California Cling Peach Advisory Board
2010 Annual Report

Project Titles:	Development of New Cling Peach Varieties
Project Leaders:	Tom Gradziel
Cooperating Personnel:	M. A. Thorpe, C. Crisosto, R. Bostock , and J. Adaskaveg
Location:	Dept. of Plant Sciences, Univ. of California at Davis

Summary.

Weather conditions in the spring of 2010 were comparable to 2009 and again favorable for making controlled crosses among selected breeding parents. Over 8,000 seedlings were generated which are currently undergoing greenhouse screening. Over 3,000 seedlings will be rouged in the greenhouse with the remainder planted to field plots in March, 2011. More than half the breeding seed recovered resulted from self-pollination (either through bagging flowering branches to enforce selfing or by letting the branches self naturally and subsequently using molecular markers to rogue the occasional cross-pollination). Hybrid seed was also generated from controlled crosses between parents selected for superior processing quality, high yield potential, specific maturity season, and ability to maintain good fruit integrity for an extended period after the full-ripe date. This last ‘'long-keeper'‘ trait would enable ‘once-over’ and mechanical harvesting, and would also encourage greater individual fruit mass and so ultimately higher orchard yields as it allows the fruit additional time on the tree to accumulate carbohydrates. Over 11,000 seedlings from controlled 2009 crosses were field planted in 2010. The resultant UCD processing peach breeding population continues to exceed the targeted goals for this stage of the breeding program (Fig. 1) with the breeding population surge being in response to industry calls for more mechanical-management (i. e. harvest, pruning, thinning, etc.) amenable processing varieties maturing both throughout the traditional harvest season and possibly earlier and later than current cultivars. To better understand the factors contributing to fruit post-maturity softening and bruising several hundred fruit from selected breeding populations are being analyzed for a range of fruit traits including flesh browning potential, flesh firmness, and ease of pit removal. Preliminary results supports distinct inner and outer mesocarp components affecting processing peach fruit flesh integrity, with differing consequences on post-ripe and post-harvest softening. Molecular genetic analysis of individuals from greatly expanded populations is being pursued in order to identify markers which could improve the breeding efficiency for these and other commercially important traits. Drastic improvements in breeding program efficiency are required to adjust to massive University cuts.

Despite ongoing and extensive University cutbacks in field support, UCD Processing Peach Breeding progeny population sizes have increased with over 9000 individual progeny trees evaluated in 2010 from the 2008 (planting year) Block, compared with 6800 progeny trees planted in 2007. Final evaluation of 2005 Block breeding progeny trees (initially 6500 trees, but rogued out by approximately 50% in each intervening year) were also completed in 2010 with most of the block now scheduled to be bulldozed. (In previous years, breeding progeny blocks were maintained an additional 1 to 2 years to allow vegetative propagation of the most promising individuals, however, beginning in 2010 promising selections are dormant budded by Duarte Nurseries - allowing timely block removal with considerable savings in field costs). Over 11,700 trees from breeding program crosses were planted in 2009 with over 10,000 planted in 2010. At the same time, field costs have been dramatically reduced by eliminating virtually all hand labor, utilizing a combination of mechanical, chemical, and cultural management for maintaining desired tree size, structure and productivity. The DeJong Peach Development Model has been used extensively to maintain desired tree size/structure and to interpret fruit production potential based on different fruit-thinning levels. Because most of our processing peach breeding lines, including those derived from more exotic European, Brazilian, South African and interspecies (almond, etc.) germplasm, have now progressed to more traditional, California-adapted peach fruit/tree types, field evaluations can be more efficiently focused on multiple commercial traits such as fruit size, quality, disease resistance and productivity. In addition, a greater use of self-pollinations versus the more tedious and costly cross-pollinations, are being employed to more rapidly sort out the best individuals within these advanced breeding lines.

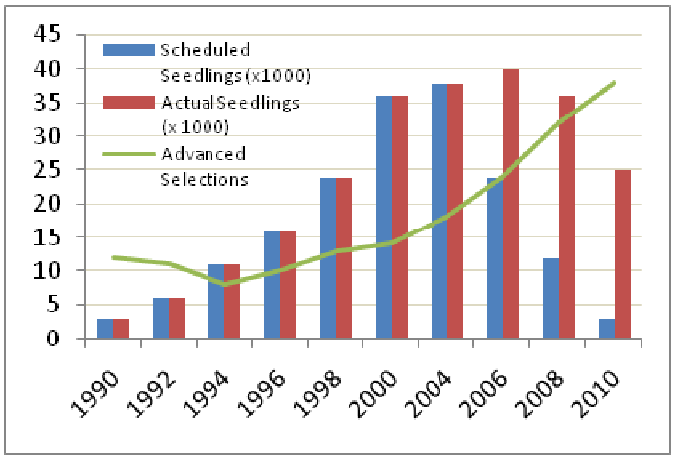


Fig. 1. Initial breeding projections vs. actual counts.

Starting in 2010, detailed information on fruit and tree characteristics for over 330 UCD breeding parents and progeny were collected in collaboration with Dr. Crisosto's lab to complement the high-resolution genetic mapping of these individuals to be completed in 2012 as part of the $14 million RosBreed project. Correlations between specific fruit/tree traits and specific DNA-based molecular markers would then be determined using specialized software to facilitate a more efficient marker-assisted-selection of these traits in the future. Because RosBreed is a multi-state, multi-crop project (also including peach breeding programs in Texas, Arkansas and North Carolina as well as breeding programs for apples, cherries, and strawberries) the initially targeted traits are fruit quality traits such as fruit size, ripe-date, soluble solids and acidity, which while allowing a broad consensus among researchers may not provide a high degree of success for peaches because of the strong confounding affect of

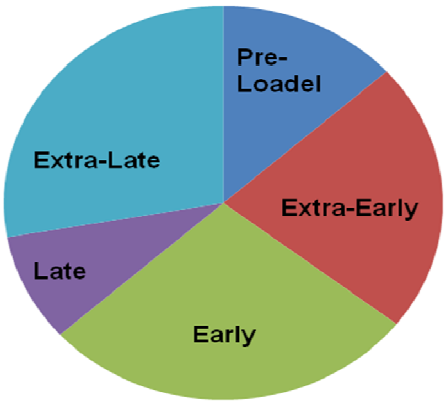


Fig. 2. Proportion of breeding efforts currently targeting different crop maturity periods. .

environment on final expression of these traits. However, we are also taking data on the range of other traits including color, bruising/browning and disease susceptibility, flesh firmness, and tree structure, which should (based on our previous breeding experience) provide more reliable markers to assist both public and private California breeding efforts.

In addition to the 330 UCD selections evaluated for the RosBreed project, 260 breeding selections were evaluated at the UCD Cruise Hall Fruit/Vegetable Processing Pilot Plant Facility. While not included in the original planning, UCD Processing Peach Breeding Program equipment, including an industry-standard Atlas torque-pitter, a custom lye-peel line and rotary cooker are currently being reassembled at the state-of-the-art Moldavi Food Science complex south of campus (due in large part to the efforts of the new Pilot Plant Manager, Molly Lear) .

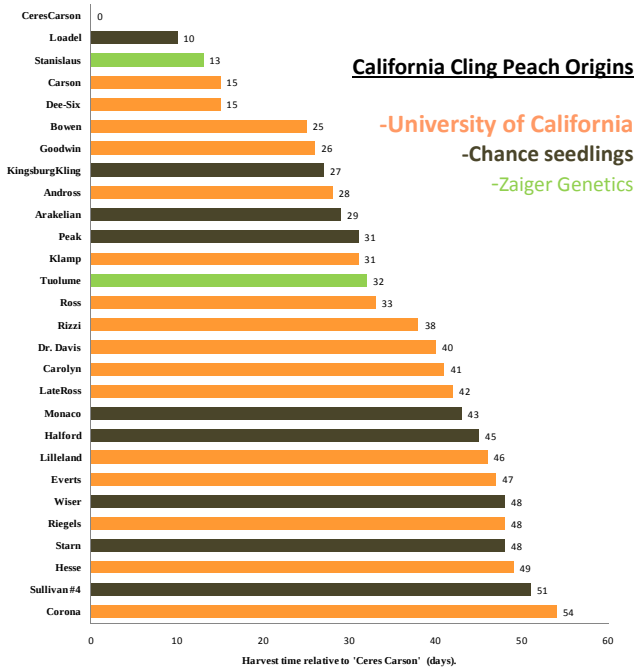


Fig. 3. California processing cling peach cultivars sorted on maturity in breeding program origin.

