Final report

A. Project title: **Survey, characterization and biological properties of a California isolate of *Squash vein yellowing virus* (SqVYV)**

B. Proposed for one year beginning January 1, 2017

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F. Work was performed at the University of California-Davis, USDA-ARS laboratories in Salinas, CA, DREC in Holtville, CA, and cucurbit fields in the Imperial Valley and other production regions

1. Introduction

Squash vein yellowing virus (SqVYV) is a new type of whitefly-transmitted virus that was first identified infecting melons and other cucurbits in California in 2014 and appears to have become established in the Imperial Valley. Thus, it now needs to be added to the list of known insect-transmitted viruses that can impact cucurbit production in California. SqYVV is particularly concerning because of the potential to cause the devastating watermelon vine decline disease, a disease that is currently causing economic losses in Florida and Central America (Guatemala and Honduras). The other insect-transmitted viruses that can affect production of melons and other cucurbits in the Imperial Valley and other regions in the desert southwest of the United States and Northern Mexico include the whitefly (*Bemisia tabaci*)-transmitted viruses *Cucurbit*

yellow stunting disorder virus (CYSDV, genus *Crinivirus*) and *Cucurbit leaf crumple virus* (CuLCrV) and *Squash leaf curl virus* (SLCV, genus *Begomovirus*); and the aphid-transmitted viruses *Watermelon mosaic virus* (WMV), *Papaya ringspot virus* (PRSV) and *Zucchini yellow mosaic virus* (ZYMV, genus *Potyvirus*) and *Cucumber mosaic virus* (CMV, genus *Cucumovirus*).

In the fall of 2014, pumpkin plants growing at the Desert Research Extension Center (DREC) showed unusually severe symptoms of stunting and leaf yellowing, crumpling and epinasty. In a nearby melon plot in DREC with a wide range of germ plasm being grown as part of a CYSDV resistance breeding program, some plants were observed with a yellow mosaic-mottling symptom that was distinct from the typical interveinal yellowing induced by CYSDV. Not unexpectedly, analysis of leaves of these plants revealed infection with CYSDV and SLCV. However, RT-PCR analysis of these leaves for potyvirus infection with the cylindrical inclusion (CI) and HC-Pro degenerate primer pairs revealed the expected-size DNA fragment only with the CI primer pair (in contrast to the expected fragments for both primer pairs that is obtained with the typical potyviruses, e.g. PRSV, WMV and ZYMV). Sequence analysis of the fragment amplified with the CI primer pair revealed 84% identity with the CI gene of SqVYV from Florida. This was the first time that a SqYVV-like virus was detected in California.

Subsequent surveys conducted for SqVYV in cucurbits in California in 2015, 2016 and 2017 revealed infections in California in both growing seasons, although most infections were detected in late-planted cucurbits and all infections were in the Imperial Valley. Therefore, it appears that SqVYV has become established in California. However, to date, there has been no evidence of economic losses due to SqVYV infection in California.

2. Objectives

2.1 Long-range objectives

Because SqVYV is a new virus in California and is presently causing economic losses in Florida and Central America, it is important to continue to survey for the incidence and severity in California to know if it is going to spread and increase in

importance here. We also want to further determine the biological properties of a California isolate of SqVYV, including the host range and symptoms induced in different cucurbits and in other plant species. The determination of the complete nucleotide sequence of a SqVYV isolate from California will provide insight into the relationship of the California isolate with isolates from Florida and Central America, and possibly how and from where the virus was introduced into California. Ultimately, we want to know if SqVYV poses a serious threat to commercial production of melons, watermelons or other cucurbits in the Imperial Valley and other melon-growing regions. Finally, we want to develop a series of tools for diagnostics and resistance breeding for SqVYV to allow us to gain a better understanding of the distribution of the virus, its biological properties and the potential for management by disease resistance.

2.2 Specific objectives

a. Conduct early and late season surveys of cucurbit plantings at DREC and commercial melon and watermelon fields in the Imperial Valley and other regions in California for SqVYV and other viruses in 2017

b. Complete the genetic sequence of a SqVYV isolate from California

c. Generate an infectious clone of a California isolate of SqVYV

2.3 Progress on Objectives to Date:

a. Conduct early and late season surveys of cucurbit plantings at DREC and commercial melon and watermelon fields in the Imperial Valley and other regions in California for SqVYV and other viruses in 2017

An early and a later survey of cucurbits (watermelon, melons and cucumber) was conducted in Imperial Valley and Fresno County, respectively. A late season survey was not performed in the Imperial Valley because of a lack of cucurbits fields. The total RNA of the samples was extracted and used in RT-PCR to detect SqVYV and other RNA viruses including CYSDV and potyviruses. SqVYV was only detected in the early survey

and in low incidences. Thus, only two of 16 samples with virus symptoms were positive for infection with SqVYV (Table 1). In the early survey, a total of 16 samples were collected in seven fields in the Imperial Valley, five of eight melon samples were positive for CYSDV and all samples were negative for SqVYV. Three of seven watermelon samples were positive for CYSDV and two for SqVYV; one of these was in mixed infection.

