Interspecific Breeding Germplasm for Rootstock Research and Development

Project No.:   **13-HORT10-Gradziel**

Project Leader: Tom Gradziel
Department of Plant Sciences
UC Davis
One Shields Ave.
Davis, CA 95616
530.752.1575
tmgradziel@ucdavis.edu

Project Cooperators: M.A. Thorpe, J. Fresnedo, S. Overstreet, B. Lampinen,
J. Adaskaveg, J.Connell, S. Metcalf, C. Crisosto, J. Preece,
T. Michailides, M. Aradhya, C. Ledbetter, G. Browne, C. Peace,
and J. Slaughter

Objectives:

A. Generate, catalog and compile targeted interspecific breeding populations currently available for dissemination to interested public and private researchers.

B. Propagate promising selections for distribution to interested researchers and/or USDA Prunus Germplasm Repository for long-term maintenance and public-domain access.

C. Begin a preliminary analysis of the value of marker assisted selection (MAS) in characterizing and prioritizing populations of differing interspecific backgrounds.
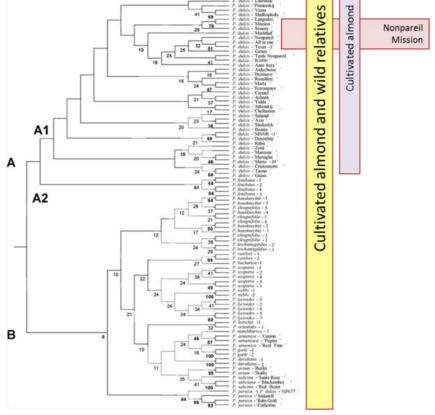
Interpretive Summary:

Interspecific germplasm sources within the UC Davis (UCD) almond and peach breeding program which show value for use in rootstock genetic improvement are being identified, catalogued and propagated. Detailed pedigree relationships as well as trait expression data has been transferred to the RosBEED website (http://www.rosbreed.org/) to allow end-user analysis. Germplasm represented by these populations demonstrate extensive diversity both genetically and in the range of new traits that they bring to rootstock improvement programs. This germplasm includes 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 and progeny from 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 where appropriate, by seed propagation, for distribution to USDA germplasm repository and interested researchers. Several progeny populations are being maintained as ungerminated seed and limited sample-size plantings with more promising breeding lines being planted in 2014/15 to better assess final rootstock breeding value. Disease and nematode evaluation plots are concurrently being developed in cooperation with UCD, USDA, nursery and grower cooperators. The genetic

characterization of principle species and species-hybrid parents and, when possible, breeding progeny is continuing with the genotyping for over 500 molecular markers showing a fairly uniform distribution over all eight of the Prunus chromosomes with results made available at the RosBREED website (http://www.rosbreed.org/). Inheritance studies further indicate that some markers may be unreliable as indicators for specific trait selection, presumably a consequence genetic masking resulting from the inter-species origin. Based on both field expression of tree traits and earlier molecular marker success for trait prediction in a diverse peach breeding material, a subset of species and interspecies germplasm showing promise for rootstock development was further evaluated using an Illumina 9K SNP mini-array. While inheritance studies for certain traits again showed inconsistencies, particularly in genotypes with interspecies origin, general inheritance patterns both within and among chromosomes suggested opportunities for more efficient characterization as well as selection of important rootstock traits such as vegetative vigor and tree architecture.

