Developing Self-compatibility in 'Nonpareil' Almond

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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 through the interaction of specific pistil and pollen S-genes. The objective of this study is to identify a potential region of the almond genome that may confer self-compatibility. An understanding of the mechanism of SI and how the S-genes interact 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 (self-fruitful) cultivars will mean that no pollinator trees or bees would be required to set fruit, creating a more economical and efficient process. In the short term the discovery of individual genes involved in self-incompatibility provides a robust set of diagnostic tools for growers, nurseries and breeders to identify cross incompatibility genotypes among existing cultivars and new varieties rapidly and efficiently.

This study has two main objectives:

1. To identify potential regions within the S-genes that confers self-compatibility
2. Continue to classify current and new almond varieties into cross incompatibility groups (CIG) genotypes.

To identify potential regions within the S-genes that confers self-compatibility

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-alleles. The pistil S-alleles encode S-RNase proteins (an enzyme that destroys RNA) that are expressed in stylar tissue and that are responsible for the inactivation of 'self' pollen growth through interaction with a "pollen component" via an as yet unidentified mechanism. When pollen containing the same S-allele as the pistil begins to grow down through the style, the interaction between the pistil and pollen S-alleles trigger the self-incompatibility mechanism, thus aborting pollen tube growth and inhibiting fertilization. Therefore, unless the plant is cross-pollinated there is greatly reduced fruit set. Almond cultivars in California have 4 predominant pistil-S alleles, designated, Sa, Sb, Sc and Sd as defined by crossing studies (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). From these initial four pistil alleles, additional S-RNases have been identified. The enlarged number of alleles has in turn led to an expanded CIG classification as explained below (Tao et al., 1997, Ushijima et al., 1998). Two of the newly identified S-alleles, Sj and Si, are

expressed in varieties 'Winters' and 'UCD 25-75' respectively. These two varieties have shown self-compatibility in field trials (Gradziel, unpublished). The Sj allele from 'Winters' was compared to parental self-incompatible varieties, 'Harriot' and 'Jordanolo', and analyzed for sequence differences. Allele Si from 'UCD 25-75' was compared to its self-incompatible parent, 'Arbuckle', and also analyzed for sequence differences. In both cases, exon and intron II sequence differed slightly. However, the intron I sequence for both alleles varied between parent and progeny varieties. 'Winters' was found to express two intron I sequences which varied considerably in length and sequence while still maintaining primer sequences and intron/exon boundaries. In the case of 'UCD 25-75' and 'Arbuckle', the intron I sequence varied only slightly in length and sequence. These differences between self-compatible and self-incompatible varieties may indicate a region conferring self-compatibility. Further investigation of these compared varieties may help shed light on the mechanism of self-incompatibility.

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 almond cross-incompatibility groups (CIG) for each of the commercially grown cultivars. 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 the 6 CIG groups defined earlier 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. Currently, there are now 21 CIG groups including the original 6. Newly added varieties which have been added to the CIG grouping include Folsom, Blue Gum, and Galaxy. Over the last two years our task has been to identify new pistil-S alleles that would permit the assignment of the almond cultivars. Another intron sequence has been previously identified and is located just down stream from the secretory leader peptide sequence. Size polymorphism also exists for this intron sequence creating another method for genotyping almond varieties. This intron has now been sequenced for all known S-alleles. Furthermore, 'Carrion', prior to this year, was believed to contain only a Sa allele. Current data now shows that 'Carrion' contains an Sj allele as well. Our goals for this year are to continue to investigate the differences between self-compatible and self-incompatible varieties, and to identify additional pollen S-gene alleles. Hopefully these may provide additional markers for CIG grouping as well as provide additional insight into the mechanism of self-incompatibility.

References

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