Breeding Progress: 2010-11

A major objective of the UCD Processing Peach Breeding Program is the replacement of inferior varieties- particularly in the *Dixon-Andross* and *Halford-Starn* harvest time as well as season extension earlier than *Loadel* and later than *Corona*. In addition to the basic requirements for commercial productivity in California's Central Valleys, new cultivars need to possess high processing quality and field and case-yield potential, improve disease resistance , and an ability to maintain good on- tree fruit integrity for an extended period after the full-ripe date. The suppression of fruit drop and maintenance of fruit firmness and quality following tree ripening, known as the 'long-keeper' trait is needed for once-over hand and or mechanical harvesting, and would also encourage greater final fruit mass and so ultimately higher orchard yields (as it allows the fruit additional time on the tree to accumulate carbohydrates). The proportion of breeding efforts for specific harvest seasons is presented in Figure 2 and remain similar to those from previous years. While the Late-Harvest season already contains high-quality, high yielding cultivars such as *Ross*, *Dr. Davis*, and *Late-Ross* (Figure 3), some limited breeding Late-season efforts continue to target new cultivars amenable to once-over and mechanical harvest. While the California processing peach industry is dominated by UCD cultivars(including UC Berkeley/USDA-when Davis was the UC Berkeley Agriculture Farm) , most early cultivars were the result of interbreeding within traditional California processing peach varieties. *Ross* and *Dr. Davis*, released in the mid-1980s from Dr. LD Davis's UCD breeding program, are exceptional both in their high quality and yields but also in that they resulted from the incorporation of novel germplasm (Figures 4 and 5). While the intervening years has (arguably) shown *Ross* to be the

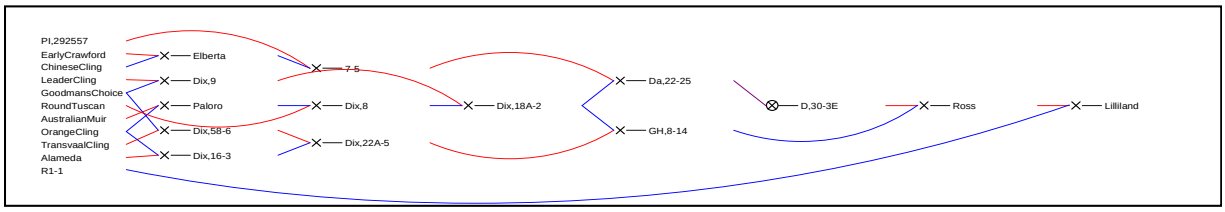


Fig. 4. Lineage of the Ross variety showing the introgression the unique germplasm PI xxx as well as its subsequent parental contributions to the variety Lilleland.

superior variety of the two, it has not been found to be the superior parent for breeding (despite being the parent of the recently released *Lilleland* variety and the source of the budsport mutation *Late-Ross* variety). *Ross* is very difficult to use as a breeding parent because it gives very low seed set in crosses and progeny are generally of low quality-indicating that the quality in *Ross* is controlled by a large number of genes with relatively small individual effect and subsequently a small probability of recombining most quality genes into individual progeny. *Dr. Davis*, however, has been found to be a promising parent as it confers a number of desirable traits to progeny, including fruit firmness, size, flavor, brown rot resistance, and color quality. In addition, genetic control of these traits appears to be through relatively major genes allowing us to map their position on a genetic linkage map as that shown in Figure 7 for a *Georga Belle* by

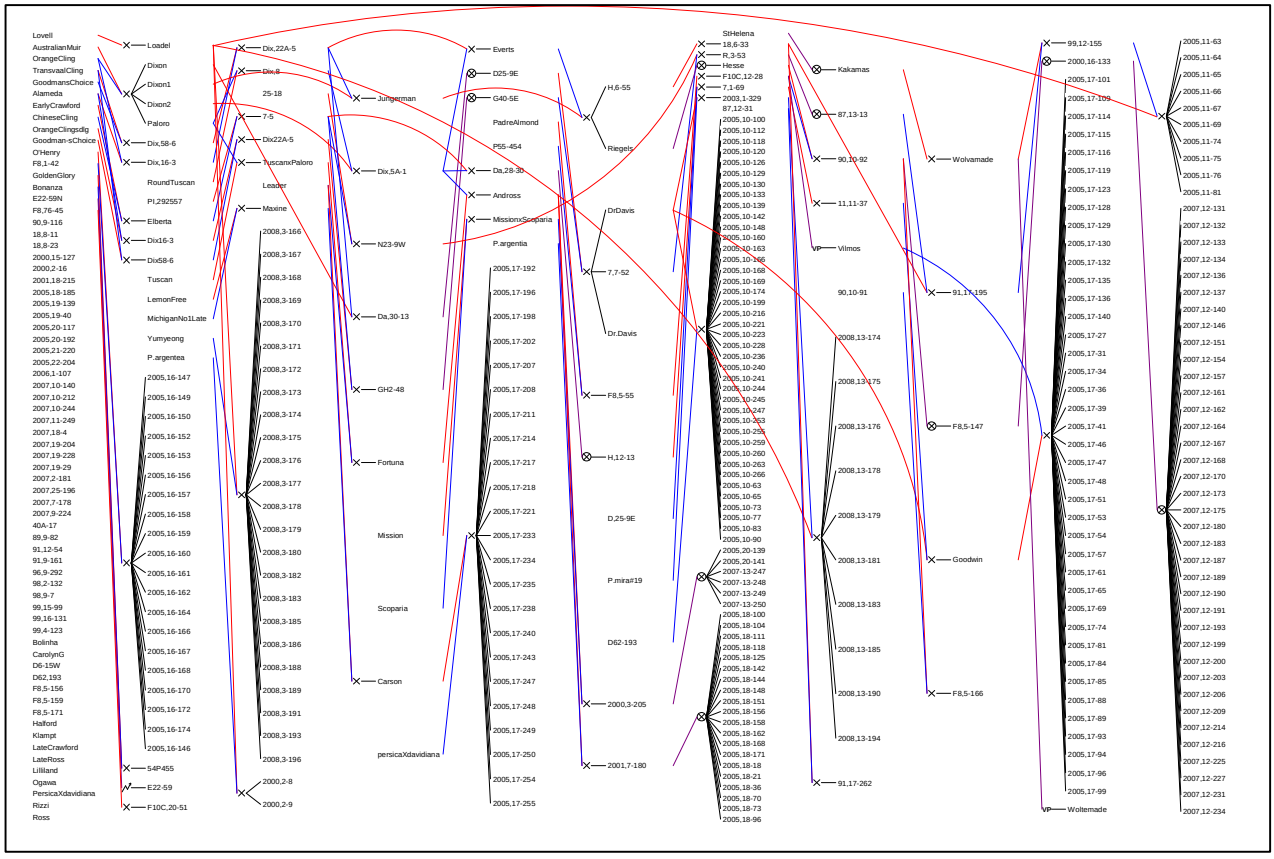


Fig. 5. Lineage of the Dr. Davis variety as well as important lineages contributing to current breeding program objectives.

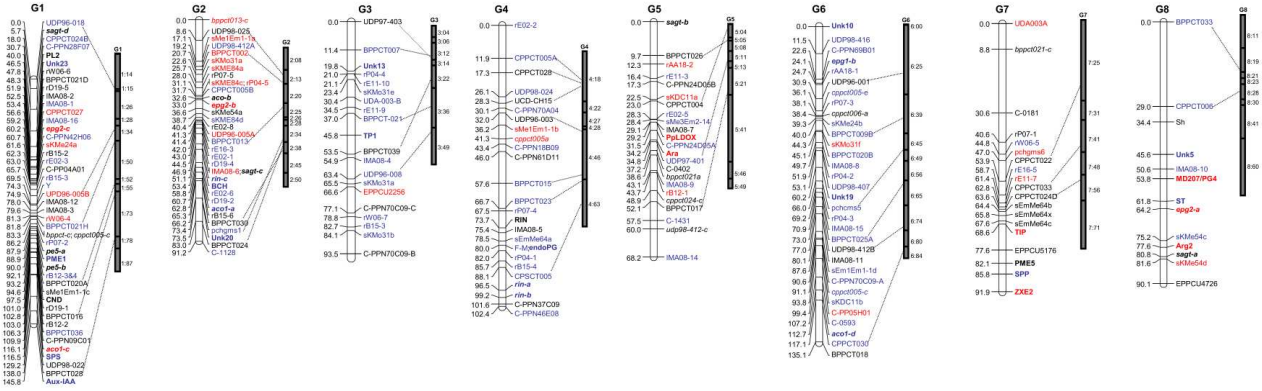


Figure 6. Genetic linkage map of Pop-DG with fruit texture, flavor, pigment, and CI resistance genes. Pop-DG = 'Dr. Davis × 'Georgia Belle'. Open vertical bars represent linkage groups. Vertical solid bars represent linkage groups of the T × E Prunus reference map (Dirlewanger et al. 2004; Howard et al. 2005) showing the bins and anchored with linkage groups of Pop-DG. Positions of SSR markers on the T × E map corresponding to the Pop-DG map are connected by dotted lines. Genetic markers are to the right side of each linkage group of Pop-DG, genetic distances (cM) are to the left. Markers in bold are fruit texture, pigment, flavor, and CI resistance candidate genes. Markers with prefix 'C-' are novel Prunus EST-SSRs obtained from the ChillPeach database (Ogundiwin et al. 2008). RAF and SRAP markers start with prefixes 'r' and 's', respectively. Accessory markers are italicized. Markers in blue fonts were heterozygous in 'Georgia Belle' only, markers in red fonts were heterozygous in 'Dr. Davis' only, and all other markers were heterozygous in both parents.

