Development of Citrus Cultivars with Reduced Juvenility

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The overall objective of this project has been to develop technology that will allow for the creation of commercially important citrus species that have reduced juvenility. Since most citrus improvement programs involve the use of seedlings, plants with reduced juvenility will prove invaluable for expediting work that is based in conventional breeding, biotechnology, or a combination of the two due to a shorter time period required for screening, evaluations and/or subsequent crosses.

The hypothesis that formed the basis of this work was that transgenic citrus species over-expressing a genetic regulator of flowering response from *Arabidopsis thaliana* or its ortholog from Washington navel orange would have reduced juvenility characteristics with respect to flowering. As a first step, a cDNA clone encoding the gene *FLOWERING TIME T (FT)* was obtained from the Arabidopsis Biological Resource Center and subsequently placed under the control of both a strong (CaMV35S) and relatively weaker (Ubi3) promoter. The resulting expression cassettes were then moved into transformation vectors for *Agrobacterium*-mediated transformation of Carrizo citrange, Eureka lemon, tobacco and tomato. Work on the construction of these transformation vectors was completed during the summer of 2004.

Work completed during the 2004-2005 funding cycle:

Propagation and evaluation of transgenic plant material overexpressing *FT:* Approximately 135 Carrizo citrange plants and 85 Eureka lemon plants were recovered from transformation experiments with vectors containing either the CaMV35S*FT* or UBi3*FT* expression cassettes. They are currently in various stages of growth and should be ready for propagation in the greenhouse early in 2006.

Expression Cassette	Flowering Dates (2005)	# Independent Lines
Empty Vector (control)	7/24-8/10	13
CaMV35S-FT	7/14-8/1	11
Ubi3-FT	7/1-7/10	10

Table 1. Over-expression of *FT* in Greenhouse-grown Transgenic Tobacco Resulted in Early Flowering.

The two *FT*-containing vectors were also used to transform tomato and tobacco. This work was performed to test the function of the constructs in systems that produce plants more quickly. Approximately thirty transgenic tobacco plants were recovered and moved to the greenhouse for growth and evaluation. One third of the plants were transformed with CaMV35S*FT*, another third with UBi3*FT*, and the remainder with an empty vector as control. Nearly all of the plants transformed with UBi3*FT* produced flowers at least one month earlier than the control group whereas the lines transformed with CaMV35S*FT* generally bloomed two weeks earlier (Table 1).

In summary, the early results with the *FT* coding sequence from *Arabidopsis* are very positive in that it appears that the expression cassettes are not only functional but result in plants with reduced juvenility and otherwise normal morphology (Figure 1).

Isolate and clone the *FT* cDNA ortholog from a Washington navel cDNA library and test its functionality in transgenic tobacco and tomato plants: The citrus EST databases being assembled at both UC Riverside and UC Davis were searched for sequences with similarity to *Arabidopsis FT* and revealed several full-length sequences with a relatively high degree of homology (approximately 75% with respect to *FT*). Three full-length cDNA sequences from three separate libraries prepared from Washington navel peel tissue and archived at the UC Davis Core Genomics Facility were selected for further analyses. Results from sequencing studies eliminated one putative gene as a possible candidate. The remaining two were incorporated into expression cassettes under the control of a CaMV35S promoter and used in experiments to transform tobacco in order to test for biological activity during year three of the project.

Figure 1. Over-expression of FT in transgenic tobacco induced early flowering. The plant *shown on the left was transformed with an empty vector as control whereas the one on the right was transformed with a vector containing Ubi3*FT.