Introduction:

A new generation of resistant and compatible rootstocks are required to meet changes in almond cropping, soil pests, and cultural management patterns. To meet 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 greatest opportunity for new trait introduction as well the ability to leverage interspecies 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

Genetic variability in almond & relatives

Figure 1. Revised genomic relationships among species evaluated (Ref. #6) demonstrating the large germplasm diversity exploitable for rootstock breeding.

including *P. argentea, P. bucharica, P. davidiana, P. fenzliana, P. domestica, P. mira, P. orthosepala P. scoparia, P. tangutica* and *P. webbii*. Early selections within this germplasm have demonstrated traits which would be desirable for rootstock/interstock traits including

drought and disease tolerance. Because of large and continuing cutbacks in UCD field support, germplasm not directly applicable to the almond and peach cultivar breeding program is being for removed. During the first year of this project germplasm having an interspecies origin has been catalogued (several thousand genotypes) and we are now propagating the more promising selections to make them available to interested researchers. In addition, field crosses in 2012 have generated over 3000 seed (some material is being saved as seed for later germination screens) and/or seedling of almond by peach (F1, F2,), almond by Nemaguard, almond by 40A17 (nematode resistant source), peach by *Prunus mira*, (F1, BC1), peach by *P. tangutica* (F1, F2), peach by *P. davidiana* (F1, F2), peach by Nemaguard, peach by *P. argentea* (F2, BC1), peach by almond by *P. scoparia* (F2, BC1). Progeny population sizes range from only a few to several hundred individuals depending upon the difficulty of the cross. Field crosses in 2013 have generated an additional 2000 seed and/or seedling of almond by peach (F2,), Nemaguard by CP45 (almond parent of Nickels rootstock), almond by 40A17 (nematode resistant source), peach by *Prunus mira*, (BC1), peach by *P. tangutica* (F2).

Materials and Methods:

A. Generate, catalog and compile targeted interspecific breeding populations currently available 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 self-pollinations to generate seed populations of 100-200 individuals for germination/greenhouse planting or seed storage for later germination assays. Crossing targets for 2013 include *P. tangutica, P. scoparia, P. davidiana* and *P. dulcis* interspecies F2's and backcrosses.

C. Use RosBEED developed molecular markers (utilizing 2013 RosBEED SNP arrays when necessary) test whether molecular markers assort normally from parent to progeny in interspecies crosses or whether aberrant segregation patterns (due to chromosomal rearrangements such as translocations/inversions and associated gene methylation) are evident. Some peach by almond lineages in the initial RosBEED analysis showed strong distortions from expected patterns, undermining the predictive ability of marker assisted selection (MAS) in these relatively closely related interspecies crosses.

Results and Discussion:

A. Generate, catalog and compile targeted interspecific breeding populations.
Species represented within this germplasm (**Figure 1**) *include Prunus tangutica, P persica, P davidiana, P mira, the argentea, P scoparia, P dulcis,* and *P webbii.* An extensive diversity is also captured for each species within introgression lineages. There are a range of interspecies hybrids

Figure 2. Characteristic tree and fruit traits typical for the primal-group of interspecies introgression genotypes.

with a well-established rootstock value owing to their typical high vigor and disease/stress resistance and ability to modify scion architecture and physiology. Also included are increasingly advanced introgressed individuals who tend to lose this interspecific robustness but gain in specific scion compatibility and, in some cases, more compact tree structure. A very rare but intriguing introgression-type which combines the vigor and growth habit typically found in interspecies hybrids with greater genomic uniformity is the primal-type. Typically found in advanced introgression lines at very low frequencies (1/500-1/1000) these rare individuals appear to be reversions to a more primal, undomesticated phenotype which typically displays the almond-like leaf, tree and fruit traits characteristic of wild Prunus (**Figure 2**). This reversion-type also typically shows augmentation of tree vigor and tree architecture commonly associated with interspecies hybrids (**Figure 3a**). Although rare, the size and genetic diversity of UCD breeding program has allowed the collection of over 30 primal genotypes, most often in peach-almond and in peach-mira introgression lines. In some advanced introgression material, primals have been selected which display a high quality, sweet kernel in an otherwise hybrid vigor background and so may have unique opportunities for commercial almond production under high disease/environmental stress conditions (**Figure 3b**). Because of their rarity there is virtually no information concerning such primals in the literature. Based on the UCD experience, such next interspecies material may be relatively common in domesticated almond (see reference 8, Gradziel and Martinez-Gomez) and some of the plums. Peach-almond reversion lines are particularly useful because they are easy to identify given the distinctive tree/leaf/fruit characteristics for peach, and because that specific species hybrid group has proven particularly valuable for rootstock development.

