Almond Variety Development

Project No.: 12-HORT1-Gradziel

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Objectives:

The key goals of this project are: (1) improved pollinizers for Nonpareil, and ultimately, (2) varieties that possess self-fertility and improved market value and resistance to disease, insects and environmental stress. Specific objectives for 2012-2013 include:

- A. Identify effective predictors of yield potential (annual and cumulative) to assess opportunities/limitations of traditional and new biotech approaches including molecular marker-assisted-selection (MAS).
- B. Generate 10,000 new seedling progeny [offspring] from controlled crosses between elite parents. Prioritize traits required for commercial success in partnership with growers, handlers and processors. Evaluate ~28,000 trees currently in breeding progeny plots and grower trials. Identify candidates for new Regional Variety Trials (RVT).
- C. Streamline the variety development cycle through more efficient controlled hybridizations and screening of progeny trees for self-compatibility, tree productivity, kernel quality, and resistance to key pests, diseases and environmental stresses, including water use efficiency.

Interpretive Summary:

Project goals include the identification and transfer of new germplasm conferring desired traits, incorporation of these new traits into advanced breeding lines well-adapted to California growing conditions, the selection of the most promising genotypes for advanced regional testing, and ultimately, the release of regionally-tested and proven new varieties. Breeding progress is shown in **Figure 1** where major breeding lineages have been plotted. Advanced selections [selection showing sufficient promise to move regional grower trials] demonstrating good kernel quality and adaptability to California conditions have been

derived from a range of germplasm including European and Asian almonds, wild species and cultivated peach. A dozen advanced selections, representing the breadth of this genetic diversity now becoming available for California almond improvement, are currently being propagated for the next round of Regional Variety Trials. Elite selection UCD2-19E, which has demonstrated exceptional productivity following long-term, regional variety testing and which strongly complements the Nonpareil variety in pollen crossincompatibility, bloom-overlap, cultural management practices as well as shell and kernel qualities, is currently in the process of patenting and release to the industry. Breeding efforts continue using this and other advanced selections as parents to further incorporate improved productivity, self-compatibility and disease/pest resistance into new varieties. Despite University cutbacks, cultural management improvements and, to a lesser extent, molecular markers are allowing an increase in breeding program size as well as an acceleration of breeding progress.

Figure 1. Updated lineage of principal UCD breeding lines. Circled clusters have advanced selections entering into the new Regional Variety Trials.

Materials and Methods:

A. Identify effective predictors of cultivar performance, particularly yield potential.

A consequence of the successful transfer or introgression of new germplasm into the UC Davis (UCD) Almond Variety Development program (see **Figure 1**) (as well as the

corresponding introgression of almond germplasm into the UCD Processing Peach Variety Development program) has been a rich diversity of genes and associated traits in advanced breeding lines. This diversity not only provides needed new options for increased crop performance and disease/pest resistance, but also permits the development of unique molecular markers (see **Figure 2**) for those traits which could potentially allow their selection at the seedling stage of the breeding program (and so ultimately reduce the need for the traditionally large-scale, multi-year seedling tree selection process). While industry funds are used entirely for applied breeding objectives, the genetic uniqueness of the resultant populations has allowed us to successfully secure outside state and federal funding to pursue more basic research in

Figure 2. Almond–Peach high density map [from citations 10, 12, 13]. Note the large number of markers on chromosome 7 (G7) and the dearth of markers on chromosome 8 (G8).

areas such as marker-assisted-selection (MAS) as demonstrated by the recently completed SCRI RosBreed project where the *Prunus* component was heavily dependent on our diverse almond by peach germplasm [citation 9, 18].

Figure 3. Plot demonstrating a strong correlation between molecular markers on chromosome 7 and increase s in soluble solid content as indicated by the strong association peak (red arrow) at that location.

