UTILIZING WILD *CAPSICUM ANNUUM* (CHILE PEPPER) FOR BREEDING *BEET CURLY TOP VIRUS* RESISTANCE IN CULTIVATED HOT PEPPERS

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Geminiviruses are the largest family of viruses threatening global vegetable production. Additionally, *Beet curly top virus* (BCTV) is one of the most damaging geminivirus of chili pepper (*Capsicum annuum*) in the United States that can result in yield losses ranging from 20-80%. BCTV is transmitted by leafhoppers (*Circulifer tenellus*) and infect a wide range of plants, such as pepper, bean, sugar beet, tomato, cucurbits and spinach. Both the virus and the insect vector continue to be difficult to control.

Our goal is to investigate germplasm sources from landraces collected in Mexico, where virus is prevalent, as well as 10 lines from the literature for resistance to BCTV. To identify sources of resistance, we utilize a rapid *Agrobacterium*-mediated inoculation assay. Interestingly, 20% of the accessions from the literature were susceptible to BCTV, while only 26% of the wild accessions appeared to be susceptible. Resistance has been confirmed using a leafhopper assay for some of the wild accessions.

Several accessions identified as resistant have been crossed into a cultivated, susceptible jalapeño variety to generate and test populations segregating for BCTV resistance and favorable agronomic traits. Wild accessions were preferentially selected based on traits such as seed production, fruit type, and the ability to cross with other *C. annuum*. These populations are being used to determine the genetics of BCTV resistance in pepper. Our long-term goals are to develop and release pepper breeding lines that combine resistance from wild pepper germplasm to BCTV, as well as to determine the genetic basis of this resistance. Identifying genetic resistance from multiple sources is the key to integrated management programs to protect yield and quality in pepper and other crops.

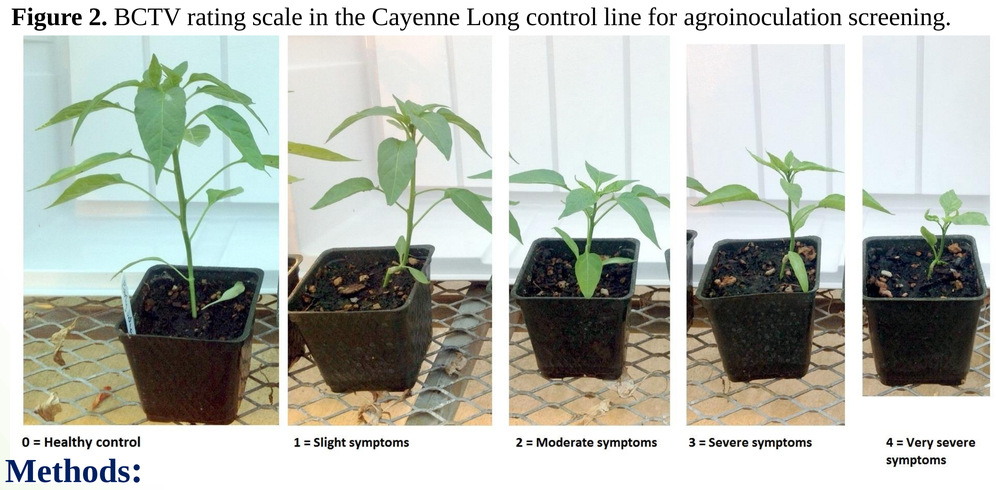
Introduction and Background:

Beet curly top virus (BCTV) is a *Curtovirus* (Family: *Geminiviridae)* that was first reported in California in the 1890's and now occurs throughout California's vegetable producing regions. Management of this disease in complicated due to its broad host range, and its persistence in the field over winter due to bridge species such as weeds like Sheppard's purse as well as crops like radicchio. There have been some landraces reported to have BCTV resistance as well as one of the only commercially resistant to BCTV available from New Mexico State University (NMSU). However, the resistance from NMSU was found serendipitously after a severe loss to BCTV, and the genes underlying this resistance have remained unidentified.

Our lab has 100+ wild *Capsicum sp.* accessions that were collected in Mexico (**Figure 1**) where Geminiviruses are prevalent, and 30 have been screened for resistance to BCTV. Some wild accessions have been used as donor parents, as well as be genotyped using genotyping-by-sequencing. Populations segregating for BCTV resistance are undergoing screening. A bulk segregant analysis will be performed on the populations generated from the wild*cultivated crosses to determine the genomic region(s) responsible for BCTV. In this presentation, we present the accessions and screening methods that have been used to identify wild pepper accessions that show tolerance/resistance to BCTV – *Pepper curly top virus* primarily using an agroinoculation screening technique as well as preliminary results on population screens.

Figure 1. Wild accessions, parents, and crosses grown in the greenhouse and the field at UC Davis in 2015-16.

Figure 2. BCTV rating scale in the Cayenne Long control line for agroinoculation screening.



