California Cling Peach Advisory Board
2009 Annual Report

Project Titles: Development of New Cling Peach Varieties

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Synopsis.
Weather conditions in the spring of 2009 were favorable for making controlled crosses among selected breeding parents. Over 9,000 seedlings were generated which are currently undergoing greenhouse screening. Approximately 3,000 seedlings will be rouged in the greenhouse with the remainder planted to field plots in April, 2010. Roughly 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). In addition, hybrid seed was 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 8,000 seedlings from controlled 2008 crosses were field planted in 2009. The resultant final UCD processing peach breeding population greatly exceeded 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 torque force required for pit removal. Preliminary results supports distinct inner and outer mesocarp components affecting processing peach fruit

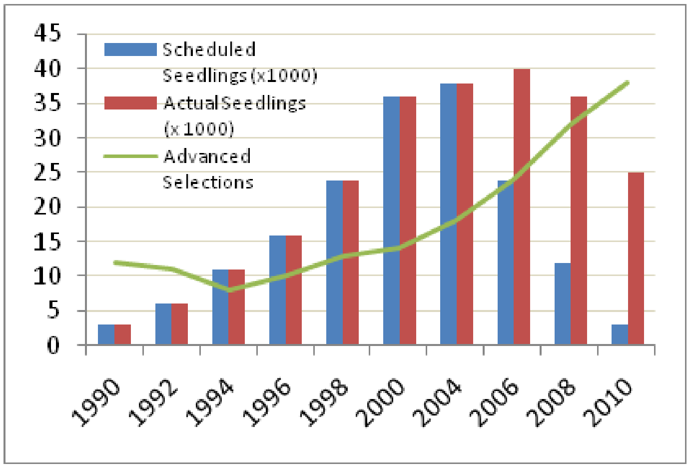


Fig. 1. Initial breeding projections vs. actual.

flesh integrity, with differing consequences on post-ripe and post-harvest softening. Molecular genetic analysis of individuals from these populations is being pursued in order to identify molecular markers which could improve the breeding efficiency for these traits. Drastic improvements in breeding program efficiency required in order to bring the rapidly escalating field and lab costs (resulting from dramatic reductions in University support) under control (see Figure 2). Concurrently the breeding program strategy is evolving from parallel programs for a) gene discovery and transfer to California adapted breeding lines and b) recombining traditional with introgressesed germplasm to develop new processing peach varieties containing desired new genes (and traits) with the traditional and proven genes for California adaptation and fruit quality. Promising advanced selections from diverse lineages and maturing in the Extra-Early, Early, Late, and Extra-Late harvest season have been selected which demonstrate good fruit quality combined with good flesh integrity both at full-ripe stage and continuing for an additional 10 to 20 days. Large progeny populations have been developed between these elite parents and more traditional California varieties to improve overall fruit quality and integrity in future varieties and to establish segregating populations for genetic analysis and trait dissection. In addition, breeding selections demonstrating good commercial quality with improved fruit-rot resistance have been identified in the Extra Early, Early, and Late harvest seasons to complement earlier fruit brown-rot resistant candidates ripening in the Ultra-Early and Extra-Late harvest season (which are currently in grower testing, -see 2009 Regional testing report).

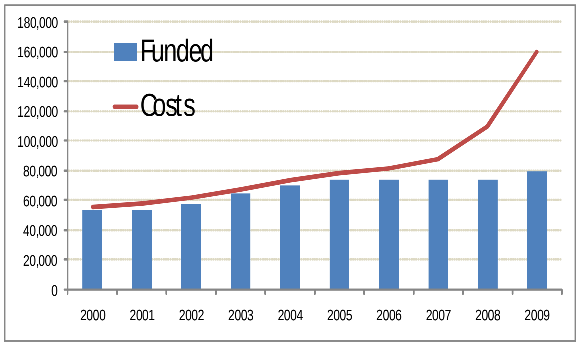


Fig. 2. Breeding program costs vs. funding by year.

Breeding summary.

Processing peach breeding efforts have been traditionally divided into specific goals, such as germplasm improvement, disease resistance, fruit integrity and longevity, as well as efforts towards the ultimate goal of recombining genes from different sources to achieve regionally adapted cultivars with high productivity and processing quality. A pie-chart has often been presented to conveniently convey the proportion efforts towards each specific goal. The breeding program has now evolved to where the new germplasm containing the desired genes has been identified and transferred to more mainstream breeding populations. Consequently, the estimation of breeding effort proportion is now presented solely in terms of the targeted harvest season (Figure 3) as efforts to transfer improved fruit integrity, disease resistance, productivity, processed quality, etc. are being made for all harvest season groups (although fruit brown rot resistance is being pursued more aggressively in the Extra-Early and Extra-Late maturity groups owing for greater vulnerability during these times). Since previous annual reports have provided details on specific breeding approaches, (such as exotic gene introgression, brown rot resistance, fruit integrity and longevity, and use molecular marker assisted breeding), the intent of this report will be to provide a larger overview of the diverse breeding populations and their use in developing improved Californian cultivars. Overviews will be provided for a) the relationship of our processing peach breeding lines with the larger fresh market germplasm (including the opportunities for cross feeding between market types in terms of universally useful genes, as well as molecular genetic information), b) the relationship of different populations within processing peach breeding program, c) to transfer and recombination of genes among UCD populations to achieve specific goals, and, d) the characterization of breeding value of germplasm from differing sources for achieving specific goals.