Dr. Davis cross. This mapping information can then be used to more efficiently identify the best parents for crossing as well as identifying traits most amenable to recombination. For example, in Figure 6 the traits controlling both freestones/clingstone (F) and melting/non-melting (M) are found very close together or tightly linked at the endoPG locus on the lower part of linkage group 4. This very tight physical linkage on the controlling DNA results in these traits rarely being found separately (i.e. recombination). This is why freestone peaches are almost always melting flesh while clingstone peaches almost always non-melting flesh. Characteristic fruit samples of some of the most promising UCD advanced selections currently in grower testing (and also breeding lines showing high value for new variety development) are shown in Figure 7. These samples represent a very diverse, new germplasm with origins in South Africa, Brazil, China , budsport mutations, and even genes from cultivated and wild almonds, and so represent even greater diversity than the novel plant introductions (for example, PI292557 in Figure 4) used by LD Davis in developing the *Dr. Davis* and *Ross* varieties.

Fig. 7. Promising UCD advanced selections and breeding parents currently in grower testing

Because of the novelty of the material, it took LD Davis thousands of crosses and several decades to transfer the desirable quality traits to a California adapted background. If, prior to planting, LD could have determine the presence of desirable and undesirable genes in each seedling, he could very quickly focus on the most promising material and so dramatically improve his breeding efficiency. Genetic linkage maps provide this knowledge and so are the main objective of the UCD Processing Peach Breeding Program 's collaboration with the RosBreed Project (a $14 million SCRI funded consortium to develop molecular markers for use in breeding peach and related tree crop species) [for more information see www.RosBreed.org]. To better understand both these new traits and new technology, this report will focus on four diverse breeding lines to characterize both their unique contribution in terms of processing peach quality as well as the potential value of molecular marker assisted breeding for accelerating the development of new varieties meeting California's emerging needs.

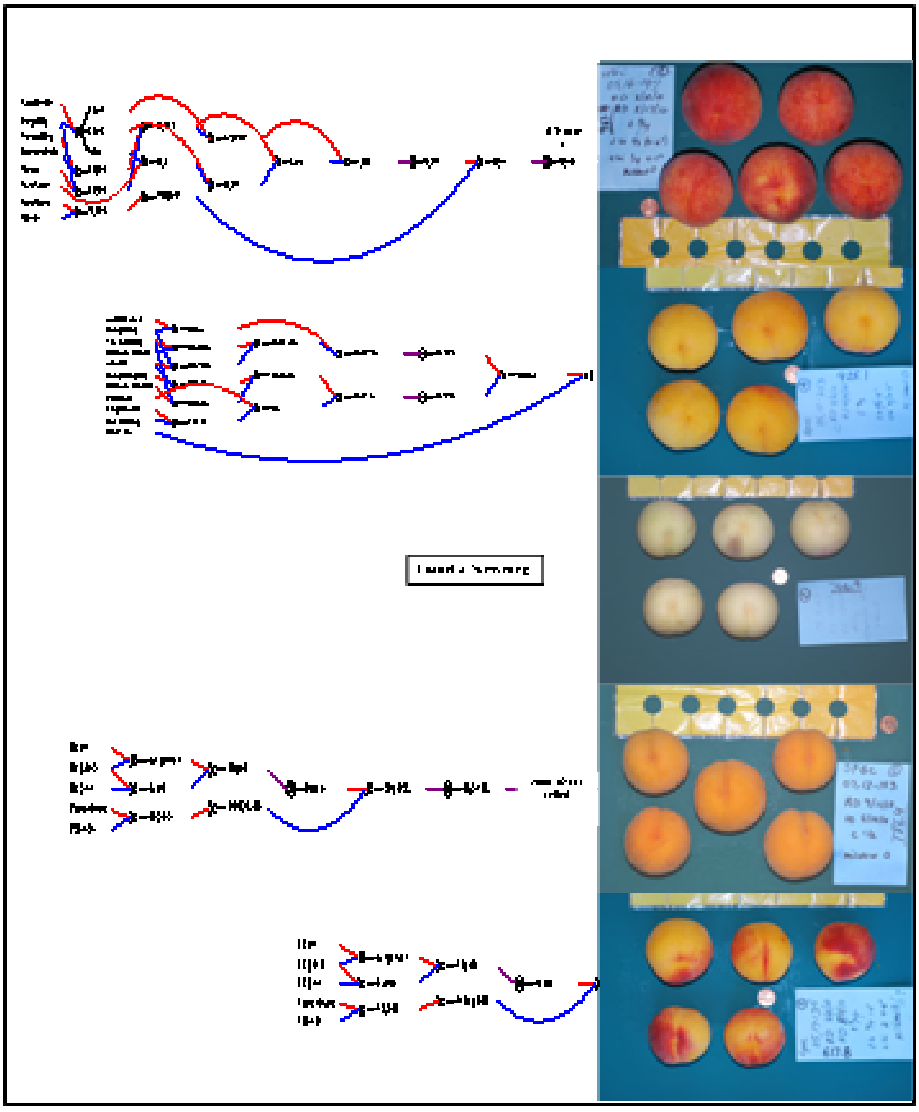


Figure 8. Breeding lineages analyzed with sample fruit of one of the more promising progeny trees presented at right. (Lineage charts show derivation of final seed parent. If no pollen parent is given it was a selfpollination). [Enlarged diagram reprinted on last page].

Analysis of processing peach breeding lineages.

Lineage diagrams for breeding lines to be analyzed as well as representative fruit samples are provided In Figure 8. (Diagrams are a subset of Figure 5 which can also be referenced to show the relatedness of different populations. Figure 5, in turn, is a subset of the more comprehensive

pedigree map provided in 2009 Annual Report). Fruit vary in size, color, flesh firmness and stone to flesh adhesion as well as numerous chemical properties (acidity, soluble solids, etc.). [In 2010, extensive data was collected from over 300 selected genotypes from over 20 breeding populations for the traits Ground Color L*(C) avg.), (Ground Color a*(C) average), (Ground Color b*(C) average), RipDate (Julian), Harv.Date (Julian), Crop, Weight , Pubescence , Mildew, Blush %, Ground Fuzz Color, Flesh Color, Red in Flesh, Red in Pit, Adherence to pit, Fruit Texture, Pit Split %, Flesh Firmness average , 50% Bloom Date (Julian), Brix %, pH, Malic Acid / Titratable Acidity, Pit weight, Bruise Rating, Harv, (Flesh Color L*(C) average), Flesh Color a*(C) average, Flesh Color (b*(C) average), FrmCheekAv, FrmSutAv, FrmPitAv, Chk-Chk, Sut-W, and fruit Lngth]. When fully complied, this data will be analyzed using specialized statistical software against genetic mapping data currently being developed for these 300 individuals at Washington State University. The goal will be to identify high correlations between specific plant traits and molecular marker sequences so that eventually the most promising sequences can be used as markers for those traits to facilitate future breeding (i.e. final fruit firmness or maturity could be deciphered at the seedling stage reducing the need for costly and timely field evaluation of progeny populations). Four fruit traits: firmness, size, cropping ability and right date will be sampled in this report. Approximately 20 individual progeny trees from each breeding line were evaluated with 5 fruit samples from each genotype being tested. To characterize total fruit firmness, flesh firmness at the fruit pit is plotted (on the Y- axis) against firmness of the outer fruit (after removal of the skin) on the X-axis (Figure 9). The uppermost plot in figure 9 shows the results for the cross *O'Henry* (freestone-melting flesh; see cross-sections in Figure 10) by *F8,1-42* (a unique freestone-non-melting peach developed at by our program; see Figure 5). Despite some variation in outer flesh firmness, firmness to the pit is uniformly soft, melting

