Developing Self-compatibility of 'Nonpareil' Almond

Project No.: 01-AD-00

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This project is aimed at identifying the genes that determine self-incompatibility and then to develop technologies that can be used to 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 and fruit set. 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 transformation technologies will be used to inactivate the self-incompatiblity mechanism. The development of plant transformation technologies will also provide the opportunity to introduce other useful genes into almond. Plant transformation offers a powerful approach to 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 productivity and quality characteristics.

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. Our major objective has been to develop DNA tools to identify these genes 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 are confirming these new S-alleles by identifying the corresponding cDNA, this effort is ongoing with most being identified. Recently we have also been able 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.

Transformation of Nonpareil

The focus of the transformation experiments is still on the tissue culture phase involving callus induction and regeneration of shoots from the callus on leaf segments. In addition we have initiated somatic embryos from almond zygotic embryos. Immature zygotic embryos were isolated from Nonpareil nuts collected in June (2000) when the endosperm was in the jelly phase and the embryo was not fully expanded. Somatic embryo cultures were successfully initiated from these embryos. Individual somatic embryo lines have been established and are being examined for repetitive embryogenesis, good multiplication rate *in vitro,* and the formation of normal embryos. Germination studies are underway to see if these somatic embryos will germinate normally. This year we have also begun to initiate somatic embryo lines from dihaploid almonds. If successful these dihaploid lines will be very useful for the breeding program.

Conclusion and Future Directions

We can conclude from these experiments that we need to keep trying different approaches and strategies to isolate somatic embryos from almond. We plan to continue this effort this year. Another major success has been to further the development of identifying all of the S-alleles of almond varieties and selections in CA. We have identified 7 new S-alleles that have been confirmed by cloning and DNA sequence analysis. In most cases we have been successful in identifying the corresponding cDNA. A major discovery is the identification of the genetic defect in cv Jeffries that involves a deletion of ~200 kb of genomic DNA including and surrounding the Sc loci.

Publications

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