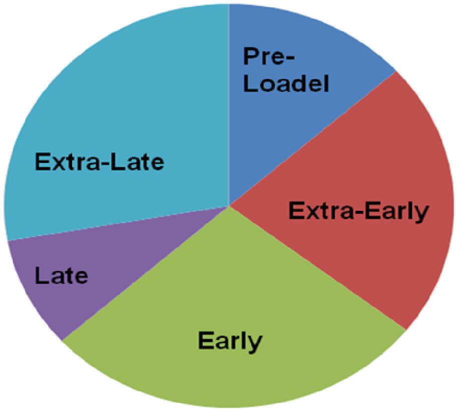
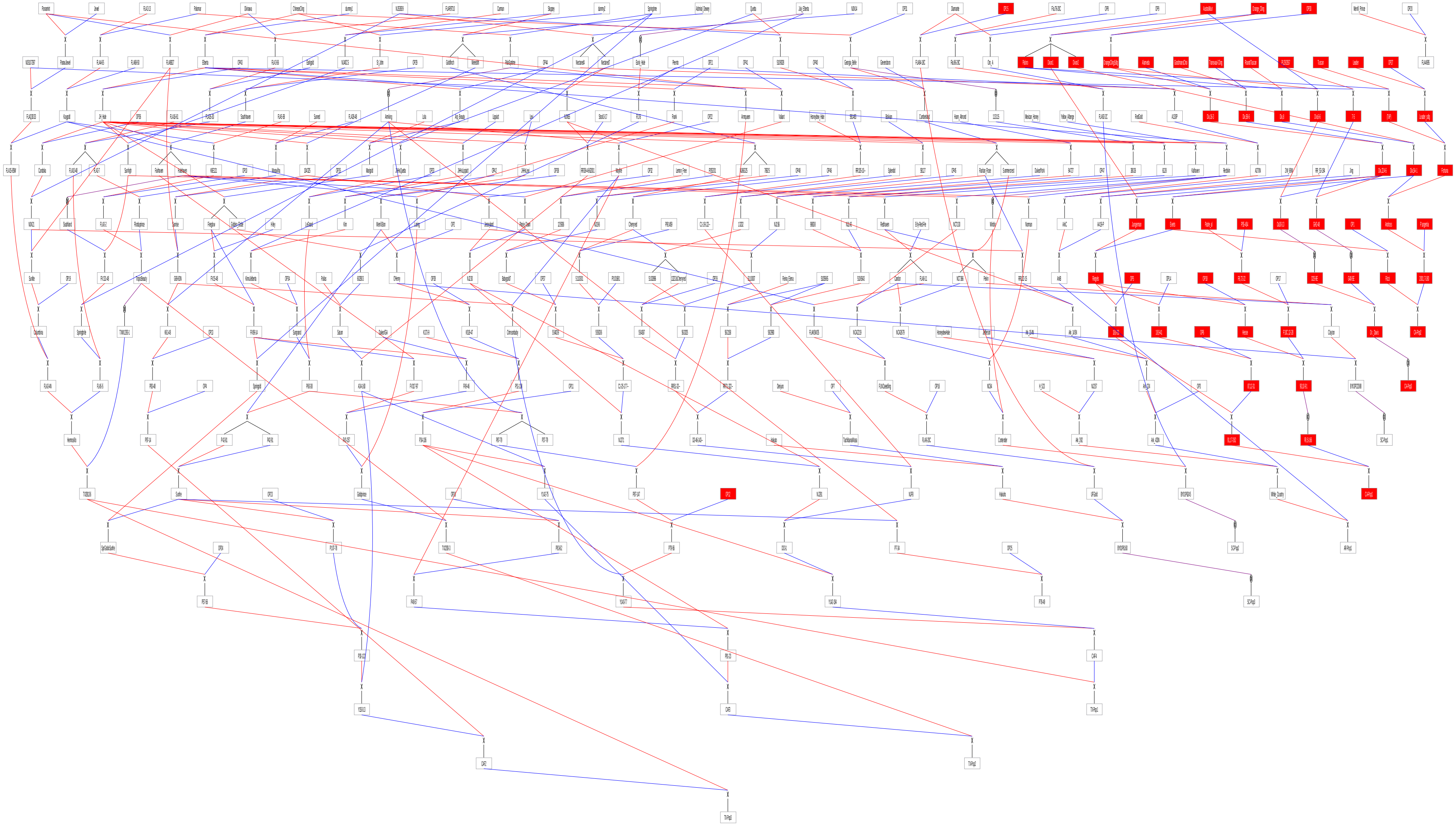


Fig. 3. Proportion of breeding efforts currently targeting different crop maturity periods. .