Recent work by graduate student Jonathan Fresnedo has found a strong correlation between molecular markers on chromosome 7 and increased soluble solid contents (**Figure 3**). Thus, by selecting for these molecular markers at even the seedling stage, we may be able to increase the sugar content of the fruit/hull and/or kernel. Related work on the SCRI RosBreed project by Fresnedo has also indicated that genetic variation within the chromosome can be significantly suppressed or enhanced (depending upon cross and chromosome position) in these interspecific introgression lines [breeding lines of interspecific origin which are being crossed back to almond to combine new traits such as self compatibility into a commercial almond background]. This is shown in **Figure 4** where the almond-peach derived breeding line F8,1-42 shows unusually high genetic variation at the central area of scaffold (chromosome) 2 and end of chromosome 8, though with almost complete suppression of genetic variation (and hence recombination) at the end of chromosomes 2 and 3 when compared to modern variety *Dr. Davis* in the heirloom variety *Georgia Belle*. Results, which are consistent with our previous findings, indicate that interspecies introgression lines may effectively

Figure 4. Comparison in the distribution of genetic variants along each scaffold in each genotype for the interspecies introgression line F8,1-42 compared to standard peach cultivars. The frequency is given in number of variants per 100 Kb for a particular position in the scaffold.

open up more recombination in certain areas while suppressing it in others. More recombination would be desirable for separating desired exotic genes from undesirable wild species germplasm, while less recombination would act to protect desirable genetic combinations from reshuffling (as might be useful for developing seedling propagated rootstocks). Another consequence of such aberrant segregation patterns would be that the molecular markers may often be relevant only for the specific lineage in which they were identified and may not be as effective in even closely related lineages. Thus, since our advanced almond breeding lines represent a complex and diverse lineage, markers developed in one population may not be effective in other populations and may overlook positive genetic contributions for that trait from other lineages. Interestingly, in certain introgression lines, the value of using molecular markers to identify unique and desirable genes appears to fail entirely. **Figure 5** shows the pedigree of an almond by peach hybrid which was subsequently backcrossed (introgressed) to peach. The upper colored chart summarizes the recombination/segregation of over 500 markers covering all eight chromosomes (top colored bands) in 40 progeny from the self-pollination of A5,16-133. Surprisingly, very little recombination (circled) was observed even though many of the loci [the locations of a [gene](http://www.biology-online.org/dictionary/Gene) (or of a significant [sequence\)](http://www.biology-online.org/dictionary/Sequence) on a [chromosome\]](http://www.biology-online.org/dictionary/Chromosome) were heterozygous in the parent and progeny (identified by pink arrows at the bottom of the chart) and so should show extensive recombination. Such findings further reinforce the conclusion that MAS may be effective only in limited populations and that a strong

dependence on this selection approach may actually limit breeding progress in crops such as almond, where a strong genetic/genomic diversity is desirable [3, 7, 11, 15]. However, as discussed in the 2010 and 2011 reports, markers which directly utilize the coding DNA for the trait (rather than association by

Figure 5. Pedigree of an almond by peach hybrid which was subsequently backcrossed to peach. The upper color-banded chart shows the recombination/segregation of over 500 markers covering all eight chromosomes (top colored bands) in 40 progeny from the self-pollination of A5,16-133. Very little recombination (circled) was observed even though many of the loci were heterozygous in the parent and progeny (identified by pink arrows at the bottom of the chart) and so should show extensive recombination in the progeny. Left-center image shows distinct almond-type individuals which can very rarely be found in these otherwise strongly peach-type progeny populations. Results indicate a strong suppression of recombination in certain introgression lines, or a possible inherent failure in the molecular techniques used to develop molecular marker data for these introgression lines.

proximity DNA) can be particularly useful across breeding lineages, as shown by the successful selection of self-compatible forms of the S-gene in almond [7, 15, and 2012 *Interspecific Germplasm for Rootstock Breeding* Report]. These S-allele PCR[polymer chain reaction]-based selection strategies, which are based on the actual DNA sequence of the targeted gene, have proven very effective for selecting and transferring selfcompatible genes from peach and wild almond relatives to advanced UCD almond breeding lines as documented in earlier reports.