Figure 3. (a) Comparison of relative vigor of smaller peach and larger peach-almond primal-type of identical tree age (left photo). (b) Primal selection 2008, 19-76 combining sweet, good commercial quality kernels in a fruit and tree type more characteristic of interspecies hybrids (right photo)**.**

B. Propagate promising selections.

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 4000 new seed/seedling genotypes in 2013/14 for use in trait/molecular marker segregation projects. Germplasm now available in our core collection as clonal material and/or seed/seedling populations is shown in **Tables 1** and **2**. Advanced seedling

progeny generations of this material as well as novel germplasm, including primals, currently in the hybridization program is summarized in **Table 3**.

Table 1. Species hybrids and introgression lines presently available in our core collection. Items which have been vegetatively propagated in 2013 are highlighted in yellow while lineages maintained through seed propagation followed by field planting in 2013/14 are highlighted in blue.

Species	F1	F2	BC1	Other
Peach x P. argentea	2	130	85	150
Peach x P. scoparia	1	10	410	100
Peach x P. dulcis	90	420	80	800
Peach x P. mira	3	150	200	400
Peach x P. davidiana	1	90	40	80
Peach x P. tangutica	1	50		
Peach x plum	2			
Almond x P.persica	240	210	5	1400

Table 2. UCD peach and almond species germplasm selections for which detailed molecular and phenotype information is now available through the RosBREED website (http://www.rosbreed.org/). Items which have been vegetatively propagated in 2013 are highlighted in yellow while lineages maintained through seed propagation followed by field planting in 2013/14 are highlighted in blue.

Selection	Parent1	Parent2	Source	Number of Selections, F1 or F2s
2008_3_196	Loadel	Yumyeong	*P.persica*	22
2005_17_1	Loadel	Vilmos	*P. dulcis*	42
2005_17_255	Carson	persXdavidiana	*P.davidiana*	23
2005_17_155	Loadel	persXdavidiana	*P.davidiana*	1
2006_1_107	18_8_11	P_Tang_Mix	*P.tangutica*	1
2003_1_329	DrDavis	P.mira19	*P. dulcis*	1
P.mira#19	P.mira	P.mira	*P.mira*	1
2001_7_180	Andross	P.argentea	*P.argentea*	1
2000_2_8	Loadel	P.argentea	*P.argentea*	2
2005_20_192	91_16_154	Ogawa	*P. dulcis*	1
Hansen1	Almondseed2	Nemaguard	*P.davidiana*	1
Nickels	CP_5_33	Nemaguard	*P. dulcis*	1
2000_3_205	Andross	MissionxScoparia	*P.scoparia*	1
Carmel	Nonpareil	Mission	*P. dulcis*	1
Jordanolo	Nonpareil	Harriott	*P. dulcis*	1
Panamint	BabcockxBoston	GoldminexRioOsoGem	*P.persica*	1
2000_2_16	Loadel	F8_5_166	*P. dulcis*	1
2005_22_204	91_17_195	F8_5_159	*P. dulcis*	2
98_2_132	Pallas	F8_1_96	*P. dulcis*	1
2005_16_172	OHenry	F8_1_42	*P. dulcis*	19
99_16_131	F8_1_121	F8_1_121	*P. dulcis*	1
2005_17_5	Loadel	F10C_12_28	*P. dulcis*	1
Woltemade	Kakamas	F_Wolvamade	*P.persica*	1
Stukey_ 6_9BF	Nonpareil	F_Stukey69BF	*P. dulcis*	1
Stukey_ 6_8	Nonpareil	F_Stukey68	*P. dulcis*	1
Stukey_6_27H	Nonpareil	F_Stukey627H	*P. dulcis*	1
Stukey_6_27	Nonpareil	F_Stukey627	*P. dulcis*	1
St_John	ChineseCling	F_StJohn	*P. dulcis*	1
Sonora	Nonpareil	F_Sonora	*P. dulcis*	1
F5C_6_9BF	Nonpareil	F_F5C_6_9BF	*P. dulcis*	1
F5C_6_8	Nonpareil	F_F5C_6_8	*P. dulcis*	1
F10C_20_51	F8_76_45	F_F10C_20_51	*P. dulcis*	1
F10C_12_28	F8_72_33	F_F10C_12_28	*P. dulcis*	1
2007_12_209	2000_16_133		*P. dulcis*	1

