Project title: **Characterization and assessment of the potential importance of a new whitefly-transmitted virus infecting cucurbits in the Imperial Valley of California: *Squash vein yellowing virus* (SqVYV)**

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Summary

In the fall 2014, the whitefly-transmitted ipomovirus, *Squash vein yellowing virus* (SqVYV), was detected in cucurbits at the Desert Research and Extension Center (DREC) and a few commercial melon fields in the Imperial County, California. This was the first finding of this virus in California. Surveys conducted in cucurbit fields in Imperial and Fresno Counties in 2015 indicated that SqVYV overwintered in 2014-15 and infected cucurbits in 2015, but it was not detected in cucurbits samples from Fresno County. In 2016, surveys were conducted early and late in the growing season. For the early season samples (collected from April-July), a small number of cucurbit samples from the Imperial Valley (5/120, ~4%) were positive for infection by SqVYV, and all were from the Imperial Valley. A higher rate of SqVYV infection (6/40, 15%) was detected in late season cucurbit samples from the Imperial Valley, and many were in mixed infections with *Cucurbit yellow stunting disorder virus* (CYSDV). Thus, our 2016 survey results clearly indicated that SqVYV overwintered in 2015-16, and infected cucurbit plants in fields during the 2016 growing season. The failure to detect SqVYV in samples from outside of Imperial County in 2016 was encouraging as it indicated that it had not spread long distance in California. Thus, it continues to appear that SqVYV has become established in the Imperial Valley, although there is no evidence that it has caused economic losses. To determine the relationship between isolates of SqVYV from California and Florida, we are determining the complete nucleotide sequence of a California isolate. A new technique, called Gibson Assembly, was used to successfully amplify the ~9800 nt genome of an isolate of SqVYV from the Imperial Valley of California. Because of the large size of the viral genome, the genome was amplified in two pieces. These two pieces are now being cloned into a vector, which will facilitate the sequencing of the complete genome of this California isolate of SqVYV.

Objectives

1. To determine if the newly introduced SqVYV will become established in the Imperial Valley, spread to other cucurbit production region and pose a threat to commercial production of melons, watermelons or other cucurbits.

2. Determine the complete nucleotide sequence of a California isolate of SqVYV in order to gain insight into origin of the virus and to develop better detection tools and information on the biology of the virus

Results

In 2016, we worked together with the Imperial Valley Agricultural Commissioner and identified commercial melon and watermelon plantings in the spring and in the fall growing seasons in the Imperial Valley. We also identified all cucurbit plantings in the DREC. Surveys of these fields were conducted for plants showing symptoms of virus infection. Our early season (spring-summer) surveys were conducted from April through July in 32 fields/locations in the Imperial Valley (98 samples), and an additional 22 samples were received from cucurbit fields in Fresno and Yolo Counties. Fall surveys were conducted in October-November and all of the 40 samples came from the Imperial Valley and had symptoms of virus infection. These samples were tested for SqVYV infection with RT-PCR and capsid protein (CP)-gene specific primers. In some instances, additional RT-PCR/PCR tests with primers for other cucurbit-infecting viruses that are known to occur in cucurbits in California were used. In the case of the late season samples, many of which had symptoms of infection with CYSDV, all samples were tested for this virus.

For the spring-summer samples, a total of 5 of 120 cucurbit samples (4%; mostly melon) tested positive for SqVYV. All of the positive samples were from the Imperial Valley (5%, 5/98), and all were detected with the more sensitive nested PCR method. All of the 22 samples from Fresno and Yolo Counties were negative for SqVYV, and the symptoms in these samples were due to infection with the potyvirus, *Watermelon mosaic virus*. We obtained similar results in 2015, indicating that SqVYV had not spread out of the Imperial Valley.

For the late season samples, which were all from Imperial Valley, we detected more SqVYV-infected samples, and these were all detected with the conventional PCR method. Thus, of the 40 samples that were collected, 6 were positive for infection with SqVYV (15%). Most of these were melons (5/39) and one was a watermelon sample (1/1). Of these 40 samples, 21 (53%) were positive for infection with CYSDV. The SqVYV-positive melon samples had symptoms of yellowing and green spots, which is more typical of CYSDV infection and, consistent with this, CYSDV was also detected in all of these samples. We had previously detected mixed infections of these viruses in 2015, and this is not surprising as both a transmitted by *B. tabaci* and in a semi-persistent manner. In the cases of the single watermelon sample in which SqVYV was detected, the sample did not show obvious disease symptoms (including vine decline) nor was CYSDV detected in this sample.

Two of the SqVYV CP DNA fragments, amplified from the survey samples by RT-PCR, were sequenced. The sequences of both fragments were 98% identical to known sequences of SqVYV. These results provide further evidence that SqVYV was infecting the cucurbits in the Imperial Valley in 2016 and that the virus has become established.

Together, our survey results indicate that SqVYV overwintered during 2015-16 and infected cucurbits in the field during the 2016 growing season. As was observed in 2015, the greatest number of samples infected with SqVYV were detected in the late season samples. This is likely due to the need for whitefly vector populations to increase, to acquire the virus from reservoir hosts and introduce and spread it to cucurbits. The failure to find SqVYV in samples from Fresno County and Yolo Counties in 2016 was encouraging and indicated it had not spread long distance in California. We found no clear evidence of economic damage by SqVYV in the

surveyed fields in 2016, similar to 2015. This was in part due to the fact that nearly all infections were in melons, in which SqVYV induces relatively mild symptoms, and that late season samples had mixed infections of SqVYV and CYSDV. Thus, because many of these samples also were infected with CYSDV, it is not clear exactly what symptoms were due to SqVYV. Nonetheless, the potential remains for SqVYV to become more widely established in the Imperial Valley, which could impact watermelon, where it is known to cause vine decline. It could also potentially increase the severity of viral disease in melons, as part of mixed infections with CYSDV and other viruses.

2. Complete the genetic sequence of the SqVYV isolate(s) from California and precisely ascertain the relationship with SqVYV isolates from Florida

We are in the process of completing the sequence of a California isolate of SqVYV. The finding of 11 SqVYV-positive cucurbit samples in 2016 (all from the Imperial Valley), indicates that the virus is persisting in the Imperial Valley and the continued importance of completing the sequence of a California isolate. Moreover, the potential for SqVYV spreading to other regions, e.g., Coachella Valley or Yuma, AZ, remains a possibility. The finding that the CP sequences of 2016 isolates of SqVYV were 98% identical to isolates from 2014 and 2015 in the Imperial Valley indicates that the SqVYV isolates in California are closely related and that sequencing of any of the isolates should provide a sequence representative of the virus in California.

We recently cloned the complete genome of a large RNA virus with a new method called Gibson Assembly. Therefore, we have used this method to amplify the complete genome of a SqVYV isolate from California (2016 isolate from Imperial Valley). Because of the size of the viral genome (~9,800 nucleotides), the genome was amplified in two pieces. Both fragments of the genome have been now successfully amplified, and the cloning step in which both fragments are pasted together in a cloning vector is underway.

As we previously found, there is genetic diversity between isolates of SqVYV isolates from California and Florida and it not clear if the California SqVYV was introduced from Florida. Here, it is worth emphasizing that SqVYV has now emerged in Guatemala and Honduras, where it is causing serious economic losses to watermelon production. It is also not known where the isolates of SqVYV in Central America have come from. Thus, the analysis of the complete sequence of the California isolate of SqYVV should provide some insight into where the California isolates of SqVYV came from.