Utilizing wild *Capsicum annuum* germplasm for breeding *Curtovirus* resistance in cultivated chile pepper

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Abstract:

The US Pepper industry was valued at $802.6 M grown on 78,700 acres in 2012. Geminiviruses are the largest family of viruses threatening vegetable production, and *Beet curly top virus* (BCTV) is one of the most damaging geminivirus of pepper in the US. Curly top disease, which results from infection by viruses in the genus *Curtovirus* (family *Geminiviridae*), affects >300 plant species from 44 different families and are transmitted by the beet leafhopper (*Circulifer tenellus*). BCTV affects several economically important crops such as pepper, sugar beet, tomato and spinach, and it significantly impacted California vegetable production in 2013 due to unusually large leafhopper vector populations.

Little is known about resistance to BCTV, and only a few sources of moderate resistance have been serendipitously identified with the most resistant sources being wild accessions. Several pepper lines have been screened for BCTV resistance using an *Agrobacterium*--mediated screen. Two of the reportedly resistant lines were shown to be susceptible to BCTV, as well as the commercial lines. Lines showing resistance will be re-screened with leafhoppers to confirm true resistance. It is likely that resistance genes to BCTV are present in wild accessions, and the long-term goal of this project is to introgress resistance to BCTV from wild pepper germplasm into a cultivated background using marker assisted selection. Integrating BCTV resistance into a cultivated background will be useful for pepper production as well as understanding the genetic mechanism of BCTV resistance.

Introduction and Background:

Beet curly top virus (BCTV) is a *Curtovirus* (Family: *Geminiviridae)* that occurs throughout California's vegetable producing regions. Management of this disease in complicated due to its broad host range, including pepper, tomato, and spinach, and 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) shown in **Table 1**. 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 has150 wild *Capsicum annuum* accessions that were collected in western Mexico (**Figures 1 and 2**) where these viruses are prevalent, and they are being screened for resistance to BCTV. After screening is complete, wild accessions will be used as donor parents, as well as be genotyped using genotyping-by-sequencing. A bulk segregant analysis will be performed to determine the region(s) responsible for BCTV, and background selection using markers will be done to maximize the percent of the cultivated parent genome after each backcross. In this report, we present the accessions and screening methods that potentially can be used in a breeding program to develop new BCTV resistant chile varieties.

Table 1. BCTV germplasm with reported resistance

Line	Species	Source	Resistance
PI 257053	*C. annuum*	Wild	BCTV unspecified
PI 281297	*C. annuum*	Wild	BCTV unspecified
PI 288938*	*C. annuum*	Wild	BCTV unspecified
PI 357522*	*C. annuum*	Wild	BCTV unspecified
NuMex Las Cruces' cayenne	*C. annuum*	Cultivated	BMCTV,BSCTV, BCTV
NuMex Bailey Piquin	*C. annuum*	Wild	BCTV unspecified
NuMex Twilight	*C. annuum*	Wild	BCTV unspecified
USDA-Grif 9322	*C. frutescens*	Wild	BCTV unspecified
PI 241675	*C. frutescens*	Wild	BCTV unspecified
Tabasco	*C. frutescens*	Wild	BCTV unspecified
USDA-Grif 9303	*C. chinense*	Wild	BCTV unspecified
PI 273419	*C. chacoense*	Wild	BCTV unspecified
Population from Mexico (150) ???	*C. annuum*	Wild	Uncharacterized

Figure 1. Wild accessions grown in the greenhouse and the field at UC Davis in 2013.

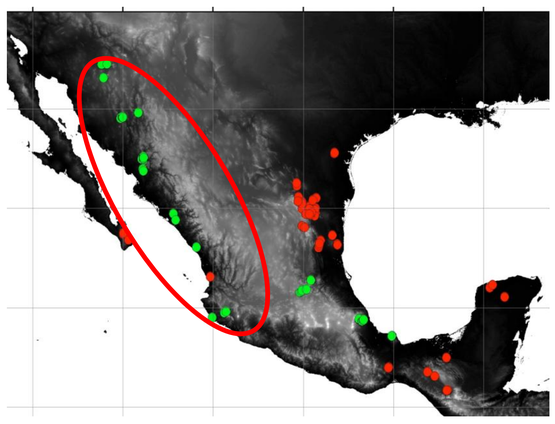


Figure 2. Collection locations of *Capsicum annuum* var. *glabriusculum* in Mexico**.** Green 2006 and red 2007 is collection.