Ultimately, the success of a new almond cultivar is determined primarily by kernel quality and yield, which are determined by a very large number of genes in addition to the selfcompatibility gene, making the identification/deployment of effective MAS markers for even a large proportion of desired genes unfeasible. Currently, the only proven approach for such selection is through replicated Regional Variety Testing (RVT). Recent McFarland RVT results from Bruce Lampinen's Field Evaluation Almond Varieties project (12-HORT2- Lampinen) are summarized in **Table 2**. Results document strong performance by UCD advanced selection 2-19E as a late-flowering pollenizer for Nonpareil, as well as good performance for the recent UCD cultivar release Sweetheart as an alternative to the premium roasting quality Spanish cultivar Marcona.

B. Generate and evaluate new genetic progeny from controlled crosses between elite parents.

The Almond Variety Development program involves an initial intercrossing between

selected parents, the identification of elite individuals in the resulting progeny populations, and a thorough testing of these elite selections [selection showing sufficiently high proven potential that is used in crossing] in long-term, replicated regional trials prior to selection and release of new, proven varieties. In 2012 over 8000 seedling progeny were generated (as described in the next section) representing the first step in a breeding pipeline. Over 450 intermediate-level selections [selection showing promise but still needing further testing] are currently being evaluated for commercial quality, self-compatibility/self-fruitfulness,

and field resistance to important diseases and pests. Twelve advanced selections are currently being propagated for inclusion in the next long-term RVT evaluations beginning in 2014 (items circled in **Figure 1** and summarized in the next section). The final step in the variety development process is the selection and release of a new variety. The UCD almond breeding program is currently in the final process of patenting and release advanced selection UCD2-19E. Its breeding history is summarized below as an example of current strategies and techniques. The recent performance of the Sweetheart variety released by UCD as a California-adapted alternative to the premium roasting quality Spanish cultivar will also be assessed.

UCD2-19E almond.

The original almond seedling population from which 2-19E was selected was produced by a cross between Tardy Nonpareil, a late blooming mutation of Nonpareil as seed parent and Arbuckle (**Figure 6**). Based on its promising kernel and yield characteristics (**Figure 7**), selection 2-19E was submitted to the Foundation Plant Service (FPS) in the 1990's for inclusion into the virus-free nursery propagation Foundation Block where it was designated by the Accession Number 03-059-01-91. In 1992, selection 2-19E was included in the semi-commercial Regional Variety Trials, supported by the Almond Board of California, which included plots in Kern County, Butte County (Chico), and San Joaquin County (Delta). Twenty-six trees each of multiple selection and varieties were included in each

plot. Data of yield, phenological [biological phenomena tied to climate, i.e., bloom] observations, and tree and nut characteristics were obtained annually from 1995 to 2004. Although selection 2-19E demonstrated very good productivity and desirable tree

Table 1. Regional Variety Trial performance of selection 2-19E relative to the Carmel standard Nonpareil pollinizer from 1996 to 2004.

L-Lovell; N- Nemaguard; ND-No data

Table 2. McFarland, CA Variety Trial performance of selection 2-19E compared with the Nonpareil quality/productivity standard and other candidate Nonpareil pollinizers from 2006 to 2012. Yield components for genotypes tested in the ongoing Kern County RVT for 2010 to 2012 (with cumulative yields from 2006). Different letters and font colors indicate statistically significant differences in these replicated trials [From Lampinen 2013 RVT Report (Field Evaluation Almond Varieties)].

In each column, values with the same letter are not significantly different (P ≤ 0.05).