In the late survey, watermelon and melon samples with virus-like symptoms were collected in Fresno County. All samples were negative for SqVYV and CYSDV, but all samples (six melon and three watermelon plants) were positive for potyvirus infection. The species of potyvirus detected in these samples was *Watermelon mosaic virus* (WMV) based upon ~97% sequence identity.

These results suggested that incidence of SqVYV in California continues to be low, it is only present in Imperial Valley and was not spread to other counties.

Table 1. Detection of SqVYV, CYSDV and WMV in cucurbit samples with virus-like symptoms

Date	County	Host	Total	SqVYV	CYSDV	WMV	Neg
Jun/2017	Imperal Valley	Melon	8	0	5	0	3
Jun/2017	Imperal Valley	Watermelon	7	2*	3*	0	2
Jun/2017	Imperal Valley	Cucumber	1	0	0	0	1
Sep/2017	Fresno	Melon	6	0	0	6	0
Sep/2017	Fresno	Watermelon	3	0	0	3	0
Total			25	2 (8%)	8 (32%)	9 (36%)	6 (24%)

*one samples in mixed infection (SqVYV + CYSDV)

b. Complete the genetic sequence of a SqVYV isolate from California

The full-length sequence of a California isolate of SqVYV was amplified with High-Fidelity DNA Polymerase (Phusion). The amplified putative full-length SqVYV genome was cloned in to the plasmid pJL89 with the Gibson Assembly cloning strategy. A putative full-length clone of SqVYV was obtained,

and the complete nucleotide sequence of this isolate of SqVYV from California (SqVYV-CA) was determined. This isolate is composed of 9912 nucleotides (nt), and is similar in size of other isolates (e.g. SqVYV-Florida 9856 nt and SqVYV-Israel 9831 nt). The complete nucleotide sequence of SqVYV-CA was more similar to an isolate from Israel (~93%) than with the isolate from Florida (~81%).

Most interestingly, we identified a recombination event of ~1000 bp in the P1a, the first gene of SqVYV-CA. The identity of this fragment was substantially lower (45%) than this part of other SqVYV isolates, and was more closely related (70%) to a potyvirus species, *Papaya ringspot virus* (PRSV). Excluding the recombinant region, the nucleotide identity of SqVYV-CA was almost identical with the isolate of SqVYV from Israel, ~98%; whereas, the identity with SqVYV-Florida remained relatively low (~84%). These data suggested that SqVYV-CA may have been introduced into California from Israel. However, the source of potyvirus parent of the recombination region of SqVYV-CA is unknown. It could be from an unknown potyvirus species from Israel, California, or even somewhere else.

To gain further insight into the relationship of these SqVYV isolates, we generated phylogenetic trees with the complete sequence of the capsid protein (CP) and P1a genes. The phylogenetic analysis with the CP nucleotide sequence placed the SqVYV-CA and the SqVYV-Israel isolate on the same branch, whereas SqVYV-Florida isolates were placed on a different branch (Figure 1). These results confirmed that SqVYV-CA is more closely related to SqVYV-Israel than the SqVYV-Florida isolates. Not surprisingly, the phylogenetic tree with the nucleotide sequence of the P1a gene placed the SqVYV-CA in a different branch from the SqVYV isolates from Israel and Florida, and on the same branch with the potyvirus species, PRSV (Figure 2). These results indicated that a true recombination event of ~1000 nt had occurred between SqVYV-CA and a potyvirus (note that these viruses are transmitted by different vectors, so mixed infections were needed for this recombinant event to have occurred).

