Developing Self-compatibility in 'Nonpareil' Almond

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Project Leader: Abhaya M. Dandekar, Dept of Pomology, UC Davis

Cooperating Personnel: Sandra Uratsu, Kristi Barckley, Hidenori Sassa and Tom Gradziel

Objectives

Almond is a self-incompatible species, thus, nut-set and yield is determined by successful pollination with non-self pollen usually coming from pollinator trees in the orchard. Self-incompatibility (SI) is a genetic system that prevents self-fertilization. The objective of this study is to understand at the genetic level how this system works in almond. An understanding of the mechanism of SI in almond will foster the development of technologies that can be used to reduce/eliminate self-incompatibility, thus, improving the efficiency of almond production. The development of self-compatible cultivars that are self-fruitful will mean that no pollinator trees or bees would be required to set fruit. In the short term the discovery of individual genes involved in self-incompatibility process provides robust diagnostic tools for growers, nursery and breeders to identify cross incompatibility genotypes among existing cultivars and new introductions rapidly and efficiently.

This study has two main objectives:

1. To identify and distinguish genes in almond responsible for self-incompatibility.
2. Develop molecular markers to distinguish all almond cross incompatibility groups (CIG) genotypes.

To identify and distinguish genes in almond responsible for self-incompatibility

The objective of this study is to identify/discover all of the S-alleles of almond encoding both pistil S-genes and pollen S-genes. Self-incompatibility (SI) is a widespread mechanism in flowering plants that prevents self-fertilization and promotes out-crossing. In almond this trait is controlled by a single locus (Bliss et al., 2002, Ushijima et al., 2001) with multiple codominant alleles (similar but not identical family of genes that perform the same function) encoding both pistil and pollen S-genes. When an almond pistil is pollinated by its own pollen interaction of the pollen and pistil S-genes leads to the triggering of SI, which results in abortion of pollen growth within the stylar tissues. Thus, unless the plant is cross pollinated there is greatly reduced fruit set. The pistil S-alleles encode SRNase proteins (an enzyme that destroys RNA) that are expressed in stylar tissue and that is responsible for the inactivation of 'self' pollen growth through interaction with an as yet unidentified "pollen component" referred to as the pollen S-gene that we have now made progress on identifying. The potential pollen component, which showed expression in pollen and linkage to the pistil component, appears to be an F-box protein (Ushijima et al., 2003). For the four predominant pistil S-alleles, a corresponding F-box protein has been observed. Further identification and characterization of the F-box proteins for other CIGs could further explain the mechanism of self-incompatibility. Regarding the pistil component we have successfully identified a family of pistil S-genes over the last several years by identifying an initial family of individual SRNase proteins (Tao et al., 1997) and mRNA

(Ushijima et al., 1998). Almond cultivars in California have 4 predominant pistil-S alleles, designated, Sa, Sb, Sc and Sd as defined by crossing (Kester et al, 1994). We have been successful in identifying the proteins encoding these alleles and the mRNA encoding these 4 pistil-S alleles through the analysis of cDNA (Tao et al., 1997; Ushijima et al., 1998; Tamura et al., 2000). As explained below we have now enlarged the set of S-alleles that we have identified from different almond cultivars.

Develop molecular markers to distinguish all almond cross incompatibility groups (CIG) genotypes

A major objective of this ongoing proposal has been to develop DNA marker based diagnostic tests to identify the cross-incompatibility groups (CIG). The DNA sequence analysis revealed similarities to other known pistil-S alleles and the N-terminal sequences we previously determined matched perfectly with the polypeptide predicted from the DNA sequence data. Additionally, these alleles correspond to the 4 predominant pistil-S alleles in California designated, Sa, Sb, Sc and Sd that correspond to 6 CIG groups defined by Kester et al., (1994). Our analysis have further shown how these gene sequences can be used to verify the CIG assignment of cultivars that belong to these six CIG groups based upon DNA sequence data. The following almond cultivars CIG assignment has been confirmed by this analysis: Nonpareil, IXL, Long IXL, Profuse, Tardy Nonpareil, Mission, Languedoc, Ballico, Thompson, Robson, Harvey, Mono, Sauret no. 2, Granada, Wood Colony, Merced, Ne Plus Ultra, Ripon, Norman, Price Cluster, Rosetta, Carmel, Carrion, Sauret no. 1, Livingston, Monarch and Monterey. However the CIG assignments of the following cannot be assigned to the known 6 CIG groups as they contain a different set or different combination of pistil S-genes and included in this group are the following cultivars: Solano, Sonora, Veista, Kapareil, Butte, Grace, Aldrich, Dottie Won, Fritz, Perl, Ruby, Padre, Tokyo, West Steyn, Durango, Le Grand, Jenette, Plateau, Avalon, Peerless, Kochi, Milo, Jordanolo, Jeffries, Johlyn, Tuono, UCD 25-75, UCD D3-25, UCD F8:7-179, UCDF8:7-161, UCD 13-1 (Winters), UCD 3-6, UCD 56-89, and Carrion. We have completed the identification of the pistil S-genes in this group through the identification of additional S-alleles that include, Se, Sf, Sg, Sh, Si, Sj and Sk. There are now 21 CIG groups including the original 6. Each of the previously mentioned cultivars has been assigned to a specific group which was determined through S-allele identification. Over the last two years our task has been to identify new pistil-S alleles that would permit the assignment of the above-mentioned cultivars. Another intron sequence has been previously identified and is located just down stream from the secretory leader peptide sequence. Current data suggests that intron size polymorphism also exists for this intron sequence. This intron sequence is currently being sequenced for the Si and Sj alleles, which have not been previously examined for this particular intron. Furthermore, Winters, prior to this year, was believed to contain only an Sj allele. Current data now shows that Winters contains an Sb allele as well. Through additional investigation of the parental lines of the Winters variety, it appears that change of the S-alleles occurs slowly, with no change occurring over three generations. In addition to the pistil S-RNase, haplotypes of the putative pollen gene are being investigated. Currently, only preliminary data has been obtained. Our goals for this year are to continue to investigate this new intron sequence and use it to authenticate our new CIG assignments, and to identify additional pollen S-gene haplotypes. Hopefully these may provide additional markers for CIG grouping as well as provide additional insight into the mechanism of self-incompatibility.

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