A) Relationship of our processing peach breeding lines with fresh market germplasm.

The principal breeding lineages within the major public peach breeding programs (Arkansas, California, Florida, North Carolina, and Texas), are presented in Figure 4. This lineage compilation is part of a larger effort among public breeders to define the genetic relationships among their programs, as the basis for developing detailed molecular markers for important fruit production and quality traits, yet applicable to all programs (assuming common origins). Previous molecular studies have supported a close genetic relatedness among many of the ‘founder’ fresh market cultivars from which most later cultivars were developed. Similar molecular studies have also shown a similar narrow genetic base for most processing peach breeding lines used in California (i. e. most cultivars can be traced back to only a few initial founder cultivars). Most previous molecular studies, however, have concluded a more distant genetic relationship between fresh market and processing peaches. This latter conclusion is supported by the lineage analysis in Figure 4, since California processing peach breeding lines (highlighted in red) appear to represent a distinct sub-population. Interestingly, a major founder parent for fresh market, freestone peaches is 'Chinese Cling', while a major founder for California processing peach is the very old variety 'Orange Cling '. [Lineage figures provided are expandable (particularly if first pasted into less restrictive formats such as PowerPoint. Upon expansion, names of individual parents are readily discernable. In expanded form, this flowchart presents a huge amount of information which allows the tracing of specific lineages within fresh market and processing populations. Even at this stage, however, it represents only a small proportion of the actual breeding lines (as it will be evident upon examination of Figure 5)]. It remains likely that both Chinese Cling and Orange Cling share a common ancestor. However, an important question is whether they are truly related and if so, whether there was a distant more recent divergence. While some incorporation of freestone peach germplasm in processing peach breeding lines is documented in the lineage chart, very little processing peach lineages are used in fresh market breeding. A major reason is that the clingstone trait is undesirable in the North

Fig. 4. Principal breeding lineages for the major North American peach breeding programs. (Blue lines connect progeny with their pollen parent while red lines identify the seed parent. California processing peach breeding lines are boxed in red).