Table 2. (continued). UCD peach and almond species germplasm selections for which detailed molecular and phenotype information is available through the RosBREED website (http://www.rosbreed.org/). Items which have been vegetatively propagated in 2013 are highlighted in yellow while lineages maintained through seed propagation followed by field planting in 2013/14 are highlighted in blue.

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
persicaXdavidiana	-	-	P.davidiana	1
Winters	-	-	P. dulcis	1
Yumyeong	-	-	P.persica	1

Table 3. Advanced introgression populations developed from core germplasm.

Primals	Seed	Pollen
2005,20-192	91,16,154	Ogawa
2007,12-209	2000,16-133	self
2009,17-302	Andross	96,9-292
2009,17-325	Andross	96, 9-292
2009,18-151	Andross	96,9-292
2009,19-331	Andross	96,9-292
2009,24-337	DRDAVIS	96,9-292
2009,25-36	DRDAVIS	96,9-292
2009,26-185	DrDAVIS	96,9-292
2009,26-205	DrDAVIS	96,9-292
2009,26-266	Dr.DAVIS	96,9-292
2008,61-38	91,17-262	E22-59
2009,19-247	Andross	96, 9-292
2009,27-175	Dr. DAVIS	96, 9-292
2009,18-87	Andross	96,9-292
2008,25-101	Andross	2000_8_157
2008,25-113	Andross	2000_8_157
2008,53-47	Riegels	00,12-110
2008,58-18	91,17-195	00,8-150
2009,17-340	Andross	96,9-292
2009,19-252	Andross	96, 9-292
2009,19-32	Andross	96, 9-292
2009,19-85	Andross	96, 9-292
2009,20-89	KLAMT	96, 9-292
2009,21-1	ROSS	98, 4-177 BRR
2009,21-168	ROSS	98, 4-177 BRR
2009,23-109	RIZZI	E 22-59
2009,23-28	RIZZI	2001_18_215
2009,28-152	18_8_11	2001_18_215
2009,29-107	Lt. ROSS	96, 9-229 BRR
2009,29-15	Everts	2001_18_215
2009,33-175	Halford	2001_18_215
Introgression lineages		
Almond x P. mira (BC1)		
Almond x P. argentea (BC1)		
Almond x P. fenzliana (BC1)		
Almond x P. mira (BC3)		
Almond x P. davidiana (BC3)		
Almond x P. argentea (BC3)		
Almond x P. mira (BC2)		
Almond x P. webbii (BC1)		
Almond x P. webbii (BC3)		
Almond x P. webbii (BC4)		
Almond x P. webbii (F2)		
P. orthosepala		
Almond x P.persica (BC3)		
Almond x P.persica (BC4)		
Almond x P. bucharica		
Almond x P. webbii x P.persica		

Figure 4. Representative RosBreed marker patterns for each of the eight Prunus chromosomes analyzed (colored bars in the top row). [While only about 30 markers are shown for each chromosome, they are representative of the over 500 markers analyzed.

C. Preliminary analysis of the value of marker assisted selection (MAS) in characterizing and prioritizing populations of differing interspecific backgrounds.