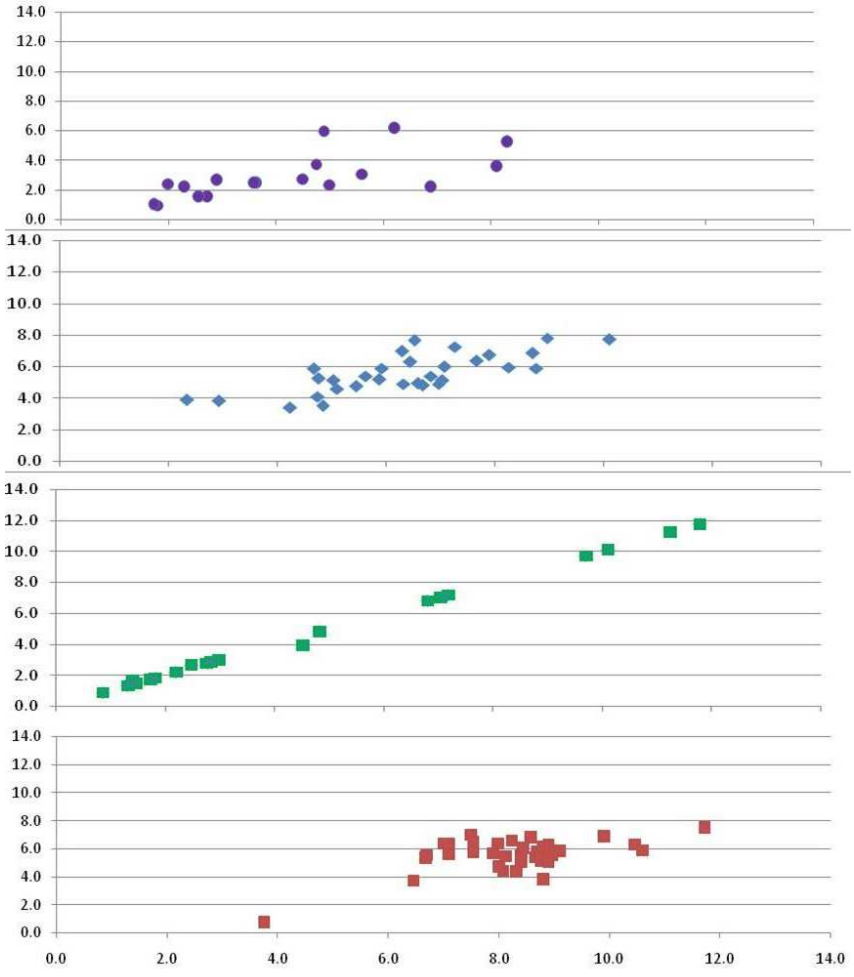


Fig. 9. Fruit texture as characterized by (X-axis) firmness of the outer flesh (after slicing away the skin layer) versus (Y-axis) firmness of the inner flesh adjacent to the pit.

(characteristic of melting-freestones because of the disintegration of the cell wall structure) with a few notable exceptions. [Exceptions may reflect the unique characteristic of F8,1-42 since stone adhesion is free but the flesh at the pit remains non-melting]. The plot immediately below shows the outer to inner firmness relationship for the population *Dr. Davis by Ultra-Early#1* and is typical for crosses between traditional clingstone-non-melting types (see cross-sections in Figure 10). Flesh firmness at the pit is generally well correlated (though lower) with firmness at the outer flesh with moderate levels of variation observed for both coordinates. The inner flesh is softer as it is composed primarily of cell strands radiating out from the pit. In the outer fruit layers these strands tend to intertwine as they develop both radially and perpendicularly to the pit (see figure 10). The third plot down shows the relationship for the cross *Loadel by Yumyeong* (white peach in Figure 9 -a 'stony-hard' peach characterized by a suppression of ethylene-induced softening at ripening). A very strong correlation is seen between inner and outer flesh firmness indicating no differences with fruit ripening and so no textural changes at these two positions. While this results in a more uniformly hard fruit flesh, it also results in greater pit shattering and fragmentation; possibly because the maintenance of a strong pit to flesh adhesion at ripening. The lowest plot shows the fruit firmness relationship in one of our Long-Keeper populations which is characterized by a more uniform internal fruit structure throughout the fruit (see Figure 10). While moderate variation is observed in the levels of outer flesh firmness, the corresponding inner flesh firmness shows notable uniformity (averaging at about 6 pounds). This higher and more uniform internal flesh firmness results in fewer pitting problems (fragments, split pits, etc.) and greater resistance to physical bruising than traditional processing peach, in addition to the ability to maintain good quality fruit on the tree for two weeks or more after the full ripe stage. While this last lineage also contained some of the largest fruit sizes (Figure 11), the average group size tended to be lower than the others, showing a distinct relationship of decreasing fruit size with increasing crop load. [The general decrease in fruit size with increasing crop load is intriguing because crop load was determined from the individual tree in general, while the fruit sampled for this study were thinned to a very low spacing (approximately 30 inches between fruit) and so should not have been affected by the heavier cropping on adjacent branches according to do the DeJong model. Part of the reason for the moderate to lower fruit sizes may be a reduced capacity to take on excess water at ripening (as a way to increase size) thus increasing its case-yield performance (high water content fruit is much more susceptible to transport and processing damage) though potentially decreasing its maximum field yield-potential. This 'water-uptake' based size increase can be readily observed in the freestone peaches in the top plot where increased size and juiciness ultimately decreased

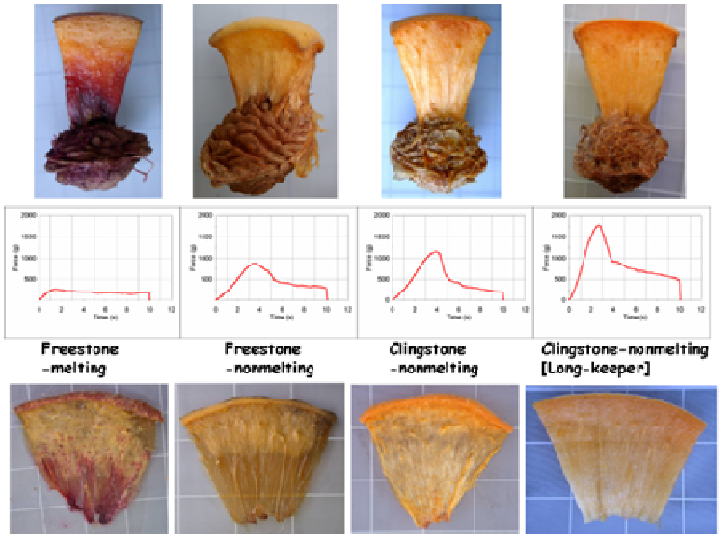


Fig. 10. Cross sections of different fruit textures. From left: melting-freestones, non-melting-freestone, nine-melting clingstone, and long-keeper type. Fruit firmness (in pounds) from surface (left) to pit (right) for each type given in central plot.

transportability. By contrast the Loadel by Yumyeong population shows very little variation in fruit size despite appreciable variations in crop load. This indicates that the genetic maximum size potential for this population is limited (part of this may be due to the still relatively unrefined nature of this germplasm (though part may be an inherent limitation of the non-ripening aspect of Yumyeong).

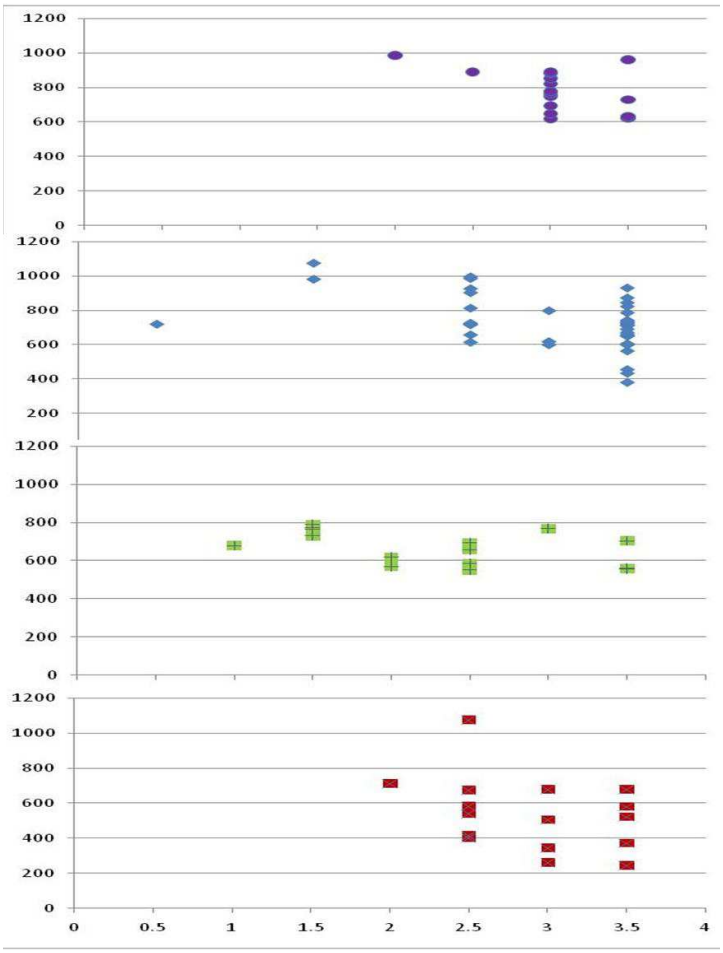


Fig. 11. Fruit crop load (X-axis) (1-low, 4-high) versus average fruit size of highly thinned fruit (Y-axis) .