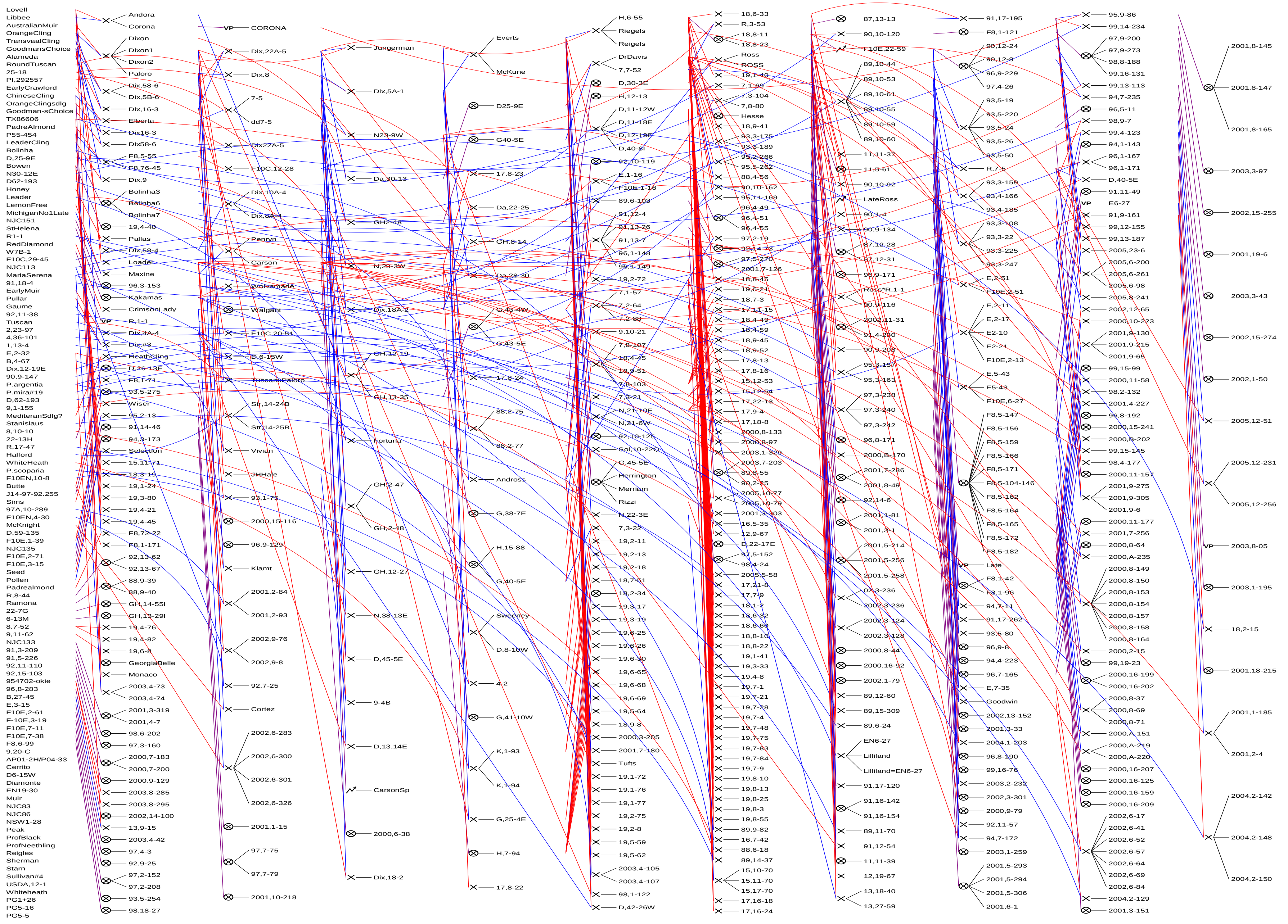


American fresh-market and the California clingstone germplasm has a reputation for being susceptible to fruit brown rot and bacterial spot. Non-melting clingstone peaches are often preferred in South American and European markets as a fresh fruit and clingstone peaches from California are in fact currently being utilized in several Central and South American peach breeding programs as well as a few disease resistance breeding programs in Europe.

B) Relationship among different populations within the processing peach breeding program.

Lineages for all current and historically important Californian processing peach varieties are plotted in Figure 5. Also shown are major breeding lines derived from those parents which constitute parents for the bulk of the seedling populations currently under evaluations in the UCD processing peach variety development program. [Since over 100,000 individual seedlings have been generated in this program during the past 2 decades, it would not be possible to show all breeding individuals, however, the majority of currently utilized cultivars as well as advanced selections in regional trials and used as breeding parents are included]. As with Figure 4, this plot can be expanded multiple-fold, allowing ready identification of specific parents. As such it represents, in a very concise format, a very detailed representation of the breeding program efforts, progress, and opportunities. For example, breeding lineages resulting in generally inferior progeny would be less likely to be represented in this chart as it contains mainly individuals demonstrating sufficiently higher levels of quality to be utilized in further crosses. Consequently, both breeding strategies as well as breeding efficiency can be inferred from general trends in this plot. Successful breeding lineages as well as the more successful parents can be visualized in the number of lines radiating from the right of these individuals to their 'successful' progeny. For example, even at low resolution, a distinct convergence of red lines is apparent about a third of the way down the second column from the left. Expansion of the chart would show that the red lines converge on two cultivars, Loadel and Carson. (Established cultivars are more likely to be used as seed parents because we would have the required larger trees available. Advanced breeding lines for brown rot resistance (for example) would often be used as the pollen parent since ample pollen is readily available from smaller seedling trees and these individuals would rarely be maintain to large tree status since at least a few of the progeny would be expected to possess similar brown rot resistance levels yet with higher fruit quality from the seed parent, (and so would be more preferable for use as a parent for the next cycle of breeding). While the charted relationships among lineages tends to be 'read' from left to right (older parents to more recent parents), it is actually constructed from right to left. That is, we started with breeding parents important at the current state of the breeding program and then determined their parents, grandparents, great grandparents, etc. until further parentage could not be determined with the information we had available. These terminal individuals would be considered 'founders' for that particular lineage and consequently located in first column. However, upon expansion, most would be found to be relatively recent additions to the program (a result of our relatively recent efforts to incorporate new germplasm into the breeding program). For this reason, approximately 85% of the individuals in the first (left) column represent recently introduced germplasm including, in some cases, recently developed cultivars

Fig. 5. Major breeding lineages involved in development of processing California peach varieties at UCD. (Blue lines connect progeny with pollen parent while red lines connect progeny with seed parent).



