Project Concluded: Final Report

Controlling Citrus Tristeza Virus by Rootstock Delivery of a CTV RNA Silencing Signal

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We completed the fourth and final year of our CRB-funded effort to develop *Citrus tristeza virus* (CTV) RNA silencing as an approach to confer systemic resistance against CTV.

We constructed artificial "genes" derived from CTV. These were designed to represent conserved genome regions found in all currently described CTV isolates. These were then engineered into *Nicotiana benthamiana* and Carrizo plants in attempts to confer systemic "RNA silencing" resistance in these plants. The resulting plants were evaluated for molecular markers associated with RNA silencing and in several cases for CTV resistance. During this effort we also identified the CTV genes that allow CTV to overcome RNA silencing. These genes encode "silencing suppressors" and we found three such genes for CTV. Finally, we also have continued efforts to develop efficient means to transform and regenerate citrus types so that not only CTV resistance, but other desirable traits also can be engineered into citrus types in the future.

Our four original objectives were: (1) Transformation and evaluation of CTV silencing using conserved CTV sequences; (2) Development of rootstock transformation systems, specifically for sour orange and Carrizo; (3) Identification of CTV silencing suppressor genes; and (4) Transformation and evaluation of a CTV replicon silencing delivery system.

Objective 1: We made excellent progress for this objective. We have made two artificial CTV RNA silencing resistance genes and these were engineered into the experimental host plant, *Nicotiana benthamiana* , and into Carrizo. We confirmed that both genes give resistance against CTV sequences in *N. benthamiana* plants. We also showed that the resistance is effective against a number of different CTV sequence variants, representing some from the world wide collection of CTV isolates maintained in the USDA Beltsville contained research facility.

Propagation and grafting of transgenic Carrizo

Plants at left are non-transgenic Madam vinous scions grafted onto transgenic Carrizo rootstocks. Plants at bottom show propagated transgenic Carrizo. At least 5 individual cuttings for each of 71 transgenic plants have been propagated for use in ongoing CTV resistance studies. Project title: **Controlling *Citrus tristeza virus* (CTV) by rootstock delivery of a CTV RNA silencing signal**. (#5200-116) Bryce W. Falk, Abhaya M. Dandekar, Shou-Wei Ding, Diane Ullman

We also identified molecular markers associated with CTV resistance in some of the Carrizo plants. All of the Carrizo plants have been vegetatively propagated by rooting cuttings, and grafting. We have made various rootstock:scion combinations using the transgenic Carrizo plus madam vinous, Washington navel and Mexican lime plants. Several plants have been challenged with CTV by aphid inoculation and plants are under evaluation for resistance to CTV infection.

Objective 2: In the Abhaya Dandekar lab (UC/Davis) we focused on the transformation/regeneration systems for Carrizo, sour orange, and more recently other citrus types. Carrizo works very well and has been adapted by others as a very useful citrus system. Sour orange and lemon are still problematic. We have a few lemon plants that have been transformed with the CTV genes and these will be evaluated during the upcoming year. We are still evaluating other citrus types, as well as attempting different transformation/regeneration strategies that may be useful for other citrus types.

Objective 3: The Shou-Wei Ding laboratory (UC/Riveside) identified that CTV contains three genes that encode for different suppressors of RNA silencing. Although silencing suppressors have been identified in other viruses, CTV is the first virus shown to contain three separate genes for this function. These are the coat protein, P20 and P23. Each gene encodes a protein that utilizes a slightly different strategy to combat the plant "RNA silencing" response against CTV. In our CTV resistance strategy, we used each of these genes as part of our artificial genes to confer RNA silencing resistance in citrus. Thus, we have directly targeted what we believe to be important CTV genes.

Objective 4: Due to technical problems, we have not yet made good progress on this long term objective. We have continued to evaluate the possibility of using other plant viruses to design replicons for use in citrus. In upcoming years we will attempt to secure additional funding to allow this work to continue.

As a final note, this funding by the CRB was extremely beneficial for us to obtain additional state and federal funds to supplement these and additional research efforts on CTV and citrus. Our work would not have been possible without the CRB and we are very appreciative of their support.