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DEVELOPING SELF-COMPATIBILITY IN 'NONPAREIL' ALMOND

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This project is aimed at developing technologies that will be used to reduce/eliminate self-incompatibility, a trait that would improve the efficiency of almond production. Unlike peaches, almonds are self-incompatible and require pollen from a heterologous source as well as an insect vector (bees) for its delivery. The development of self-compatible cultivars that are self-fruitful will mean that no pollinator trees or bees would be required to set fruit. This will be accomplished by understanding the mechanism of self-incompatibility and self-fruitfulness in almond and the development of plant transformation technologies that will be used to inactivate the self-incompatible mechanism. Plant transformation will involve the development of shoot regeneration and gene transfer techniques that will permit the introduction of novel genes into almond. Plant biotechnology offers powerful new approaches that will enhance the ongoing breeding program as it would permit very precise genetic improvement of a commercially important variety such as 'Nonpareil' with potentially minimal impact on other overall characteristics.

Discovering 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. The predominant class of SI is the gametophytic SI (GSI) that is controlled by a single locus with multiple codominant alleles referred to as S-alleles. With this type of SI, growth of a pollen tube in the recipient pistil expressing one or any number of S-alleles common to the style leads to the abortion of pollen tube in the stylar tissues. Thus, unless the plant is cross pollinated there is reduced fruit set. The S-alleles encode SRNase proteins that are present in stylar tissue and that are responsible for the inactivation of 'self' pollen tubes. Our major objective has been to identify the SRNase proteins and mRNAs. California almonds have 4 predominant S-alleles, Sa, Sb, Sc and Sd. We have been successful in identifying all four proteins and in determining their N-terminal amino acid sequence (Tao et al., 1997). The next task to be accomplished was the identification of mRNA encoding these S-

alleles through the analysis of cDNA. We have been successful in identifying cDNA in each of the 4 S-alleles with confirmation by DNA sequence analysis (Ushijima et al., 1998; Tamura et al., 2000). The DNA sequence analysis revealed similarities to other known S-allele genes and the N-terminal sequences we previously determined matched perfectly with the polypeptide predicted from the DNA sequence data (Ushijima et al., 1998).

Authentication of Almond mating genotypes using PCR of S-alleles:

We have successfully investigated an alternative approach to confirm the authenticity of our isolated S-allele cDNAs through the development of unique PCR primers based upon our DNA sequence data (Tamura et al., 1999) that rapidly identify the individual S-alleles. Using DNA from almond cultivars whose S-genotypes have been identified we analyzed different primers to identify those that will discriminate the different S-genotypes perfectly. We have been able to identify both specific and common primers that can amplify all 4 alleles to distinguish each of the SI groups. A useful application of these primers would be in the identification of unknown genotypes, in cultivar mix-ups, for paternity testing of nuts, in breeding program where they could be used to identify the S-genotype of new varieties from the UCD breeding program, Almond nursery and grower selections. Also, the genomic sequence analysis showed that both Sa- and Sb-alleles have an intron in the HV (hyper variable) region, which is common in Rosaceae (Tamura et al., 1999). Using this S-allele specific PCR system, several cultivars with unknown S-genotypes were identified: 'Butte' is SbSd, 'Grace' is ScSd, 'Aldrich' is ScSd, 'Dottie Won' is SbSd, and 'Pearl' is SbSc (Tamura et al., 2000). We have also identified two new S-alleles that we have named 'Se' and 'Sg'. The 'Se' gives a unique band and can be found in the cultivar 'Tokyo' (ScSe). The other new allele we have designated 'Sg' ('Sf' is typically the designation for a self-compatible alleles) and this allele can be found in the following cultivars 'Solano', 'Sonora', 'Vesta',and 'Kapareil' (the other allele present in these cultivars is 'Sd'). These new alleles need to be cloned and their DNA sequenced to confirm that they are indeed S-alleles.

Transformation of Nonpareil:

The focus of the transformation experiments has been on the tissue culture phase that involves callus induction and regeneration of shoots from the callus. We have developed a callus induction system using in vitro shoots. Type and

concentration of plant growth regulators and basal media types with different compositional changes have been examined.

The callus induction system was used along with infection with *Agrobacterium tumefaciens* carrying a vector that contains GFP (Green Fluorescent Protein)and GUS (β-glucuronidase] to evaluate the formation of transformed callus expressing GFP. *A. tumefaciens* EHA101 containing the binary vector pDM96.0501 was used to infect leaf disks of 'Nonpareil' and 'Jeffries'. After 5 days cocultivation, Agrobacterium was disinfested by using the antibiotic cefotaxime. Because almond leaves are very sensitive to antibiotics, no antibiotics for selection was added to callus induction media for first one month. After forming small calli, we added a low concentration of kanamycin (20 mg/l) for selection of transformants. Green fluorescence expression was observed with some 'Jeffries' callus about 2 month after infection.

Conclusion and future directions:

We can conclude from these experiments that we need to keep trying different combinations of the plant hormones to increase regeneration and transformation. We plan to continue this effort this year. Our major success has been the development of a PCR technique to identify the 4 predominant S-alleles of almond. We also have identified 2 new alleles ('Se' and 'Sg') that need to be confirmed by cloning and DNA sequence analysis. Our next task is to use this PCR technique to authenticate the S-genotype of all almond cultivars relevant to almonds production in California.

Publications:

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