(for example, Bolinha) of unknown (to us at this time) parentage. The software used to develop this chart (PediMap -developed as part of the national RosBreed Specialty Crops grant) is mathematically stringent in constructing lineages, tolerating no synonyms, duplications, spelling errors, etc. , consequently it's very difficult to load with data. (The processing peach lineage required at least 40 hours to get to this point with an additional 30 hours probably required to incorporate the final 20% of breeding selections not yet included. A highly structured and accurate lineage is crucial however, for detecting associations between specific individuals (and lineages) with desired traits as well as effective molecular markers for criticathose traits (also being developed as part of the three-year RosBreed project). Even at this early stage of development, however, the software can allow a fairly efficient dissection different lineages associated with targeted traits. This potential will be demonstrated in the next section where the Bolinha resistance source will be examined within the context of both its contribution to brown resistance as well as it's negative contribution on fruit quality.

C) The transfer and recombination of genes among populations to achieve specific goals.

A representative subset of the UCD processing peach breeding lines utilizing the Brazilian variety Bolinha as a resistance source to brown rot disease is shown in figures 6, 7, in 8. Blue lines connecting parent (left) with progeny (right) indicate that the selection served as the pollen

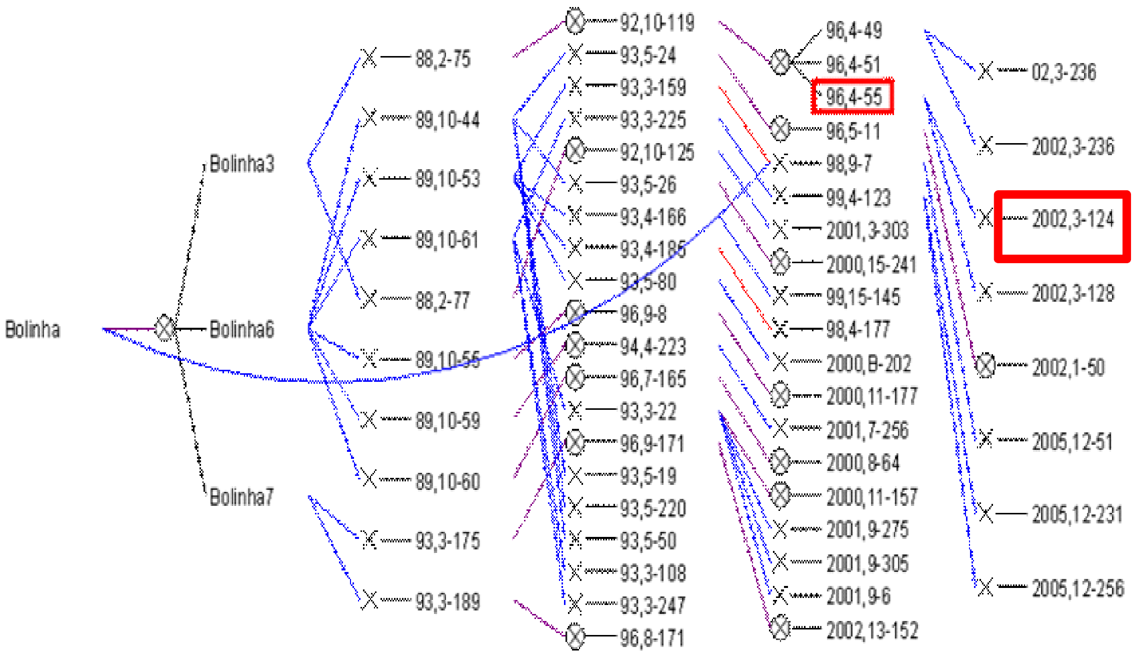


Fig. 6. Selected breeding lines derived from the Brazilian brown rot resistant variety Bolinha. [Particularly promising selections are bordered in red].

