineseCling	F_StJohn	<i>P. dulcis</i>	1
Sonora	Nonpareil	F_Sonora	<i>P. dulcis</i>	1
F5C_6_9BF	Nonpareil	F_F5C_6_9BF	<i>P. dulcis</i>	1
F5C_6_8	Nonpareil	F_F5C_6_8	<i>P. dulcis</i>	1
F10C_20_51	F8_76_45	F_F10C_20_51	<i>P. dulcis</i>	1
F10C_12_28	F8_72_33	F_F10C_12_28	<i>P. dulcis</i>	1
2007_12_209	2000_16_133		<i>P. dulcis</i>	1

Table 1 (continued). UCD peach and almond species germplasm selections for which detailed molecular and phenotype information is available through the RosBreed web-site (<http://www.rosbreed.org/>).

Name-TMG	Parent1	Parent2	Source	Number of Selections, F1 or F2s
2005_20_11	Carson	-	P.persica	1
2005_18_151	2001_7_180	-	P.argentea	1
2005_17_208	Carson	-	P. dulcis	1
2005_17_148	Loadel	-	P. dulcis	1
2008_58_18	2000_8_150	DrDavis	P.persica	1
54P455	GoldenGlory	Bonanza	P.persica	1
98_9_7	93_3_159	Bolinha	Bolinha	1
F8_7_179	Nonpareil	A80_10_22	P. dulcis	1
2009_19_18	Andross	96_9_292	P.persica	3
2005_29_95	92_14_73	92_14_73	P.persica	1
2001_18_215	91_18_6	91_18_6	P.persica	1
Ogawa	90_10_91	90_10_91	P. dulcis	6
F8_1_42	90_1_4	90_1_4	P. dulcis	1
2008_13_194	Loadel	2003_1_329	P.mira	11
2005_19_40	19_2_72	2001_7_180	P.scoparia	1
2005_18_244	Rizzi	2001_7_180	P.argentia	20
2009-28-152	18_8_11	2001_18_215	P.persica	1
2007_10_244	2000_8_150	2000_8_150	P. dulcis	3
2005_19_139	19_2_72	2000_3_205	P.scoparia	1
2005_20_117	Carson	2000_3_205	P.argentia	7
2005_18_191	2000_2_8	2000_2_8	P.argentia	3
2007_12_234	2000_16_133	2000_16_133	P. dulcis	35
2007_11_249	2000_15_122	2000_15_122	P. dulcis	1
Vilmos	F10C_12_28	*VP	P. dulcis	1
Mission_BF	Mission	*MUT	P. dulcis	1
NonpareilBF	Nonpareil	*MUT	P. dulcis	1
TardyNonpareil	Nonpareil	*MUT	P. dulcis	1
40A_17	-	-	P.persica	1
Hansen536	-	-	P. dulcis	1
Mission	-	-	P. dulcis	1
Nemaguard	-	-	P. dulcis	1
Nonpareil	-	-	P. dulcis	1
persicaXdauidiana	-	-	P.dauidiana	1
Winters	-	-	P. dulcis	1
Yumyeong	-	-	P.persica	1