The the distribution for ripening dates for individuals progeny is also informative for the different populations studied. The *O'Henry* by F8,1-42 population is skewed towards the early-season and not showing the expected bell shaped curve for this type of population (Figure 12, though interesting outliers are present both very early and very late for the season). This may indicate a bias in our sampling procedures towards later season items (perhaps because of their more desirable larger fruit size). A more typical bell-shaped curve is seen in the Dr. Davis by D,62-193 (i.e. Ultra-Early#1) population (Figure 13) though with a distinct gap in the early season distribution which may represent a developmental delay caused by pit-hardening. Otherwise the Bell curve is fairly uniform and relatively compact indicating a wide distribution of ripening dates which would be expected given the distinctly different ripening times of the parents. This distribution pattern also indicates that a relatively low number of genes are responsible for determining ripe-date in this lineage. Consistent with its previous unusual performances, the population Loadel by Yumyeong (Figure 14) shows a distinctly flat curve even though the parent ripe-dates are closer than in the previous Dr. Davis by D,62-193 population. This may indicate that a larger number of genes with relatively small effect are contributing to ripe-date, which would also suggest that the

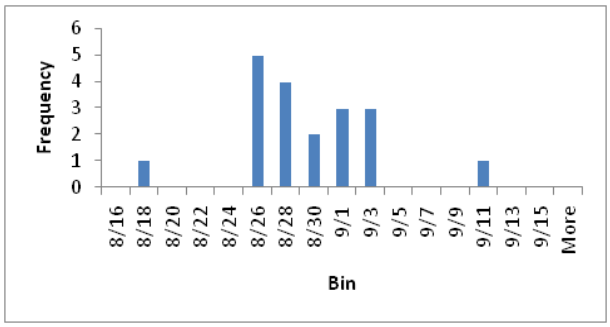


Fig. 12. Distribution of ripening dates for cross O'Henry by F8,1-42.

Yumyeong peach, which is native to China, is genetically more distinct (i.e. has distinctly different ripening genes) than the more traditional processing peach germplasm. A clear example of such a flat distribution resulting from high genetic diversity is seen in Figure 16 for the variety *Goodwin* crossed with F10C, 12-28 , and almond derived peach breeding line (see also lineage in fruit samples in Figure 8). Here, the recombination of the diverse almond with peach genes for fruit ripening results in a much more variable genetic control for this trait and consequently a wide unfocused distribution. [The mid August peak in this distribution may result from a more uniform 'hull-split'type of ripening of the more almond-like types in this population]. In contrast, a very focused distribution is seen with a population 2000,16-133 selfed (Figure 15). Although also having almond in its lineage (Figures 5 and 8), this lineage is more inbred and so less variable. For example, the selfing rather than out-crossing in the final generations eliminates genes from the population, leaving fewer genes affecting ripening. This population demonstrates how, with proper selection and self-pollination, populations can be more narrowly focused to desired traits (in this case ripening in the Extra-Late season). This same type of trait-focusing using self-pollination can be applied to most traits controlled by multiple genes. The initial breeding program emphasis was on cross hybridizations between diverse parents in order to capture the desired traits of one parent and recombined them with the greater local adaptability of the other parent; resulting in a highly reshuffled and diverse progeny (as in Figure 16). However, only two generations of selfing has resulted in a powerful trait-focusing for that population, allowing much faster breeding progress. [For example, most of the individuals in a population depicted in Figure 16 would be undesirable resulting in a low proportion of progeny having individual desired traits and an even lower proportion of progeny having multiple desired traits. In contrast, most progeny will have the desired ripening trait that population depicted in Figure 15 and will be more focused for the other desirable traits as well. Since most of our advanced breeding lines are now entering this more focused selfing generations, (a major transition occurred in 2009) we anticipate a much higher proportion of the subsequent progeny to show high commercial quality. A very distinct focusing of the desired trait as shown in Figure 15 also indicates that the genetic control is by

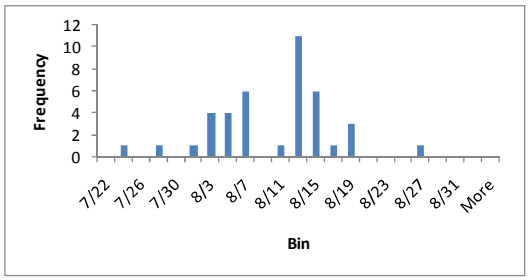


Fig. 13. Distribution of ripening dates for cross Dr.Davis by D,62-193

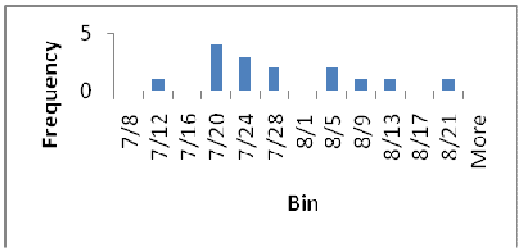


Fig. 14. Distribution of ripening dates for cross Loadel by Yumyeong.

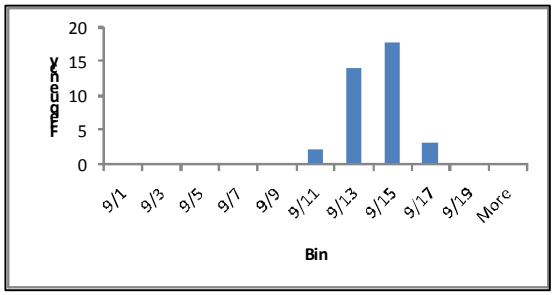


Fig. 15 Distribution of ripening dates for cross 2000,16-133 selfed.

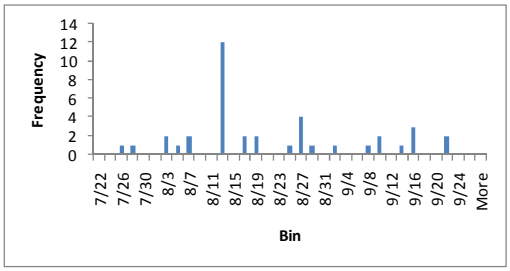


Fig. 16. Distribution of ripening dates for cross Goodwin by F10C,12-28.

relatively few genes making this trait more amenable for genetic marker development (as demonstrated in Figure 6) for use in marker assisted selection and marker assisted breeding. Too much focusing, however, can greatly reduce the discriminating power of molecular markers (as explained in the next section) making the current generations our best opportunity for molecular marker discovery/manipulation.

Table 1: Features of candidate and cold responsive genes mapped to Pop-DG

LG	Marker Code	Functional Annotation	Clone/Accession #	EST Source	CG type[a]
1	PL2	Pectate lyase	BU041363	GDR	Texture
	Unk23	similar to F19P19.4 protein related cluster	PP1004A08-T7_c_s	ChillPeach	CIRG
	PME1	pectinesterase, putative	BU043277	GDR	Texture
	CND	Chloroplast nucleoid DNA binding protein related cluster	PPN018D10-T7_c_s	ChillPeach	CIRG
	SPS	Sucrose phosphate synthase	DY653691	GDR	Flavor
	Aux-IAA	Aux/IAA protein related cluster	CL78Contig1	ChillPeach	CIRG
2	BCH	Beta-carotene-hydroxylase	BU044761	GDR	Pigment
	Unk20	OSJNBb0039L24.13 protein	CL1095Contig1	ChillPeach	CIRG
3	Unk13	highly similar to OSJNBb0004A17.4 protein related cluster	CL32Contig2	ChillPeach	CIRG
	TP1	Thaumatin-like protein 1 precursor	PPN003H07-T7_c_s	ChillPeach	CIRG
4	RIN	Similar to *Solanum lycopersicum* MADS-RIN MADS box transcription factor	BU045116	GDR	Texture
	endoPG	endopolygalacturonase	BU040689	GDR	Texture
5	PpLDOX	Leucoanthocyanidin dioxygenase	EU292217	Ogundiwin et al., 2008	Pigment
	Ara	Alpha-L-arabinofuranosidase	DQ486870	NCBI	Texture
6	Unk10	No annotation available	PP1005B10-T7_c_s	ChillPeach	CIRG
	Unk19	No annotation available	PPN024C05-T7_c_s	ChillPeach	CIRG
7	SPP	Serine protease-like protein related cluster	PPN007C09-T7_c_s	ChillPeach	CIRG
	PME5	pectin methylesterase - like protein	BU044844	GDR	Texture
	TIP	Tonoplast intrinsic protein related cluster	PP1003C07-T7_c_s	ChillPeach	ChillPeach
	ZXE2	Zeaxanthin epoxidase	CL377Contig1	ChillPeach	Pigment
8	Unk5	No annotation available	PP1004F11-T7_c_s	ChillPeach	CIRG
	PG4	*P. persica* PG gene	X77231	NCBI	Texture
	ST	Sulfate transporter 3.1	PPN065F08-T7_c_s	ChillPeach	CIRG
	Arg2	Indole-3-acetic acid-induced protein ARG2 related cluster	CL704Contig1	ChillPeach	CIRG