parent, while red lines indicate the selection was used as the seed parent. In this subgroup, individuals with promising levels of disease resistance in fruit quality were either self-pollinated (circled 'x') -to sort out desirable from undesirable genes) or backcrossed (usually to established cultivars with high fruit quality). Backcross parents are not shown in these figures to simplify the visualization of resistance transfer. The flowchart in figure 6 is developed with PediMap software using a database containing for each individual, its seed and pollen parent. By also including phenotypic or trait data in this database, the software is able to plot both the lineage flowchart and, through the use of user definable color codes, the degree of phenotypic expression for each plotted individual. In figure 7, a top-down lineage is plotted in which the degree of brown rot resistance is shown by the relative background color of each text box (red= highly resistant, blue= susceptible). A lineage chart constructed in this manner allows a rapid analysis of complex interactions. For example, it can be readily observed that the very high level of brown rot resistance in the Bolinha parent is not recovered in any of the progeny, though certain individuals have recovered relatively high resistance levels despite recurrent backcrosses to susceptible California cultivars. Seedling selection 2002,3-124, from the advanced breeding line 96,4-55 shows particular promise for level of brown rot resistance as well as improved fruit quality (Figure 8). The same flowchart, but now color-coded for level or fruit quality (Figure 8) shows that while Bolinha and its immediate progeny tend to segregate for brown rot resistance, they show low levels of fruit quality (typically small, green and easily bruised fruit, with heavy preharvest fruit crop). On average, improvements occur with each generation of recurrent back-crossing to high-quality established cultivars. Several individuals , including seedling selection 2002,3-124 and its pollen parent 96,4-55 show relatively good fruit quality (Figure 9) as well as relatively high levels of brown rot resistance. Even under laboratory inoculation conditions, the degree of brown rot disease development can vary significantly among replication and among years. Part of the reason for this sizable variation is that resistance in Bolinha and its progeny is controlled by multiple, possibly independent, components which can also vary with changing environments (see figure 10 -as modified from 2008 annual report). For this reason, we typically will not use even promising advanced selections as a brown rot resistant parent for further recurrent backcrossing, unless both it and its parent consistently showed resistance over several test years. This precaution inherently increases length of time between recurrent backcrossing cycles and so contributes to an increased time needed for resistance development. For example, in figure 6, it can be seen and it has taken 3 breeding cycles to achieve the level of fruit resistance and fruit quality consistently demonstrated by selection 96, 4-55 (Figure 9) and 4 cycles for selection 2002,3-124 (but resistance consistency is not yet certified by multiyear data).
While more tedious in terms of breeding cycles, this approach is ultimately is more efficient since it reduces the probability of wasting valuable field space/time with progeny from fundamentally susceptible crosses.

The complicated, multiple-component nature of resistance in even this very promising Bolinha source demonstrates the difficulties in rapidly transferring resistance to high quality, locally adapted varieties. This is particularly true when individual resistance components can vary widely depending on particular growth environment. (For example, cuticle thickness will vary from season to season depending upon temperature, relative humidity and other factors, and can vary even within an individual tree depending upon local growth conditions and level of skin abrasion). Molecular markers for this type og environmentally variable trait are particularly

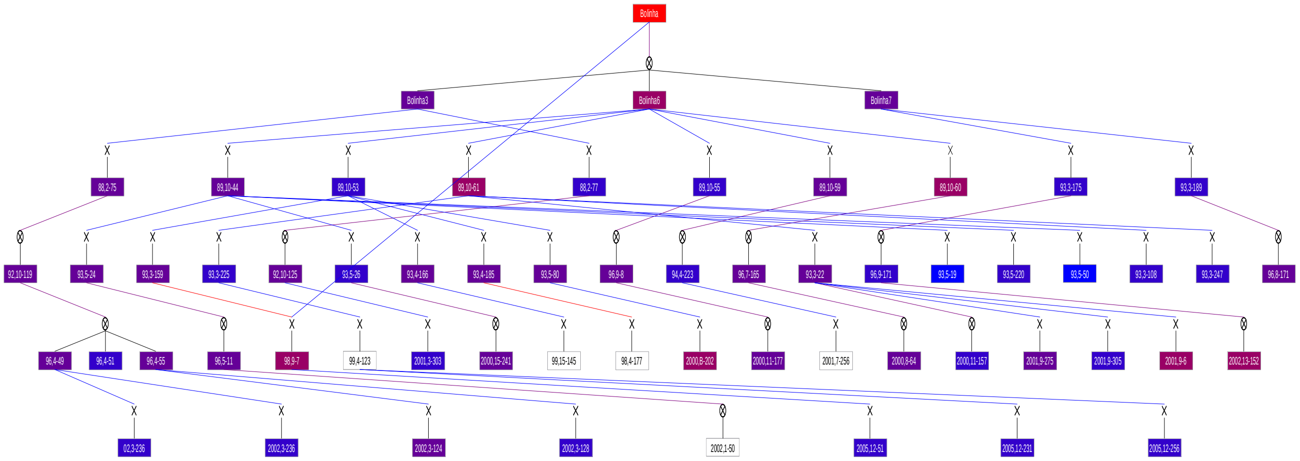


Fig. 7. A top-down lineage of Bolinha derived breeding lines , in which the degree of brown rot resistance is shown by the relative background color of each text box (red= highly resistant, blue= susceptible).

