Genetic Engineering of Citrus

Project Leaders:
Henry J. Fisk and Abhaya M. Dandekar
Plant Sciences, UC/Davis

The main goal of this work has been to develop genetic transformation methods for citrus scion cultivars important to the California industry. The cultivars investigated during the 2004-05 funding cycle included Washington navel and Valencia sweet oranges, Kinnow and Owari Satsuma mandarins, Eureka lemon and Mexican lime. Additional varieties, particularly mandarins, have been considered for future experiments.

Every plant transformation method consists of two important subsystems. The first is a regeneration system that allows for the development of organized plant tissues from single cells, usually through techniques based in tissue culture. The second is a transformation system that utilizes one of several methods to physically deliver new genetic material into plant cells.

Work to optimize conditions for both of these subsystems was conducted during previous funding cycles and resulted in the establishment of reasonably to highly efficient organogenic regeneration methods based on nucellar seedlings that produced genetically true-to-type shoots and also demonstrated efficient transformability using *Agrobacterium*-mediated transformation (Table 1).

In order to recover a transgenic plant, the new genetic material introduced during the transformation step must be delivered to the same single cells that are capable of dividing and regenerating into new organized plant structures. In other words, the regeneration and transformation subsystems must be successfully merged to create a complete transformation method. It is this step that received the most emphasis during the 2004-05 funding cycle because it remained as a limiting factor with respect to the consistent, reproducible recovery of transgenic citrus.

The transforming agent *Agrobacterium tumefaciens* demonstrated its ability to be highly effective at introducing new genetic material into citrus cells, but it also adversely impacted transgenic regeneration. Therefore, investigations aimed at mitigating the negative side effects of *A. tumefaciens* were performed using selected antioxidants, plant growth regulators and varied culture conditions. Regeneration efficiency was improved dramatically in response to some of the treatments and represented one of the most significant accomplishments of the most recent funding cycle. Post-*Agrobacterium* cocultivation efficiencies were improved to greater than 90% in some cases and represented a nearly ten-fold enhancement since the work began midway through FY ’03-’04 (Table 1). Future work in this area has been planned as a point of emphasis for the upcoming research year.

Transgenic lemon trees that were produced as a result of this program were submitted to the Citrus Clonal Protection Program for pathogen screening and are scheduled for release in 2006 and 2007. These trees have already yielded important information regarding the relationship between regenerating source material and juvenility. Subsequent field evaluations have been planned following their release.

Finally, a minor emphasis of the project has been the identification and development of useful traits that may be introduced into citrus through transformation. One trait under investigation was improved growth architecture. A transformation vector containing the *rol* genes from *A. rhizogenes* was constructed and

used to transform the highly transformable citrus rootstock Carrizo citrange. Some of the recovered trees expressing these genes displayed potentially useful horticultural characteristics such as reduced internodal distances and increased branching (Figure 1).

An unexpected and perhaps more significant outcome of these experiments was that several of the transgenic Carrizo citrange seedlings were also thornless, or nearly so (Figure 1). Future studies have been planned to test this newly created vector with the scion cultivars listed above.

Contact Citrus Research Board for table.

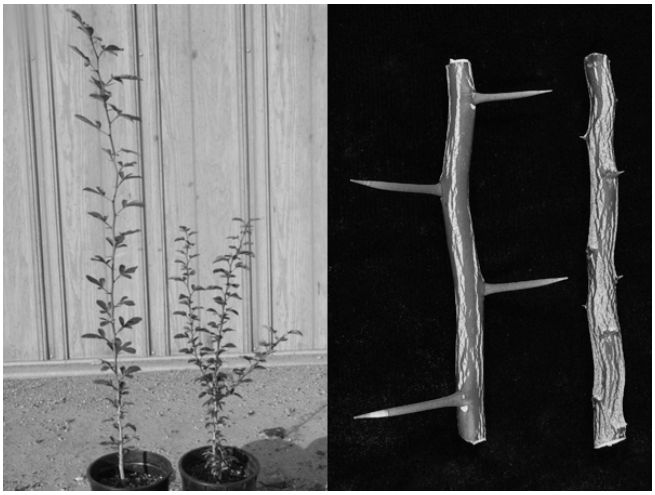

Figure 1. Expression of rol *genes in Carrizo citrange resulted in a more compact growth habit, increasing branching and thornlessness relative to the non-transgenic control plants.*