INDUCED RESISTANCE TO BEET CURLY TOP VIRUS

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Curly top has affected agriculture throughout much of the western United States for over a century, and no cost effective control methods have been developed that effectively and reliably prevent losses. The wide host range of *Beet curly top virus* (BCTV), *Beet severe curly top virus* and *Beet mild curly top virus*, the abundance of the beet leafhopper vector (*Circulifer tenellus*), and increasing acreage of uncultivated rangeland in some areas due to reduced availabilty of irrigation water and other factors, is making curly top management increasingly difficult. Affected crops include sugarbeet, tomato, pepper, bean, and cucurbits to a lesser degree. The viruses also infect a wide array of weed hosts. Present management strategies focus on the large-scale use of insecticides to control the leafhopper vector in rangeland, the use of insecticidal treatments on crops, and carefully timed planting and harvesting to reduce impact of the virus on crops.

The desire for more effective and environmentally friendly management necessitates the use of novel approaches, including biotechnology. These methods have shown promise with some related viruses in other hosts, and should be effective for curly top as well. Many plants induce a natural process known as *virus-induced gene silencing* (VIGS) upon infection by viruses. VIGS causes selective, specific degradation of RNA virus genome sequences, as well as any additional sequences inserted into it. This can occur either during or after production of RNA. A number of different structural features on nucleic acids have been implicated as possible triggers, including abnormal double stranded RNA molecules, tandem insertions of the same DNA sequence, and specific structural features on the nucleic acids to name a few. VIGS can initiate even in the first cell the virus infects, preventing whole plant infection, and silencing signals can be transmitted systemically throughout plants. Although most studies on gene silencing have been done with RNA viruses, silencing also occurs with DNA viruses, although the latter occurs through silencing of RNAs produced by the virus (post-transcriptional gene silencing or PTGS). BCTV is a DNA virus, however it does produce RNA as a template for synthesis of virus proteins. Recent studies indicated that silencing based approaches have been effective for other geminiviruses, such as *African cassava mosaic virus* and *Tomato yellow leaf curl virus*. Our goal was to develop VIGS/PTGS for control of the predominant curtoviruses affecting agriculture in the United States. Results show promise for this method as a means for curly top control.

A PCR fragment corresponding to a 394 bp region encompassing a portion of the intergenic region and the 5' end of the C1 (Rep) gene of BSCTV was cloned into pTRV2, a virus-based vector provided by S.P. Dinesh Kumar, Yale University, and named pCFH-C1. This construct was *agro*-inoculated into curly top host *N. benthamiana* along with partner construct, pTRV1, which is also required for infection using *Agrobacterium tumefaciens* strain AGL1 when plants were at the 4-6 leaf stage using standard methods. Controls were treated with TRV constructs lacking the silencing insert.

The silencing construct, pBCTV-3HP (3-way hairpin construct) was designed in reference to the successful silencing of TYLCV in tomato by others. PCR fragments of three sections of the curtovirus genome conserved between BSCTV and BMCTV (Fig. 1A) were ligated together in both the sense and antisense directions, inserted into the agrobacterium gene expression vector pFGC5941 at positions upstream and downstream of the CHSA intron to form a large hairpin. *A. tumefaciens* strain AGL1 was also used to deliver this construct to the test plants. *N. benthamiana* was inoculated as described above and tomato was inoculated using either direct injection into the stem or the airbrush method described below.

A new method for treatment (injection) of the host plants with the silencing constructs was studied. Previously the syringe infiltration method was used (needleless syringe to inject the agrobacterium solution into the leaf). This works well with *N. benthamiana* but not with tomato. For tomato, another method was employed using an artist's airbrush to spray the agrobacterium solution onto the ventral side of the leaves from a distance of approximately 4-6 inches at 70 psi for 3-5 seconds. Initial testing in the spring of 2009 showed positive results and currently more extensive testing is in progress for this application method. A third method for the application of silencing constructs is the agrodrench method (the agrobacterium is applied directly into the soil adjacent to the crown part of the host plant). Further studies are needed to test this method. To date efforts to deliver constructs and induce silencing using these methods in sugarbeet have been unsuccessful; however, we are still working to overcome this issue.

Results demonstrated that treatment of plants with silencing construct pTRV-CFHC1 was successful at reducing the severity of curly top symptoms in *N. benthamiana* and nearly completely eliminated infection in tomato. The silencing construct pBCTV-3HP virtually eliminated infection of tomato by BSCTV when plants were inoculated 24 days after treatment. Inoculation at 14 days following treatment reduced infection by approximately 2/3. This indicates at least three weeks are required for induction of resistance in tomato. Molecular testing by ELISA and PCR confirmed the effectiveness of the both silencing constructs in the two hosts. Unfortunately, attempts to perform similar treatments on sugarbeet have been unsuccessful to date. Further research is needed to determine what is necessary to overcome roadblocks to application of this method for control of curly top (and potentially other viruses) in sugarbeet. However, engineered resistance using these constructs for sugarbeet transformation remains an option.