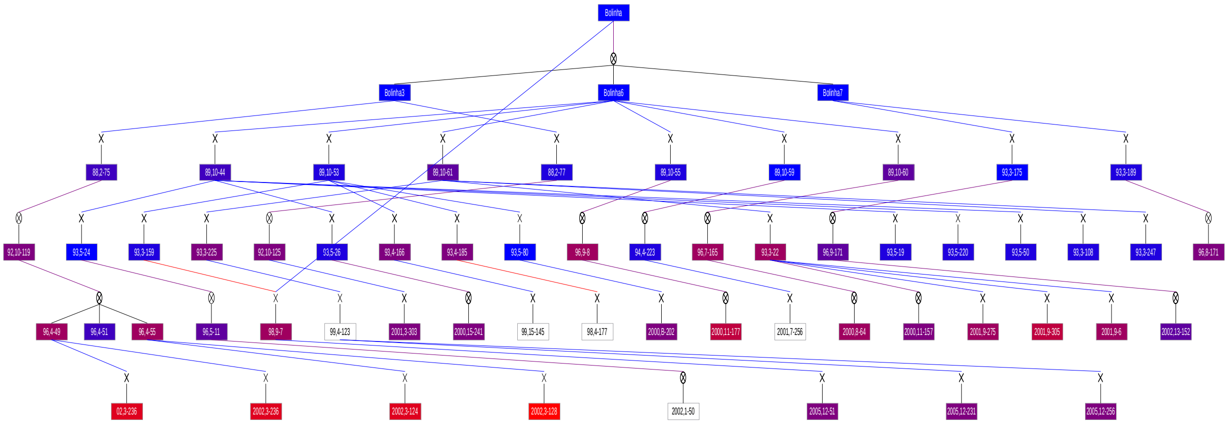


Fig. 8. A top-down lineage of Bolinha derived breeding lines , in which the level of fruit quality is indicated by the relative background color of each text box (red= high quality , blue= poor quality).

valuable in these conditions where multiple traits of varying contribution are involved, particularly when contribution will vary by environment, since it allows selection for the desired gene directly rather than indirectly through phenotypic expression. Working with Drs. Carlos Crisosto and Richard Bostock we are making progress towards the implementation of such a marker assisted selection scheme. Parallel research, implemented two years earlier by Drs. Ogundiwin, Peace and Crisosto has already resulted in the development of a peach linkage map showing potential markers for fruit integrity and post harvest longevity (Figure 11). These traits, which would be required for efficient mechanical harvest/transport are particularly difficult to breed for since there are multiple components which often interact in both positive and negative ways, depending on fruit age and environment.

Fig. 9. Advanced brown rot resistance selection 96,4-55 showing relatively good fruit quality as well as relatively high levels of brown rot resistance.

Fig. 10. Components known to be involved in fruit brown rot resistance in the Bolinha parent including (from top right) epidermis thickness.

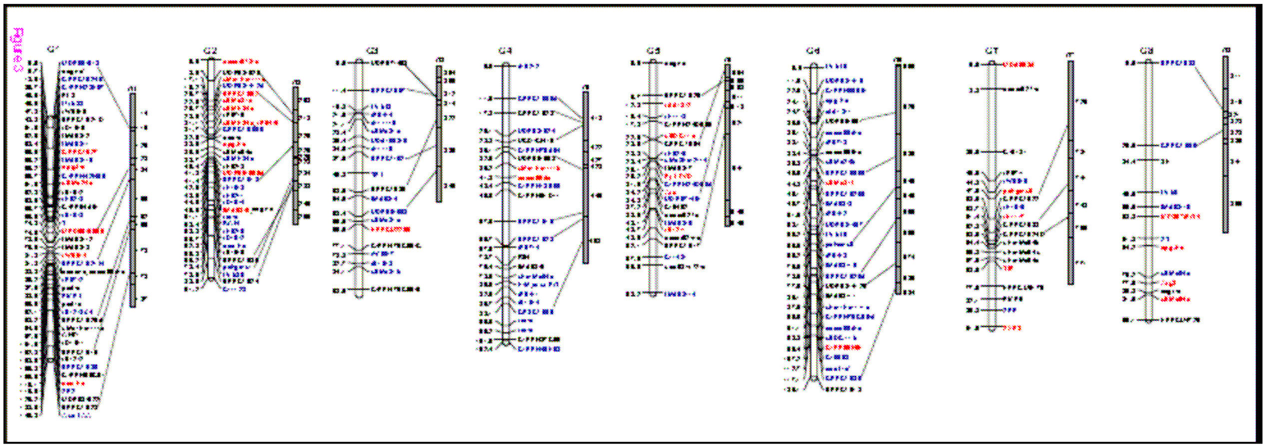


Fig. 11. Peach linkage map showing marker sites with potential for identifying major genes controlling peach fruit integrity and post harvest longevity. (Colors indicate putative mechanism of action based on similarities with other plant species).

Based on lineage, field performance, and preliminary mapping data, we have recently incorporated additional genetic sources, Late#4 and Early#6, into a more traditional California processing peach to allow a more comprehensive integration of fruit integrity genes in our ongoing breeding crosses, leading hopefully to an acceleration of breeding progress in this area

D) The characterization of breeding value from recently introgressed South African germplasm sources.