The now completed analysis of RosBREED marker data documents an extensive genetic diversity and variability within the UCD breeding program, in effect dwarfing available diversity of the three other breeding programs (Clemson, University of Arkansas, and Texas A&M) combined. As detailed in the 2012 Almond Breeding and Interspecific Breeding Germplasm report (Project 12-HORT10-Gradziel), this extensive diversity also led to a large error rate and identifying specific alleles and their associations with specific traits. This is primarily because the markers utilized were initially developed from the peach genome and while the relative position would be expected to be similar (though several exceptions in the form of translocations and inversions are presented in the 2012 reports), the specific allelic signature was often not fully recognized and so accurately recorded for many interspecific relatives and even their advanced introgression lines (see 2012 Almond Variety Development report 12-HORT1-Gradziel). Consequently, the value of individual markers for MAS needs to be determined on a lineage by individual marker basis (see discussions related to **Figure 6** below). However, the overall patterning of marker assortment appears useful for distinguishing between different interspecies lineages and, in particular, characterizing primals. **Figure 4** shows the typical patterning for an advanced almond-peach introgression line (2005-16-XXX)

as compared to some of the early primals included in the RosBREED analysis (red highlight), the almond-peach interspecific hybrids *Hansen* and *Nickels* (yellow highlight) in the various species parents (at base). Frequently, the markers identified in primals are not agreement with those of the parents (see **Figure 5**), possibly due to the previously discussed translation errors common when interspecies are analyzed. While in the interspecies hybrids *Hansen* and *Nickels* the expected heterozygosity (because one set of alleles is inherited from almond and the other from peach) is observed at most sites, a much more pronounced genetic variability is observed within the primals. Even in introgression lineage (2005-16-XXX) between parents as

Figure 5. Representative 9K SNP mini-array marker patterns for each of the eight Prunus chromosomes analyzed (colored bars in the top row). [While only about 30 markers are shown for each chromosome, they are representative of the over 1000 markers analyzed.

diverse as peach and almond, the identity by descent (common parentage) results in a generally consistent patterning over all 8 chromosomes with the expected relatively low number of discrete changes in individuals. A highly variable patterning is observed in the primals (which also argues against an accidental cross-pollination of the peach parent by outside almond pollen-which would have been simplest explanation). Each primal analyzed,

including those with a common parentage, shows a distinctive patterning arguing against a crossing error, even with the high level of translation errors expected in this material.

In 2013, additional molecular data was generated through a 9K SNP mini-array based on RosBEED markers but developed with a private service provider (Ilumina). A large number of additional primals were included in this analysis (**Figure 5**). The Ilumina array was originally pursued to see if this platform could successfully translate the trait predictions developed from the larger RosBEED data from the much more limited array results. As with the earlier RosBREED analysis, simple translation from marker presence to trait presence is not possible and it still requires molecular inbreeding expertise to identify the right markers, interpret results, and troubleshoot system glitches. Nonetheless, large haplotypic diversity in UCD peach breeding germplasm is documented. In general, this DNA test was simply predictive (easy to interpret results) for approximately 60% of germplasm but require a more careful analysis for the remainder. In particular, a large number of trait prediction failures were common in the primal material (**Figure 6**). For example, markers scored with two heterozygous groups (e.g., "AC (2)") are always for interspecies introgression populations, since they

Figure 6. Representative 9K SNP mini-array marker patterns for each of the eight Prunus chromosomes analyzed (colored bars in the top row) showing phenotypes predicted based on RosBreed marker associations with predictions highlighted in pink identifying prediction failures.

detect the presence of a third allele coming from non-peach species but cannot uniquely identify that allele.