[a]: CIRG = chilling injury resistance genes

***Marker Assisted Selection* and *Marker Assisted Breeding*.**

In marker assisted selection, a unique sequence of DNA is used as a selectable marker for a particular trait. This DNA sequence can be directly from the gene controlling that trait (as is the case for the self-compatibility S-allele markers that we have been using in almond breeding for over 10 years) or it can be from sections of DNA close enough to that trait that it essentially almost always segregates with that trait. The advantage of molecular markers is that with the proper technology one can identify the traits even at the seedling stage or even in environments where it would be otherwise difficult to identify. Markers for multiple independent traits can be managed simultaneously though it becomes very difficult to manipulate three traits are more because of the increasing complexity of the inheritance patterns involved (particularly in cultivar development where a very large number of traits such as size, shape, color, texture, flavor, etc. have to be optimized if commercial successes is to be achieved). Marker assisted breeding refers to a more general use of markers to facilitate breeding efforts. For example, in our almond breeding work for self-compatibility the ability to accurately characterize the genotype of the parents allows the selection of specific parent combinations which will ensure 100% of the progeny aare also self-compatible. This approach is particularly useful when large numbers of other traits also have to be optimized for commercial success (as explained above). Molecular

markers have also proven very useful for breeding programs in that they provide important information on genetic mechanisms, often alerting us to errors in our previously established genetic models. For example, our previous work has shown the EndoPG locus on linkage group 4 (Figures 6 and 17) to be a very good marker (and probably controlling gene) for the freestone-melting/clingstone-non-melting trait. However, breeding line F8,1-42 (see Figure 10) which shows the exceedingly rare phenotype freestone-nonmelting, appears to be controlled by a totally separate gene. The huge amount of research being carried out in other plant species and in particular Arabidopsis as a model system (as well as animal and microbial species), has developed an immense database on gene sequences which also includes putative or proven modes of action. For example, Table 2 shows the putative markers as well as the tightness of their linkage (LOD score) with several commercially important peach traits.

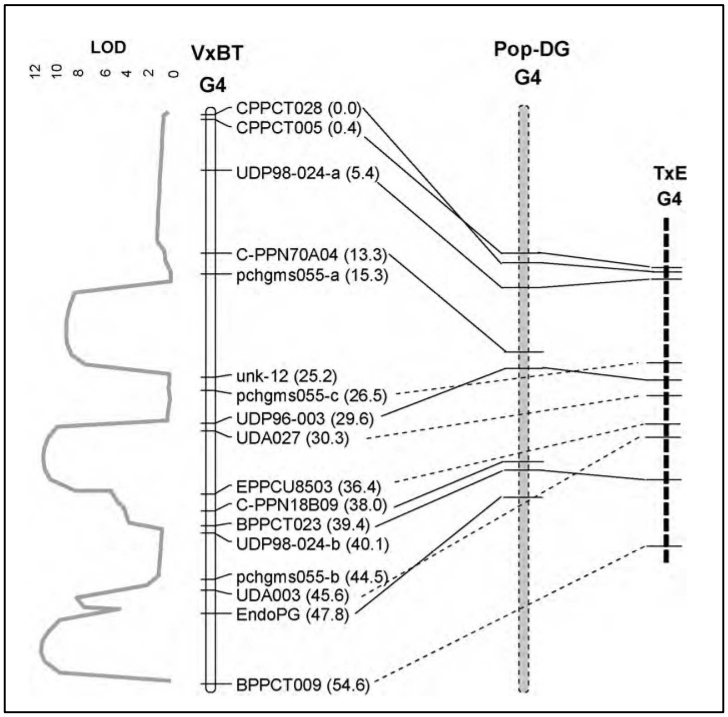


Fig. 17. Linkage group 4 map of 'Venus'×'BigTop' (V×BT) F1 progeny showing the position of DNA markers. Map distances (cM) of the markers are provided between parentheses. The QTLs detected for mealiness are shown on the left. A section of LG4 of T×E Prunus reference map (Dirlewanger et al., 2006) and a section of LG4 of the Pop-DG map (Ogundiwin et al., 2007, 2009b) are represented showing the position of common SSR markers connected with solid lines to LG4 of V×BT. Dashed lines represent common markers with the T×E map, but not with Pop-DG map.

The data is more easily visualized graphically as in Figure 17 which also shows the corresponding marker location on our *Dr. Davis* by *Georgia Belle* reference map as well as the *Texas (i.e. Mission* almond) by *Early Gold* peach interspecies map used as an international standard. While the adjacent gene positions identified have clear value as molecular markers for these traits they offer additional, and sometimes more immediate value if they help explain the mode of action. Table 1 lists a series of markers and associated *Dr. Davis* by *Georgia Belle* genes that we have recently identified as possible candidates for controlling resistance and susceptibility to cold damage and peach. The functional annotation not only informs us as to possible function of our genes but also allows us to access a much larger database from other molecular marker research as well as plant physiology studies. While several of the markers in Table 1 may prove useful as a marker for this trait, the ppLDOX gene looks particularly promising because previous work has shown its expression to be associated with conferring cold hardiness in plants. In fact, the international database on plant gene location, sequence and function, has become so sophisticated that even relatively routine queries can result in a wealth of information. Figure 19 shows the result of this type of international query using markers developed in our studies of cold damage in peach fruit. Extensive information is provided on both the general and specific

Table. 2

Nearest marker, peak position, maximum LOD score and percentage variance explained for QTLs identified on the linkage group 4 (LG4) by interval mapping in the F_1 progeny population of 'Venus' × 'BigTop'.

Trait	Nearest marker	LOD peak position (cM)	Max. LOD score	% Variance explained
SSC	pchgms055-a	17.3	14.6	82.5
pH	pchgms055-a	17.3	26.4	91.8
TA	pchgms055-a	17.3	3.2	30.9
Firmness	pchgms055-a	17.3	12.7	79.4
Height	UDA027	31.3	4.2	26.5
Harvesting date	EPPCU8503	36.4	25.9	87.2
Endocarp staining	EPPCU8503	36.4	13.1	79.6
Suture diameter	EPPCU8503	36.4	6.2	42.4
Cheek diameter	EPPCU8503	36.4	6.7	41.5
Bleeding	EPPCU8503	36.4	3.8	75.6
Fruit weight	EPPCU8503	36.5	27.0	89.6
Mealiness	BPPCT009	51.8	10.3	75.5
Graininess	BPPCT009	51.8	10.1	75.2
Leatheriness	BPPCT009	52.8	5.3	58.9
Blush	BPPCT009	52.8	5.4	68.7

Abbreviations: SSC, soluble solids content; TA, titratable acidity.

putative modes of action as well as the relative contribution to the trait of interest. To be appropriately applied, however, this information requires a fairly comprehensive understanding of the target crop physiology and development, otherwise this information has a history of providing more red herrings than actual answers. Unfortunately, the current research emphasis on molecular biology has dramatically eroded funding and so research opportunities towards the more applied aspects of plant development and function, so that the same policies that develop a potentially invaluable database simultaneously undermine our ability to appropriately mine it. Molecular biology has, however, also provided powerful tools to facilitate the needed research on plant physiology and development though the gap between these more basic studies and whole-plant application remains relatively wide. An example is seen in our recent work comparing the differential expression of genes in resistant versus susceptible genotypes (Figure 18). In this candidate-gene approach, genes that are uniquely associated with resistance are used as targets for development of molecular markers for use in breeding selections and other research. However, the difficulty of this approach is that the large numbers of genes typically identified overwhelm the research capacity, inevitably resulting in a more random 'hit or miss' final selection of gene candidates. An additional difficulty is that the genes highly expressed in resistant lines may be a consequence rather than a cause of the resistance mechanism. For example, in Figure 20 the PGIP gene is often associated with cell integrity and so frequently expressed regardless of the mode of action of resistance.

