Developing Self-compatibility in 'Nonpareil' Almond

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Objectives:

The California almond industry is the largest supplier of almonds [*Prunus dulcis* (Miller) D.A. Webb] in the United States and throughout the world. Self-incompatibility (SI) is a major issue in almond production as it greatly affects nut set. In this study, we determined full-length sequences for alleles S^a - S^l, determined the genotypes of 44 California cultivars, and assigned the cultivars to cross-incompatibility groups (CIGs). Newly identified S-alleles led to an increase in the number of CIGs. A pairwise distance tree was constructed using the aligned amino acid sequences showing their similarity. Four pairs of alleles (S^c and S^e, S^g and S^h, S^d and S^j, and S^b and S^f) showed high sequence similarity. Because of its simplicity, reproducibility, and ease of analysis, PCR is the preferred method for genotyping S-alleles.

This study has two main objectives:

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

Genetic analysis of S-RNases

We successfully identified 10 S-alleles (S^a-S^j) in all known California almond cultivars. As shown by Tamura et al. (2000), a simple PCR confirms each allele based on intron size differences. Each allele has two introns that vary in length, allowing for easy detection and confirmation. Additionally, all S-alleles have the same structural sequence motifs identified for the original four S-alleles (S^a, S^b, S^c, S^d) (Tamura et al., 2000; Ushijima et al., 1998). According to Matton et al. (1997), the HV region is required for allelic specificity and for the pollen rejection mechanism. The aligned amino acid sequences show the HV region, which is interrupted by intron II, as being highly variable among S-alleles. Therefore, amino acid changes within conserved regions should not have a negative effect on the ability to recognize "self" pollen. Sequences at the 5' end have been analyzed for almost all of the S-alleles, resulting in the identification of the signal peptide, an additional intron (intron I), and the start of the mature protein. The structure as identified by the amino acid comparisons remains similar among the ten analyzed S-alleles. The S^f allele cloned in this study is derived from 'Tuono' and has an amino acid sequence identical to the S^b allele. While our data show that the exons are the same, the introns differ in size, indicating that S^f differs from S^b. However, if the HV region is responsible for allele specificity, then S^f should be recognized as S^b as the HV regions are the same. The presence of only one allele, or one self-compatible allele, however, does indicate self-compatibility. In a self-compatible system, pollen is not recognized as "self", and therefore pollen tube growth is not terminated. The cultivar Tuono expresses the S^f allele, a self-

compatible allele (Ma and Oliveira, 2001), which has previously been shown to have the genotype S_1S^f (Bošković et al., 1997; Sanchez-Perez et al., 2004). However, according to our data, 'Tuono' does not express an S^1 allele. Additionally, the S^f allele, cloned from 'Tuono' by Ma and Oliveira (2001) differs in sequence from the S^f allele isolated in this study using the same cultivar. Therefore, it can be said that the alleles found in European and Californian cultivars are similar but not identical.

Cross-Incompatible Groups (CIGs)

This study added 15 CIGs to the six previously identified by Kester et al. (1994). The original CIGs were determined through cross-pollinations and zymograms (Kester et al. 1994). Six S-alleles (S^e-S^j) were amplified by PCR, cloned, and sequenced. In addition, RACE was performed for all S-alleles (S^a-S^j) to determine sequences at the 5' and 3' ends. Large differences in the size of the two introns were observed. These differences in intron size were important in characterization of S- alleles by PCR. A comparison of amino acid sequences was performed to determine exon differences. All the alleles maintained the overall structure of five conserved regions, two introns, and a hypervariable region, but showed individual differences, especially within the hypervariable region. More single amino acid changes seemed to occur in conserved region C4 than regions C1, 2, 3, or 5. Although there are still some unknown sequences at the 5' and 3' end of some of the alleles, the most important regions of the sequences have been confirmed in our analysis. Intron I amplification was used for additional confirmation. The PCR and sequencing method gave clear reproducible results. These methods have been used to determine S-alleles in such systems as sweet (*Prunus avium* L.)and sour cherries (*Prunus cerasus* L.) and apricot (*Prunus armeniaca* L.) (Hauck et al., 2002; Sonneveld et al., 2001; Tao et al., 2002; Wunsch and Hormaza, 2004). Because of its simplicity, reproducibility, and ease of analysis, PCR is the preferred method for genotyping S-alleles.

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