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Figure 7. Nut and tree qualities of UCD 2-19E. Nut qualities of UCD 2-19E are shown in the first two images. In the third image, note the more compact tree size of 2-19E relative to bordering Nonpareil trees (right) allowing greater light interception and so greater potential yield for the high-value Nonpareils.

and kernel characteristics at the Kern County Regional Variety Trial compared to the Carmel standard, performance at the Butte and San Joaquin County RVT sites was less promising (**Table 1**). In addition, all sites showed evidence of alternate-bearing where a high crop in one year would often be followed by a considerably lower crop the next. Both the alternate-bearing and the lower productivity in the more northern Regional Variety Trials were hypothesized to be the result of insufficient agronomic inputs for an inherently highly productive selection. Consequently, a new, replicated, semi-commercial test planting was established in McFarland, CA along with other candidate late-Nonpareilbloom pollenizers in collaboration with Bruce Lampinen and Mario Viveros,Kern County Farm Advisor. Under these orchard management conditions, selection 2-19E continued to demonstrate desirable tree and kernel characteristics (**Figure 10**) and good bloom overlap with the later Nonpareil bloom (**Figure 8**) while also demonstrating exceptional productivity without evidence for pronounced alternate bearing (**Table 2**). In addition, the high similarities between Nonpareil and 2-19E kernels may allow their mixing and subsequently higher 2-19E crop value. 2-19E harvest time occurs just after Nonpareil which allows concurrent and even simultaneous harvest (**Figure 9**). Recent field data analysis by Lampinen conclude that of the varieties tested, only UCD 2-19E approached the high, multi-year productivity of the Nonpareil standard (**Figure 11**) [16]. Based on its promising performance including consistent bloom overlap with the later bloom of Nonpareil, crosscompatibility with Nonpareil, good tree and nut qualities, and exceptional yields, selection 2-19E was selected in 2013 for patenting and release as a new UCD variety. This process should be completed by early 2014 and foundation budwood is now available for nurseries to establish their mother blocks subsequent grower propagations.

Figure 8. Average bloom data (2006 to 2012) for the McFarland Trial. Cross hatched area indicates onset of bloom to 100% petal fall. "F" indicates full bloom.

Figure 9. Average hullsplit progression by variety and Nonpareil source for 2006 to 2012 seasons at the McFarland Trial.

Figure 10. Flowering and cropping branches of selection 2-19E. Note the strong spur productivity combined with the good cropping ability on terminal branches.

Figure 11. Cumulative yield of almond selections against the Nonpareil standard showing that only UCD2-19E approaches Nonpareil's productivity in multi-year regional evaluations.

Regional performance of Sweetheart almond

Sweetheart is a recent almond cultivar from the breeding program at UC Davis. Sweetheart kernels have a distinctive cordate (heart) shape and very high oleic acid content. They are similar to the premium roasting quality Spanish cultivar Marcona but with a much higher kernel-to-nut ratio (**Figure 13**). Sweetheart shows good overlap with the economically important early bloom of cv. Nonpareil(S7S8). In addition, with a cross-

incompatibility controlling Sgenotype of S1S14, it is fully crosscompatible with Nonpareil and cross-compatible with all currently planted California almond cultivars except Winters (S1S14). Trees have an upright and open architecture which produce high yields with good year-to-year production consistency. The name Sweetheart refers to its high hearthealthy oleic acid content and its heartshaped kernel.

Figure 12. Lineage of the Sweetheart almond variety.

Figure 13. Shell and kernel characteristics of Sweetheart (left) and Marcona (right) showing similar kernel cordate shape but much higher crack-out for Sweetheart.