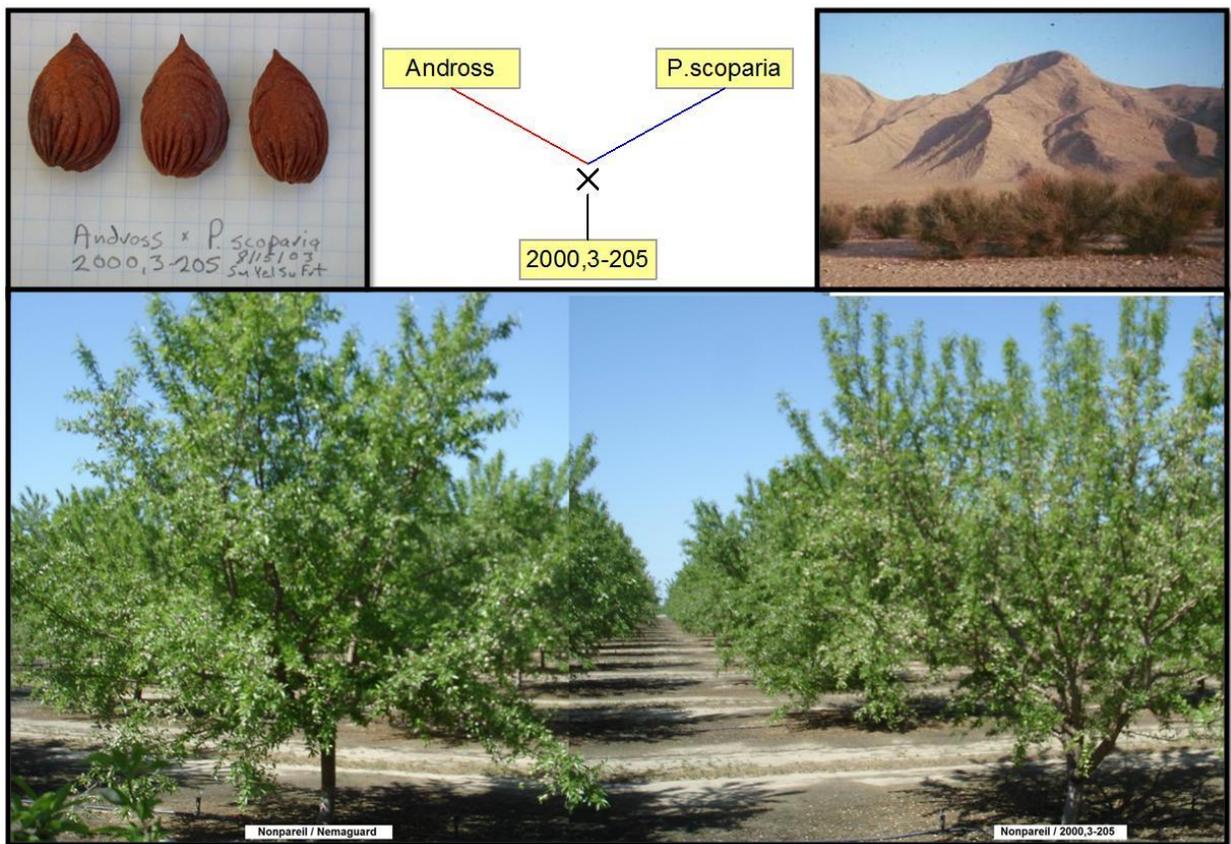


Figure 4. Origin of UCD 2000,3-205 from a cross between peach and *Prunus scoparia*, showing the harsh desert conditions the *P. scoparia* parent, the intermediate endocarp structure of the hybrid and the pronounced effect on the scion tree architecture when used as a rootstock for Nonpareil almond.

In addition, a distinctly upright and open architecture was observed when the peach x *P. scoparia* hybrid 2000,3-205 was used as rootstock for Nonpareil almonds under standard commercial culture (**Figure 4**) demonstrating the potential of rootstocks to affect aboveground growth and productivity.

Similarly, a high level of drought tolerance observed in the peach x *P. argentea* UCD selection 2001,7-180 (**Figure 5**) when irrigation was discontinued to the breeding block in anticipation of its subsequent removal. Since the original seedling tree was being evaluated, both below-ground (root) and above-ground growth would be influenced. Significantly, high fruit production was achieved in this individual despite the lack of midsummer irrigation.

Both genotypes are among those that have been characterized molecularly and phenotypically in the RosBreed database and propagated to USDA germplasm repository collection for public domain access.

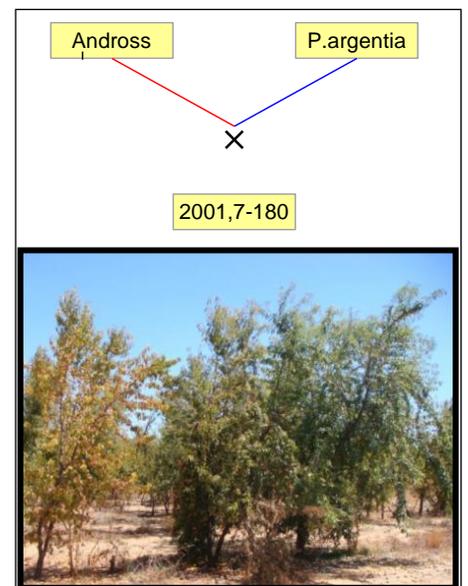


Figure 5. Demonstration of drought tolerance of original UCD 2001,7-180 seedlings derived from *P. argentea* by *P. persica* hybridization.



Figure 6. Left: An almond by peach interspecies hybrid rootstock conferring the well established and desirable strong vigor to the scion peach tree (left). Right: similar vigor observed in rare individuals of advanced introgression lines in which the expected genomic invigoration would be absent.

