DEVELOPING SELF-COMPATIBILITY IN 'NONPAREIL' ALMOND

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This project is aimed at identifying the genes that determine self-incompatibility and to try to understand the underlying mechanism that mediates this important process. A basic understanding of the process of self-incompatibility will lead to the development of new diagnostic tools to help breed new varieties that can reduce/eliminate the expression of this trait. Unlike peaches, almonds are self-incompatible and require pollen from a heterologous source as well as an insect vector (bees) for successful pollination. The development of self-compatible cultivars that are self-fruitful will mean that no pollinator trees or bees would be required to set fruit. Plant breeding and or transformation technologies can be used to inactivate the self-incompatiblity mechanism.

Identifying and distinguishing the genes in almond responsible for self-incompatibility: Self-incompatibility (SI) is a widespread mechanism in flowering plants which prevents self-fertilization and promotes out-crossing. In almond this trait is controlled by a single locus with multiple codominant alleles (similar but not identical family of genes that perform the same function) referred to as S-alleles. When an almond pistil is pollinated by its own pollen, SI is triggered which results in abortion of that pollen tube growth within the stylar tissues. Thus, unless the plant is cross pollinated there is greatly reduced fruit set. The S-alleles encode SRNase proteins (an enzyme that destroys RNA) that are expressed specifically in stylar tissue and that are responsible for the inactivation of 'self' pollen growth. The exact mechanism by which this process occurs is not understood but has been hypothesized to involve some, as yet, unidentified 'pollen component'. Our major objective has been to develop DNA tools to identify these genes and thus develop unique diagnostic tools to distinguish cross-incompatibility groups (CIGs). Almond has been shown to have 4 predominant S-alleles in California designated, Sa, Sb, Sc and Sd that correspond to 6 CIG groups (Kester et al., 1994). We have successfully developed a DNA test that can clearly distinguish almond cultivars belonging to any of these 6 CIG groups. We have authenticated our DNA test by confirming the previous CIG assignments. This test is so precise that we have expanded the list of almond cultivars that fall into these 6 CIG to include some cultivars and selections that are of current interest. In addition we have discovered 7 new S-alleles that can be categorized into 11 additional CIG groups that include cultivars and selections that did not fall into the original 6 CIG groups. We have confirmed these new S-alleles by cloning and conducting a DNA sequence analysis of the corresponding cDNA. The genes that encode these cDNA in the genomes of various almond cultivars can be distinguished by virtue of the intron sequences. Previously we had identified an intervening sequence within the HV (Hyper Variable) region, however, we have found another intron right at the start of the mature protein sequence (after the sequences that encode the leader peptide). We are cloning and sequencing these introns from genes that encode the various S-alleles from different almond cultivars. This work is ongoing. This new information will allow us to more accurately determine the size polymorphism of the different intervening sequences and thus allow us to further define the almond CIGs. We have also been able to map the SI locus in *Prunus* and to characterize the genomic region in cv Jeffries and to compare it to the corresponding region in cv Nonpareil. We now show that Jeffries is missing almost 200 kb of DNA surrounding, and including, the Sc loci.

The region surrounding Sc is important as it may contain genes that include the pollen component and we are participating in an international effort to clone these genes.

Conclusion and future directions: We can conclude from our experiments that we have successfully cloned a majority of the S-alleles of almond varieties and selections in CA. The cDNA cloning and DNA sequence analysis of mRNA coding regions and intron sequences is helping validate the discovery of 7 new S-alleles in almond. Another major discovery is the mapping of the SI locus and the identification of the genetic defect in cv Jeffries that is now providing a location on the almond genome that may provide the location of other interesting genes, e.g., the pollen component in the self-incompatibility reaction.

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