Sweetheart originated as a seedling from a cross between UCD breeding selections SB 3, 54-39E and 25-26 and was originally evaluated as selection SB13,36-52 (**Figure 12**). The seedling's pedigree includes the heavily planted California almond cultivar Nonpareil and Lukens Honey peach as a source of self-compatibility and disease resistance. Selection SB13,36-52 was selected for regional testing as an early flowering pollenizer for Nonpareil based on its desirable early bloom and high kernel quality with oleic acid contents above 70% (compared to 65% for Nonpareil under similar environments). California almond cultivars are self-sterile, requiring cross-compatible pollenizer cultivars for successful seed set. Due to its good market quality, Nonpareil currently comprises over 40% of commercial production in California. The remaining cultivars are mainly planted as pollenizers for Nonpareil with many initiating bloom after Nonpareil and thus successfully overlapping the later bloom but leaving the early bloom vulnerable. Almond flower

fecundity is higher in the early bloom, meaning early pollenizers have greater economic importance. Most early flowering pollenizers, however, show inconsistent bloom overlap with Nonpareil and a consequent lower production consistency for both cultivars. Flowering time in almonds is determined by the accumulation of sufficient chilling units to overcome flower bud dormancy followed by the accumulation of sufficient heat units for bud development to flowering. Requirements for both chilling and heat units differ among individual cultivars resulting in inconsistent year-to-year bloom overlap.

Marcona (S11S12) is a heavily planted self-sterile cultivar in Spain because of its very

high oleic acid content (approximately 70%) and subsequently high roasting quality. UCD-Marcona is the result of clonal selection of an old California Marcona accession for freedom from virus and improved adaptability to California growing conditions. UCD-Marcona produces a desirable bloom overlap with the economically important early Nonpareil bloom but has an undesirable highly lignified [woody] shell and so low kernel-to-nut ratio. Seedling SB13,36-52 (Sweetheart) along with Marcona and other breeding selections and cultivar standards were evaluated from 2005 to 2012 in replicated blocks planted in 2004 in Kern County in the southern San Joaquin Valley. Data was collected yearly on bloom and harvest period, pollen cross-compatibility, nut yield and quality, and disease and insect damage. A number of smaller grower plantings at additional sites in the Sacramento and San Joaquin Valleys were also utilized to verify RVT findings.

Figure 14. Physical appearance of Sweetheart tree showing upright growth habit and prolific bloom.

The Sweetheart tree is upright and vigorous (**Figure 14**). Production occurs on a combination of spurs and terminal

shoots. This bearing habit results in high productivity and an open tree architecture allowing greater light penetration and air circulation to the canopy interior resulting in lower foliar disease incidence.

Self-incompatibility in almond is controlled by the female gametophyte so that the cultivar Nonpareil (S7S8) will reject any pollen (which is male) with S7 or S8 genotype. Sweetheart has the S-genotype (S1S14) allowing for full cross-compatibility with Nonpareil and cross-compatibility with all commercially planted almonds except the related UCD cultivar Winters (S1S14). Self-incompatibility in almond is variable with most commercial cultivars setting less than 10% of self-pollinated flowers and so considered selfincompatible. Self-compatible cultivars need to set greater than 20% following selfpollination to be considered commercially useful. In a small number of cultivars, such as Sweetheart and Winters, the selfing percentage is between 10% and 20%, which while too low to be considered self-compatible, does confer greater crop stability were honeybee cross-pollination is limiting.

Figure 15. Average (2006 to 2012) bloom (top) and hull-split (bottom) dates for the Kern County Trial. Top: Cross hatched area indicates onset of bloom to 100% petal fall. "F" indicates full bloom. Bottom: Average hull-split progression from date of initial splitting to full-split.