The 9K SNP mini-array patterns for the over 1000 markers evaluated was highly variable for the primals, similar to that previously seen with the RosBEED markers. However, because more primal genotypes were analyzed, preliminary analysis has identified intriguing trends for a small proportion of the markers, 27 of which are summarized in **Figure 5**. These markers

clearly distinguish primals from the general peach population as well as the peach almond hybrids lineages [AxP] and almond. We are currently examining the possible significance of these marker consistencies (for example, a day from the same segments of the chromosome or involved in similar developmental processes, etc.). In addition we now have F2 populations of several primal genotypes which should be available for genetic and horticultural (disease screening, etc.) evaluation by late 2014/early 2015.

Recent Research Effort Publications:

Gradziel, T., B. Lampinen, F. Niederholzer, and M. Viveros. 2013. 'Sweetheart' Almond: a fully cross-compatible pollenizer for the early 'Nonpareil' bloom that exhibits very high 'Marcona'-type kernel quality. HortScience 48:1320–1322.

Martínez-García P., J. Fresnedo-Ramírez, D. Parfitt, T. Gradziel, C. Crisosto. 2013. Effect prediction of identified SNPs linked to fruit quality and chilling injury in peach [Prunus persica (L.) Batsch]. Plant Molecular Biology: 81:161–174. DOI 10.1007/s11103-012-9989-8.

Martínez-García, P.J., D.E. Parfitt, E.A. Ogundiwin, J. Fass, H.M. Chan, R. Ahmad, S. Lurie, A. Dandekar,T.M. Gradziel, and C. H. Crisosto. 2013. High Density SNP Mapping and QTL analysis for fruit quality characteristics in peach (Prunus persica L.) Tree Genetics and Genomes. 9:19-36 DOI 10.1007/s11295-012-0522-7.

Fresnedo-Ramírez J., P. Martínez-García, D. Parfitt, C. Crisosto, T. Gradziel. 2013. Heterogeneity in the entire genome for three genotypes of Peach [Prunus persica (L.) Batsch] as distinguished from sequence analysis of genomic variants. BMC Genomics. 2013 14:750. http://www.biomedcentral.com/1471-2164/14/750

Martínez-García P., J. Fresnedo-Ramírez, D. Parfitt, T. Gradziel, C. Crisosto. (2013) Effect prediction of identified SNPs linked to fruit quality and chilling injury in peach [Prunus persica (L.) Batsch]. Plant Molecular Biology: 81:161–174. DOI 10.1007/s11103-012-9989-8.

Rahemi, A., R. Fatahi, A. Ebadi, T. Taghavi, D. Hassani, T. Gradziel, K. Folta, & J. Chaparro. 2013. Genetic diversity of some wild almonds and related Prunus species revealed by SSR and EST-SSR molecular markers. Plant Systematics and Evolution, 298: 173-192.

Martinez Garcia, P.J., Dan E. Parfitt, Richard M. Bostock, Jonathan Fresnedo-Ramirez, Alejandra Vazquez-Lobo, Ebenezer Ogundiwin, Thomas M. Gradziel, Carlos H. Crisosto. (2014). Application of Genomic and Quantitative Genetic Tools to Identify Candidate Resistance Genes for Brown Rot Resistance in Peach. PLOS ONE.

Gradziel, T.M. & P. Martínez-Gómez. 2013. Almond Breeding. Plant Breeding Reviews 37:207-258.

Font i Forcada, C; T.M. Gradziel; C.Y. Gogorcena; M.A. Moreno. 2014. Phenotypic diversity among local Spanish and foreign peach and nectarine [Prunus persica (L.) Batsch] accessions. Euphytica 197:261–277. DOI 10.1007/s10681-014-1065-9.

Hanada, T; A. Watari, T. Kibe, H. Yamane, A. Wünsch, T.M. Gradziel, Y. Sasabe, H. Yaegaki, M. Yamaguchi and R. Tao. 2014. Two Novel Self-compatible S Haplotypes in Peach (Prunus persica). J. Japan. Soc. Hort. Sci. doi: 10.2503/jjshs1.CH-099.