In summary, extensive and powerful databases are becoming available to facilitate plant breeding and applied plant research. However, the sophistication and complexity of the technologies involved often require a high level of expertise for their appropriate application. The time required to develop this, often in-lab, expertise consequently restricts the opportunities of the individual researcher to develop the equally sophisticated though often more nuanced knowledge (gene by gene interaction, gene by environment interaction, gene by gene by environment interaction, etc.) required for successful cultivar breeding. Our approach to this problem is to assemble a team of experts in the various areas to pursue the most pragmatic solutions incorporating both traditional and molecular biology approaches. A second, possibly greater barrier to the application of molecular techniques to cultivar development, however, is that molecular biology by its nature is linear in its cause - affect approach. The major obstacles to cultivar development, however, is the complex interaction of desirable and undesirable genes,

between genes and environment, between individual genes and the larger genome, and among traits as a consequence of final gene expression. (A classic example would be the positive relationship between fruit size and tree yield for lower fruit sizes, but a negative relationship as fruit sizes increase beyond a certain threshold). Successful cultivar breeding thus requires a capacity for simultaneous and often multidimensional analysis/selection. This type of complex, multidimensional breeding approach can contribute to communication barriers with molecular researchers because of their limited experience with this type of analysis.

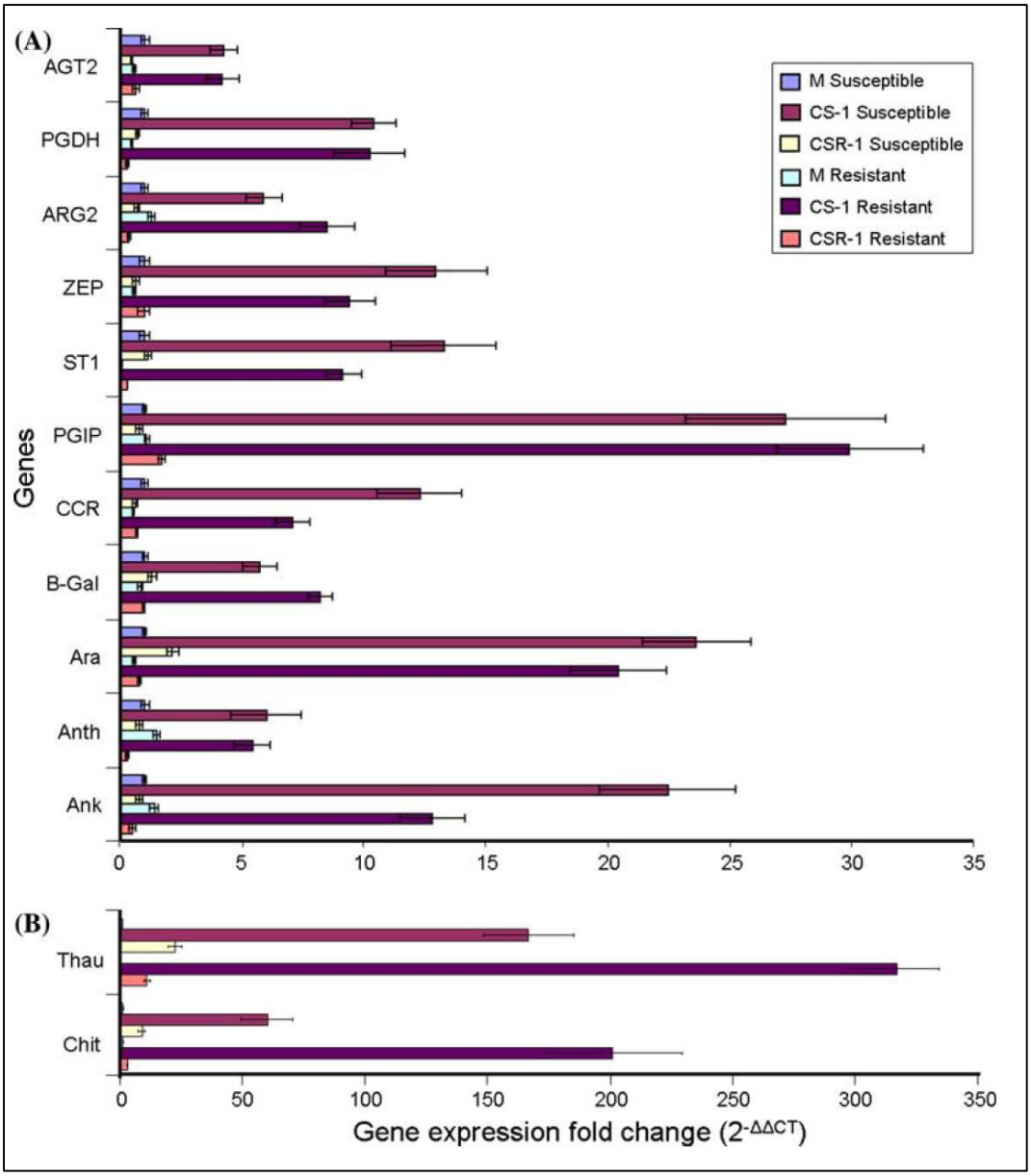
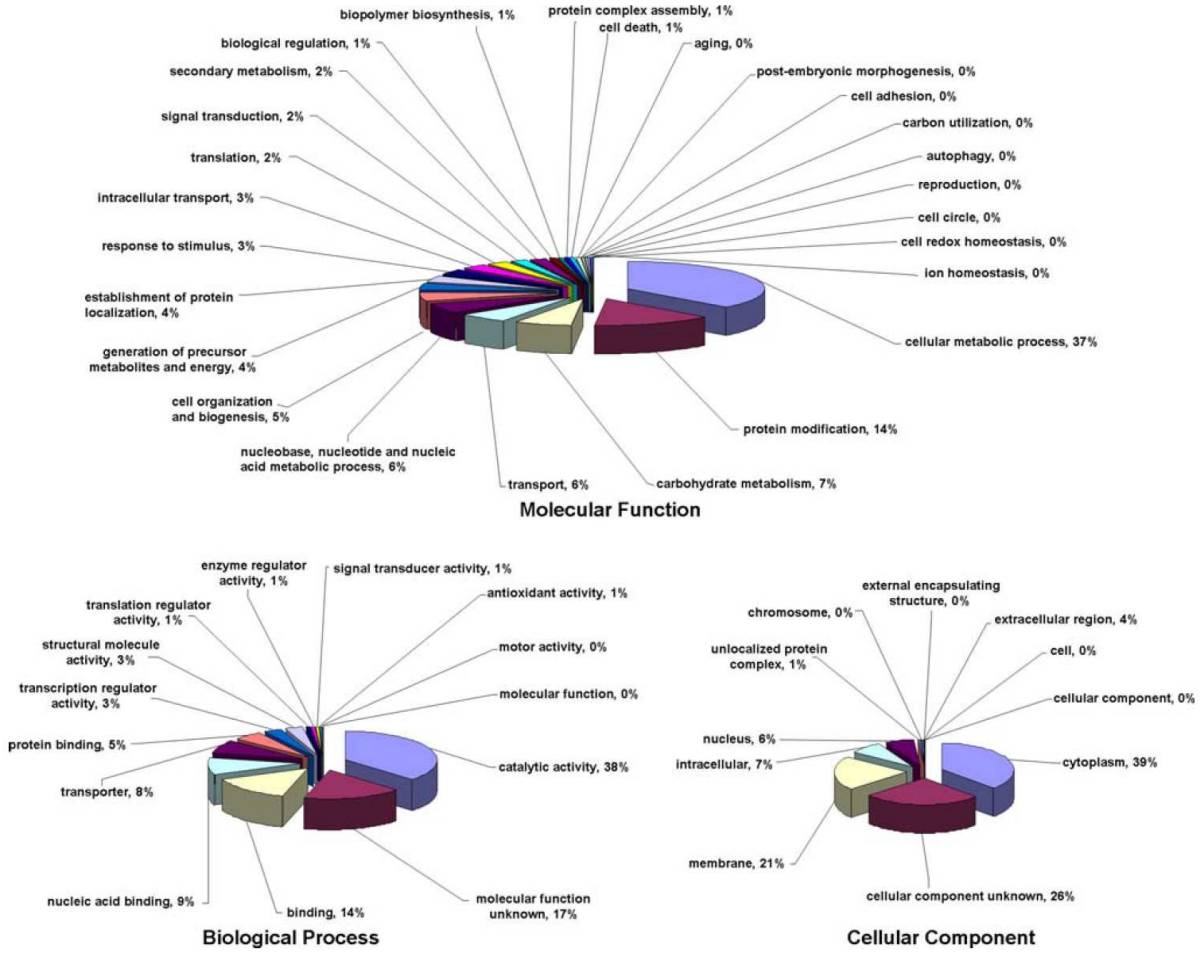
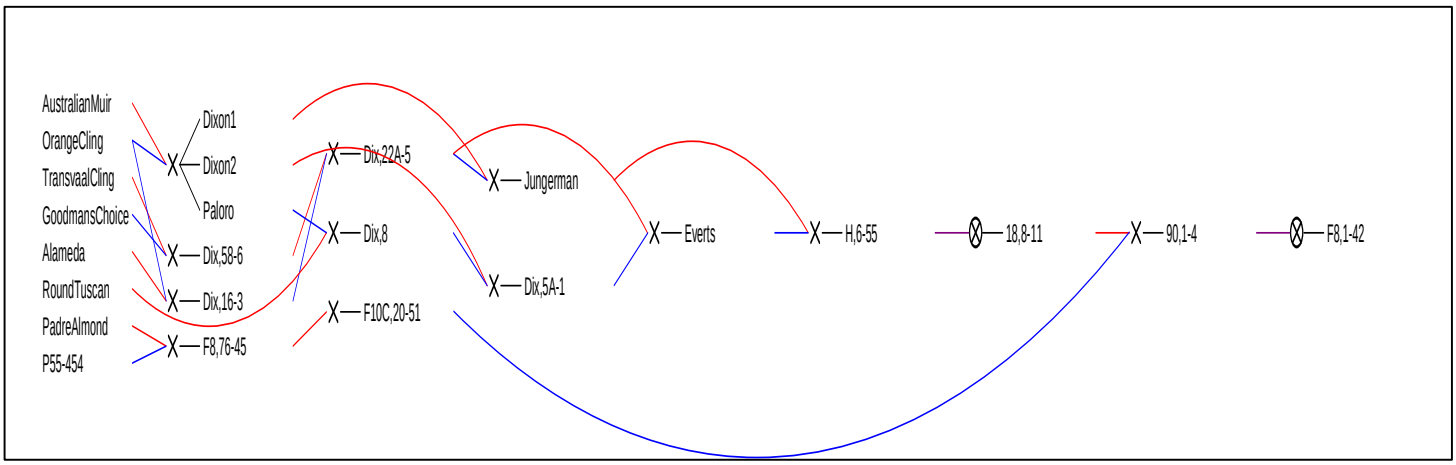
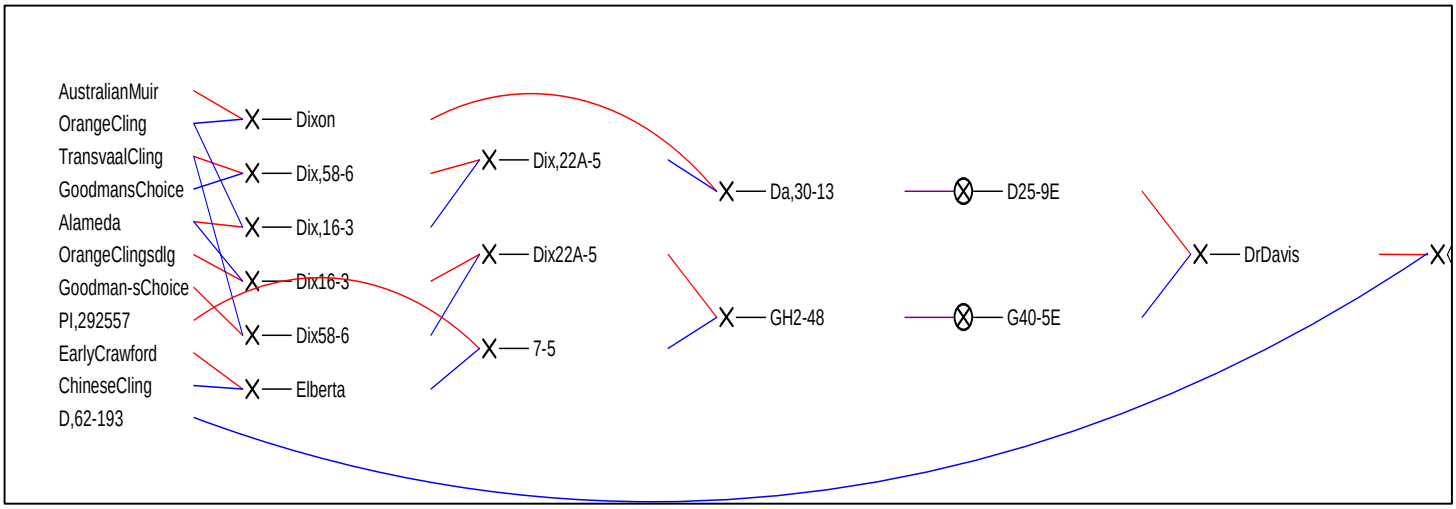