Preliminary results:

Twenty-seven lines (nine reportedly resistant lines, sixteen wild lines, and two commercial lines) have already undergone the agro-inoculation screening. Two of the reportedly resistant lines were shown to be susceptible to BCTV, as well as the commercial lines, including the jalapeño recurrent parent. Most lines screened show moderate resistance, and will need to be re-screened with leafhoppers to confirm resistance. Four of the lines tested recovered and had less severe symptoms at six week post-inoculation indicating "recovery at top' may be part of the wild lines resistance.

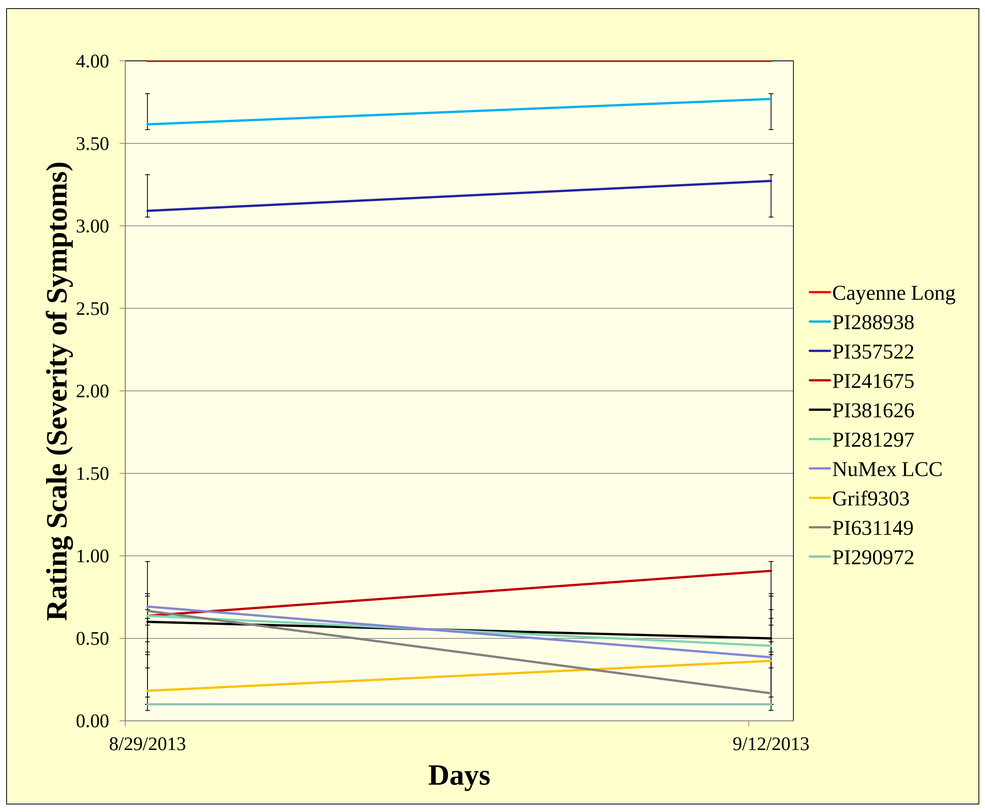
Methods:

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. 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). A variety that is highly susceptible to BCTV and has 100% agroinoculation efficiency is used as a susceptible control (**Figure 4**).

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 are fed on BCTV-infected host plants. Viruliferous leafhoppers are then placed on the pepper plants being screened for BCTV resistance in small cages and allowed to feed for a couple days, which will inoculate the plants with BCTV. The pepper plants are then evaluated for symptoms for 14-21 dpi.



Future Work:

Selected backcrosses will need to be field tested to confirm that the resistance in the greenhouse translates to resistance in the field.

Conclusions:

It is likely that resistance genes 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 4. BCTV severe symptoms in susceptible control.

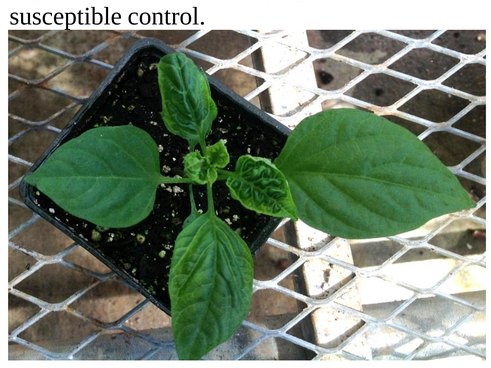


Figure 3. BCTV agroinoculation of pepper. Injection of the plant stem with hypodermic needle to introducing culture into plant phloem.

About the presenter:

My name is Randi Jimenez. 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. I am also 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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