A well-established and desirable consequence of interspecies hybrids as rootstock for *Prunus* crops is the pronounced invigoration of scion growth (**Figure 6**). This invigoration is thought to be a consequence of the unique interaction of the two distinct but related genomes (peach and almond in the above example). In advanced backcrosses of interspecies hybrids, we have documented a rare but consistent occurrence of individuals demonstrating growth vigor well beyond that of their sibs (**Figure 6**). The rarity of these individuals suggests that the invigoration is a consequence of very specific genetic rearrangements within these introgression lines. Because these genotypes would be primarily composed of the backcross parent, variability within their seedlings should be reduced resulting in more uniform progeny populations. For example, the F₂ BC 3 individual in **Figure 6** would, in theory, be > 95% almond despite the *P. mira* contribution in the original hybrid. Because of the size of the combined UCD peach and almond breeding programs, over a dozen of such rare, excessively vigorous genotypes have been identified and maintained to date. The genetic, molecular genetics and horticultural analysis of these individuals and their progeny may allow unique insights to the genetic control of above and belowground growth vigor and their effect on tolerance to diseases and environmental stress. (See section C).

B. Seed and clonal Propagation.

Key selections, representing diverse species lineages, have been propagated for distribution to interested researchers. Using both selfed pollinations as well as controlled hybridizations we have generated over 2000 new seed/seedling genotypes for use in trait/molecular marker segregation projects. Germplasm available in our core collection is shown in **Table 2**. Advanced seedling progeny generations of this material as well as novel germplasm currently in the hybridization program is summarized in **Table 3**. Seed is currently in greenhouse planting in preparation for field transplanting in late 2013 to early 2014.

Table 2. Species hybrids and introgression lines presently available in our core collection.

Species	F1	F2	BC1	Other
Peach x <i>P. argentea</i>	2	175	100	200
Peach x <i>P. scoparia</i>	1	10	500	150
Peach x <i>P. dulcis</i>	100	500	100	1000
Peach x <i>P. mira</i>	3	150	100	500
Peach x <i>P. davidiana</i>	1	100	50	100
Peach x <i>P. tangutica</i>	1	50		
Peach x plum	2			
Almond x <i>P. persica</i>	100	200	5	2000

Table 3. Advanced introgression populations developed from core germplasm.

Almond x <i>P. mira</i> (BC1)
Almond x <i>P. argentea</i> (BC1)
Almond x <i>P. fenzliana</i> (BC1)
Almond x <i>P. mira</i> (BC3)
Almond x <i>P. davidiana</i> (BC3)
Almond x <i>P. argentea</i> (BC3)
Almond x <i>P. mira</i> (BC2)
Almond x <i>P. webbii</i> (BC1)
Almond x <i>P. webbii</i> (BC3)
Almond x <i>P. webbii</i> (BC4)
Almond x <i>P. webbii</i> (F2)
<i>P. orthosepala</i>
Almond x <i>P. persica</i> (BC3)
Almond x <i>P. persica</i> (BC4)
Almond x <i>P. bucharica</i>
Almond x <i>P. webbii</i> x <i>P. persica</i>

As documented in the accompanying tables, a large amount of clonal (propagated) and seed-based interspecies germplasm has been assembled by this project and an ongoing challenge is its dispersal to interested researchers. Research momentum in rootstock and disease screening has been somewhat stalled in 2013 due to insufficient funding. Similarly, University and industry cutbacks within our genetic improvement programs have limited our ability to move forward with all promising interspecies lines. As a consequence, several progeny populations will be maintained as ungerminated seed while smaller sample-size plantings of the more promising breeding lines are being planned for 2014 in order to better assess ultimate rootstock breeding value.

C. Identify core germplasm.

A core germplasm (i.e., capturing fullest range of diversity) based on PediMap analysis is currently being developed as described in section A. The most promising material has been propagated and approx. 20 selections transferred to date to the USDA Repository for long-term maintenance and public access. Limited USDA field space, however, is focusing more attention on identifying the core genetic material with the greatest promise. While the species, themselves, would represent the greatest genetic diversity; many of the desirable aspects of rootstock performance appear to be the consequence of the unique genetic/genomic interaction typically found in interspecies hybrids. Compilations of molecular data from both the RosBreed and related UCD marker analysis are continuing, which would allow the next step of a more structured biometric analysis of the different interspecies populations. **Figure 7** shows the genomic fingerprints resulting from comparison of over 500 molecular markers for a dozen genotypes displaying the advanced introgression-invigoration described in **Figure 6**. While analysis is still at an initial stage, some initial association patterns are evident. For example, a distinct outlier from the observed common patterning (shown by blue arrows in the stacked sections of an initially continuous chart) was subsequently found to be the result of an error in leaf sampling, demonstrating, at least initially, the potential discriminating power these markers. Detailed analysis of both molecular and phenotypic data will begin in 2014 when the final compilation is complete.