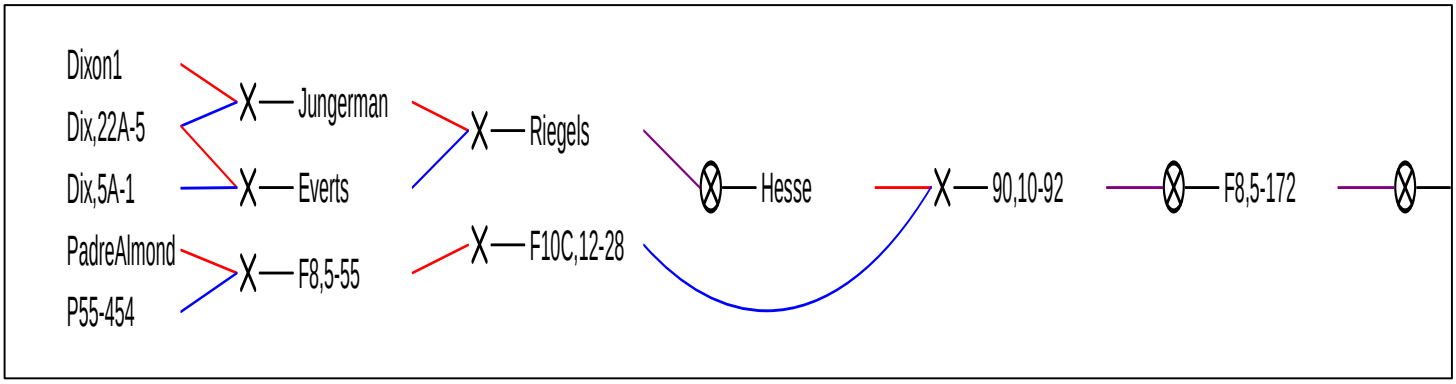
Fig. 18. Genes chosen for data validation by real time qRTPCR. Shown are relative levels of differential gene expression among treatments. Genes in group B were charted on a different scale because their expression in some of the treatments was about 10 times the expression of genes in group A. The data represented the mean of two biological and three technical replicates. Gene expression levels were normalized against peach Initiation Factor elF-4-Gamma. The level of each analyzed gene transcript in mature (M) susceptible sample was set to one and the level of the remaining sample was calculated relative to this reference. Error bars show the standard error of the mean for each treatment

Fig. 19. Proposed functional distribution of ChillPeach unigenes based on GO (statistical analysis program) functional categorization.









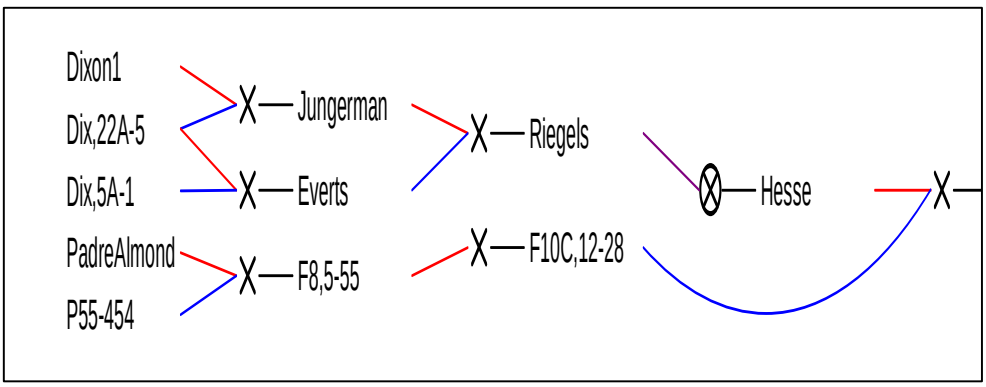


Figure 8e. Enlargeable images of breeding lineages from Fig. 8.