Results:

Forty-three lines (nine reportedly resistant lines from the literature, thirty wild lines, and four commercial lines) have undergone the agroinoculation screening. Results of some of the lines are shown in in **Figures 5.** Two of the reportedly resistant lines were shown to be susceptible to BCTV, as well as the commercial lines, including the jalapeño recurrent parent used in the crossing scheme. Several of the lines screened show moderate resistance, and will need to be re-screened with leafhoppers to confirm resistance. However, some wild lines are very susceptible to BCTV such as the line shown in F**igure 6.**

A preliminary $F_{1:2}$ population of 263 plants derived from a wild accession by jalapeno cross has been screened using the agroinoculation method. Based on the scale in **Figure 2,** 12.5% of the plants show severe to very severe symptoms, or a highly susceptible phenotype as shown in **Figure 7**. This is suggestive of two-dominant genes controlling the trait. When tested via χ^2 , the null hypothesis of a two gene model is not rejected ($p>0.05$).

Methods:

Method 1: *Agrobacterium*-mediated infection with infectious clone

Agrobacterium-mediated infection (agroinoculation) is the method of choice due to the ease and efficiency of inoculation. A commercial line that is highly susceptible to BCTV and has 100% agroinoculation efficiency is shown in **Figure 2**.

Our agroinoculation is performed with a full-length clone of a strain of BCTV isolated from pepper called *Pepper curly top virus*. Multimeric forms of full-length infectious clones are ligated into a binary vector, which is transformed into *Agrobacterium tumefaciens*. The transformed *Agrobacterium* culture is then inoculated into the plant stem using a needle puncture method (**Figure 3**), and the plant is monitored for symptoms of curly top disease, which typically occur 14-21 days post inoculation (dpi).

Figure 3. BCTV agroinoculation of pepper. Injection of the plant stem with agrobacterium containing *Pepper curly top virus* genome using hypodermic needle to introducing culture into plant phloem.

Method 2: Utilizing viruliferous leafhoppers

The leafhopper vector is used for confirmation of the agroinoculation method. Non-viruliferous leafhoppers from the colony maintained at UC Davis (**Figure 4a)** are fed on BCTV-infected host plants. Viruliferous leafhoppers are then placed on the pepper plants being screened for BCTV resistance in small cages (**Figure 4b**) and allowed to feed for 2-3 days, which will inoculate the plants with BCTV. The pepper plants are then evaluated for symptoms for 14-21 dpi.

Figure 4a. Leafhopper colonies maintained on sugar beets.

Figure 4b. Individual clip cages that affix to plant leaves and ensure leafhopper feeding on individual pepper plants.

Figure 5. Clustered bar graph showing comparison of wild accessions to susceptible and resistant control in BCTV agroinoculation screen based on symptom score. * denotes susceptibility $p< 0.01$.

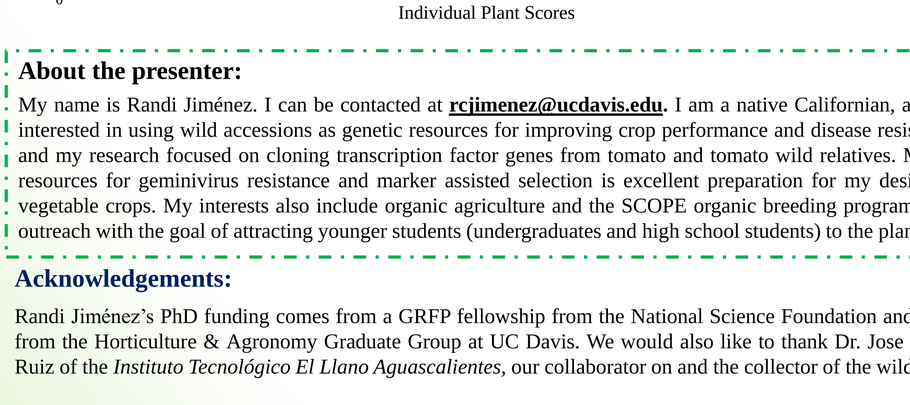


Figure 7. Scores of agroinoculation screen on $F_{1:2}$ population of 263 plants derived from a wild accession by jalapeno cross.

Future Work:

Selected $F_{2:3}$ plants will need to be screened to confirm resistance, and a Bulk Segregant analysis performed to identify QTL linked to the BCTV resistance trait.

Conclusions:

It is appears that resistance alleles are within the wild lines, and integrating it into a cultivated background through breeding will be useful for pepper production as well as understanding the genetic mechanism of BCTV resistance.

Figure 6. BCTV severe symptoms in wild *C. annuum* accession.

About the presenter:

My name is Randi Jiménez. I can be contacted at **rcjimenez@ucdavis.edu.** I am a native Californian, and I have always been interested in plants. I am interested in using wild accessions as genetic resources for improving crop performance and disease resistance. I received my MSc in 2012 at UC Davis, and my research focused on cloning transcription factor genes from tomato and tomato wild relatives. My PhD project using wild pepper accessions as resources for geminivirus resistance and marker assisted selection is excellent preparation for my desired career of being a plant breeder working in vegetable crops. My interests also include organic agriculture and the SCOPE organic breeding program at UC Davis, and I am extensively involved in outreach with the goal of attracting younger students (undergraduates and high school students) to the plant sciences, especially plant breeding.

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