Sweetheart consistently showed good overlap with early Nonpareil bloom in all years and sites evaluated (**Figure 15**), including the northern Sacramento Valley where the more variable winter and spring temperatures frequently result in erratic bloom patterns. The combination of open tree architecture, high flower-density, good bloom overlap with Nonpareil, and partial self-compatibility has resulted in consistently high productivity over the years tested (**Table 3**). Sweetheart has shown complete freedom from Noninfectious Bud-failure syndrome which has plagued Nonpareil and many of its pollenizers. The more moderate shell-seal of Sweetheart make kernels more susceptible than Marcona to damage by navel orangeworm (*Amyelois transitella*) but damage remains lower than Nonpareil. The kernel/(kernel plus shell) 'crack-out' proportion of approximately 0.6, while much lower than Marcona (**Table 3**) is well within the desired range for California cultivars. The incidence of undesirable kernel types, particularly double-kernels and kernels having multiple embryos was low, being similar to Nonpareil. The development of creases or folding of the kernel seedcoat, which is undesirable in fresh market as well as blanched kernels, is very low in Sweetheart relative to Marcona and other cultivars tested. Kernels of Sweetheart are also similar to Marcona in size and cordate-shape and but possess a paper shell rather than the highly lignified peach shell of Marcona (**Table 3, Figure 13**). Under intensive California cultivation, Sweetheart produces a slightly smaller kernel than Marcona (**Table 3**). Harvest of Sweetheart just after Nonpareil promotes efficient use of harvesting equipment.

Table 3. Average 2010-2012 production data for early blooming almond cultivars compared to Nonpareilin a 2004 grower test planting in Kern County. Cumulative (2006-12) kernel yield presented in last column.

same letter are not significantly different.

C. Streamline the variety development cycle.

The long-term objective of the UCD Almond Breeding Program has been the incorporation of new genes for economically important traits such as self-compatibility and disease/pest resistance, the transfer of this new germplasm into a California adapted genetic background, and the generation of improved varieties containing the best traits from both novel and traditional germplasm. Because of the exotic interspecies origin of many of these new traits, such as self-compatibility, early breeding efforts were focused on trait identification and transfer to a commercial-type background. As documented in **Figure 1**, current advanced breeding parents demonstrate good kernel and tree characteristics while possessing novel traits such as self-compatibility, resistance, and improved kernel quality. Moving forward, these new traits need to be incorporated into an elite genetic background possessing desirable traits/genes for the range of characteristics required for commercially successful variety. Because of the low probability of combining all of these elite traits into a single individual, future breeding strategies will require very large progeny population sizes at a time when University resources, including land and labor, are rapidly decreasing. Typical breeding methods employed in the past involved controlled crosses between selected parents, stratifying resulting and the typically low number (10 to hundreds) seed in petri dishes, germinating and growing seed for one year in the greenhouse, acclimatizing seed in lathe-houses, followed by hand-transplanting to field and hand training and hand-weeding. To streamline the breeding process while allowing even larger populations, we are implementing the use of caged or isolated parent blocks where the desired cross is achieved by bringing in bees and the desired pollen. The bulk stratification of resultant and typically large (100 to thousands of seed) populations directly plant germinating seed in the field, and use of herbicides and other non-labor intensive methods for weed control. The inherent lack of precise control over critical factors such as weather, disease/pest, and the soil/weed environments have resulted in several failed experiments over the past two years with a loss of approximately 3000 (low priority,

experimental) seed. However, at this time over 2000 seed have been successfully planted through this streamlined method with an additional 3000 seed expected to be planted by late summer. Over 3000 seed from higher value crosses has been stratified and germinated by traditional methods and is currently growing in greenhouses and lathehouses for transplanting in the spring of 2014. More efficient streamlined methods are also being pursued for field evaluation of seedling performance including self-compatibility (using bagged-branches self pollinated with artificial wind generators, i.e. leaf blowers), disease resistance (using field disease blocks in collaboration with Jim Adaskaveg), and tree productivity (using photosynthetic PAR measurements with Bruce Lampinen). Currently, our principal breeding parents are characterized by good tree and kernel qualities (as documented in **Figure 1**), adaptedness to California growing conditions, and possessing new traits (self-compatibility, disease resistance, pest resistance, kernel quality, etc.). The most promising of these genetically diverse items (**Table 4**) are currently being propagated for inclusion in the next RVT trials. In addition to assessing the potential of these exotically-derived selections as new California varieties, these trials will also identify both desirable and undesirable characteristics of these breeding lineages which can be targeted for improvement in future crosses.

Table 4. Promising UCD advanced almond selections currently being propagated for inclusion in the next RVT trials.

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