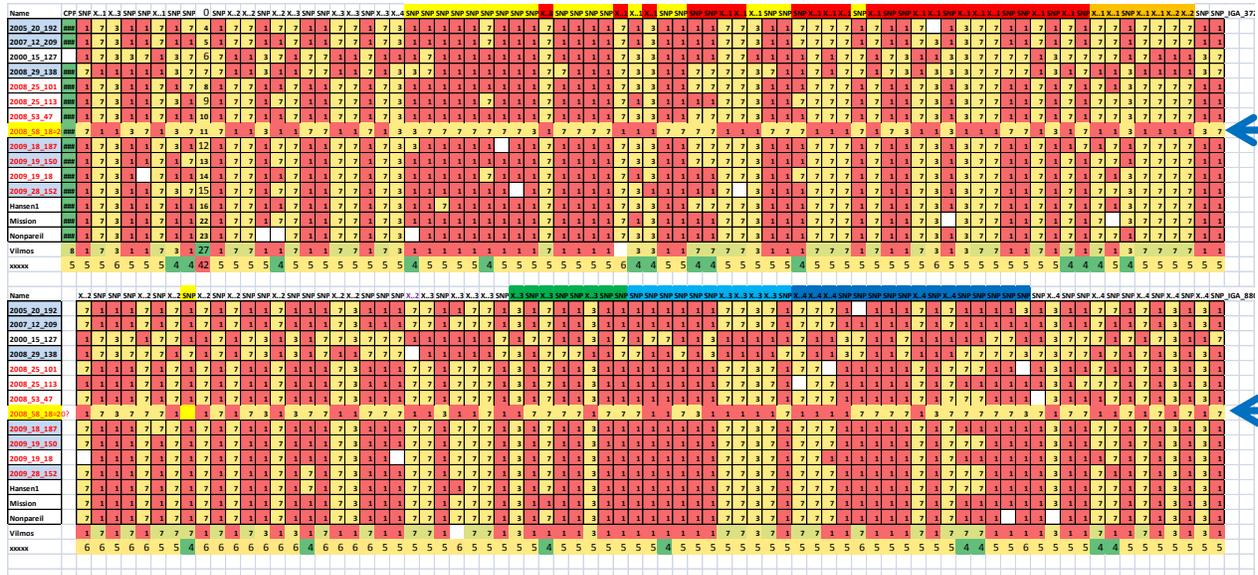


Figure 7. Summary of segregation patterns for over 500 molecular markers covering all 8 *Prunus* chromosomes (top colored bands) for sampled introgression-invigoration lines described in **Figure 6** allowing identification of a sampling error (blue arrows).

Recent Research Effort Publications:

Sorkheh, K., Shiran, B., Asadi, E., Jahanbazei, H., Gradziel, T. M., Mohammady, SH 2011. Evaluation of Genetic Variation, Relatedness, and Testing The Genetic Origin of Cultivated Genotypes and Wild Species of Almond using AFLP Markers, *Scientific Journal of Agriculture*.

Sorkheh, K., B. Shiran, R. Ahmad, P. Martinez-Gomez, T.M. Gradziel, N.Amirbakhtiar 2011. Analysis of Almond [*Prunus dulcis* (Miller) D. A. Webb] cultivars and related wild species using SSR and AFLP markers. *Journal of Horticultural and biotechnology*.

Sánchez-Pérez R, M Zeinalabedini, M. Khayam-Nekoui, V Grigorian, T.M. Gradziel, and P Martínez-Gómez. 2011. The origin and dissemination of the cultivated almond as determined by nuclear and chloroplast SSR marker analysis. *Scientia Horticulturae*.

Shiran, B. Sorkheh, K., V. Rouhi, T. M. Gradziel, B. K. Epperson, P. Martinez-Gomez 2011. Molecular characterization of Iranian almond cultivars and related wild species using amplified fragment-length polymorphisms (AFLPs). Zaragoza (Spain), 16-20 September. *Acta Horticulture*

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Socias i Company, R., J.M. Alonso, O. Kodad and T.M. Gradziel. 2011. Almonds. In: M.L. Badenes and D.H. Byrne (eds.), *Fruit Breeding, Handbook of Plant Breeding 8*. Springer N.Y. pg. 697-728.

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