Late#4. The molecular study summarized in figure 11 identified multiple components in the processing peach cultivar Dr. Davis contributing to fruit firmness and, in particular, aspects of texture integrity and longevity crucial for the long-keeper capacity required for once-over and mechanical harvest. Results confirmed previous breeding studies which showed that progeny resulting from the self-pollination of Dr. Davis would segregate (although in a rather complex manner) for long-keeper ability. Genetic sources for this trait expressing even higher levels field performance have been identified and introduced. However since this germplasm is derived from more exotic sources (eastern Europe for *Extra-Early* season sources and almond, as a source for *Extra-Late* season expression), multiple backcrosses to locally adapted parents has been required to make this material accessible for California processing peach improvement using traditional breeding methods. Advanced selections from this aspect of the breeding program are currently in regional grower trials (Ultra-Early #1, and Extra-Late #4 through 7; see 2009 Regional Testing Report). Promising breeding parents representing a third source for long-keeper capacity, targeting mid-season breeding material has recently been derived from South African germplasm as characterized by the old South African variety Kakamas. Initial evaluations in the mid-1990's identified good fruit quality and productivity potential in Kakamas

Fig. 12. Fruit of Late #4 harvested 4 days before full-ripe showing good fruit quality and firmness as well as good flesh color, even for green fruit owing to its precocious expression of a yellow-gold flesh color.

Fig. 13. Fruit of Late #4 harvested at 18 days passed the full tree-ripe date showing the maintenance of good fruit and pit characteristics including flesh color and canning quality.

but early fruit-drop an soft fruit were also common in progeny, diminishing its genetic promise. Certain crossing combination, however, resulted in very good levels long-keeper ability, with one of the most promising selections being designated *Late #4*. Late #4 typically ripens between Dr. Davis and Monaco, but will hold on tree until after Halford. Fruit are large with a medium sized and somewhat ragged pit (Figure 12). Flesh is a uniform yellow-gold to orange-gold with a clean pit . Trees are very productive and amenable to mechanical harvest with low flesh bruising and low fruit brown rot. Flesh continued to be firm even with increasing age. Fruit harvested 18 days passed the full-ripe date in 2009 continued to show good fruit quality (Figure 13) and firmness (Figure 14). Late #4 has recently been advanced to regional grower testing, where its greatest promise appears to be as a mid-season option for growers planning to mechanically harvest their peaches. It's yellow-gold to orange-gold flesh color, while allowing some early harvest without green-fruit penalties as well as resistance to flesh bruising, may present a problem for processors when mixed with lighter colored fruit.

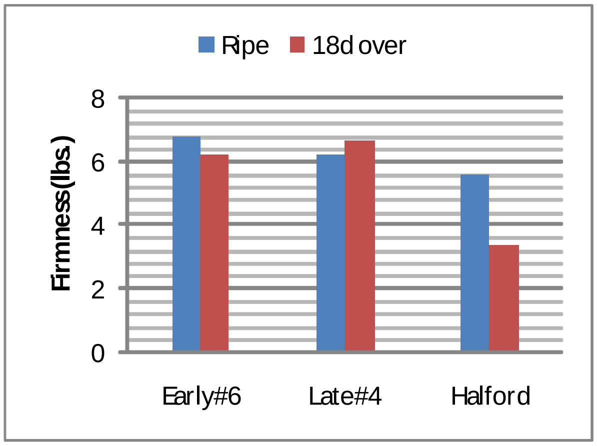


Fig. 14. Fruit firmness (as measured internally at the pit cavity following mechanical torque pitting) for South African derived processing peach selections, at their full tree-ripe date and when harvested 18 days passed the tree-ripe date.

Early#6. Recently an improved selection derived from the South African germplasm and combining the long-keeper potential of Late#4 with a more traditional golden-yellow flesh color, and maturity time within the crucial Dixon-Andross season has been identified. This selection has consistently shown superior fruit productivity, size, color (Figure 15) and harvest and post-harvest firmness (Figure 14) over a multi-year test period. Fruit show no red blush on the skin and, more importantly, no red stain development in the fruit pit

Fig. 12. Fruit of Early #6 harvested at full-ripe showing good fruit size, shape pit quality, firmness with a more traditional flesh color of yellow-gold.

cavity even up to two weeks passed the full-ripe date (Figure 16). Fruit ripen just before Dixon and because of the ability of ripe fruit to hang on the tree for extended periods can be harvested with or up to a few days after Andross. The tree is productive with low pre-harvest drop and moderate to good levels of field-resistance to fruit brown-rot. This selection is being introduced for grower testing as ***Early#6*** with initial plantings occurring in 2009 and propagations for more extensive plantings taking place in 2010.

Fig. 12. Fruit of Early #6 harvested at 18 days past full-ripe showing maintenance of fruit and pit pit quality, and a clean pit cavity with no evidence of red pit staining common for this maturity period.