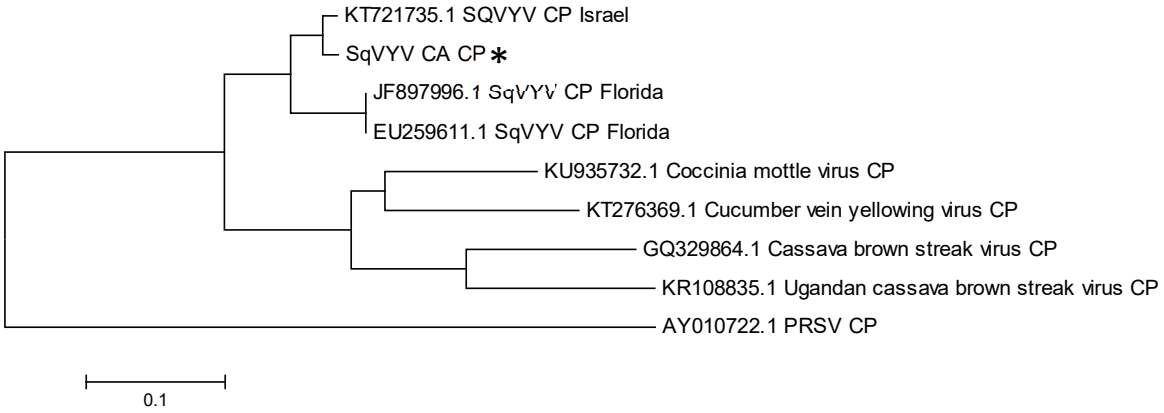

Figure 1. Phylogenetic tree showing the relationship of the complete nucleotide sequence of the capsid protein (CP) of the California SqVYV isolate (*) and nucleotide sequences from the CP genes of SqVYV isolates from Florida and Israel, other ipomoviruses and the potyvirus *Papaya ringspot virus*.

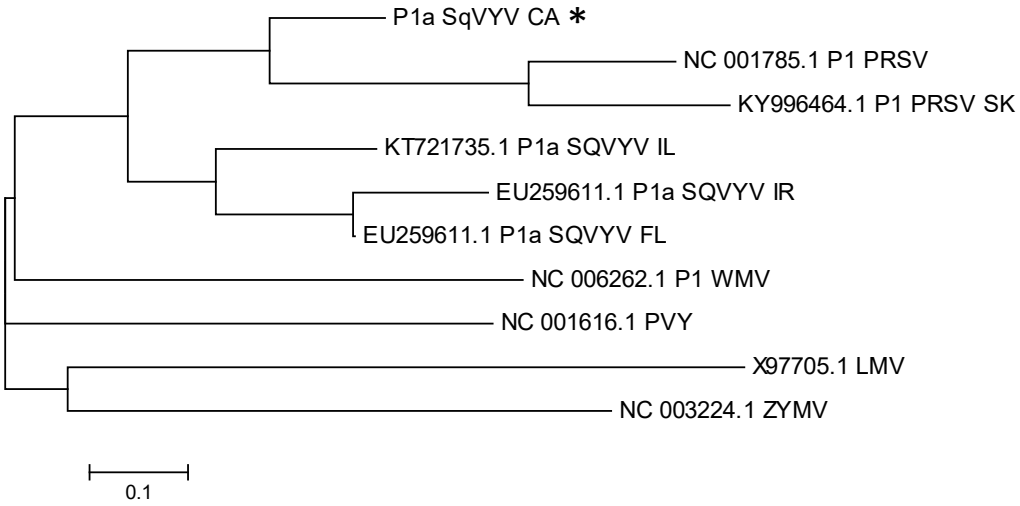

Figure 2. Phylogenetic tree showing the relationship of the complete nucleotide sequence of the P1a protein (P1a) of California SqVYV isolate (*) and nucleotide sequences from P1a genes of other SqVYV isolates and the P1 from potyviruses species.

c. Generate an infectious clone of a California isolate of SqVYV

A putative infectious clone of SqVYV-CA was obtained with the Gibson assembly strategy. The clone was then transformed into *A. tumefaciens,* and pumpkin plants cv. Sugar Pie were agroinoculated to test the infectivity of the clone. Symptoms of vein yellowing were observed ~21 days post inoculation (dpi) (Figure 3A), suggesting that the clone was infections. To confirm this, the total RNA from symptomatic plants was extracted and RT-PCR with specific primers for SqVYV CP were used. The expected size DNA fragment (1071 nt) was obtained, and DNA sequencing confirmed it was SqVYV. This established the infectivity of the SqVYV-CA clone.

The full-length sequence of SqVYV-CA was compared to Florida and Israel isolates and had higher sequence identity with SqVYV-Israel (93%) than SqVYV-Florida (81%). Taken together with the diversity among SqVYV isolates and the recombinant region in SqVYV-CA, it is important to determine the host range of SqVYV-CA. Thus, a partial host range was performed, in which SqVYV-CA was agroinoculated into two watermelon varieties (Chaleston Gray and Sugar Baby), pumpkin cv. Sugar Pie, squash cv. Heirloom, zucchini cv. Jade and cucumber cv. Poinsett 76. Most of the inoculated plants did not show clear symptoms at 21 dpi; however, pumpkin cv. Sugar Pie and zucchini cv. Jade showed clear vein yellowing symptoms, and zucchini cv. Jade also showed leaf deformation (Figure 3B). Symptomless infections in two watermelon cvs. Chaleston Gray and cv. Sugar Baby, squash cv. Heirloom and cucumber cv. Poinsett 76 were confirmed by RT-PCR with specific primers. We will inoculate pumpkin, squash, melon, watermelon and, cucumber varieties to finish the host range of the SqVYV-CA. Three independent experiments will be performed.

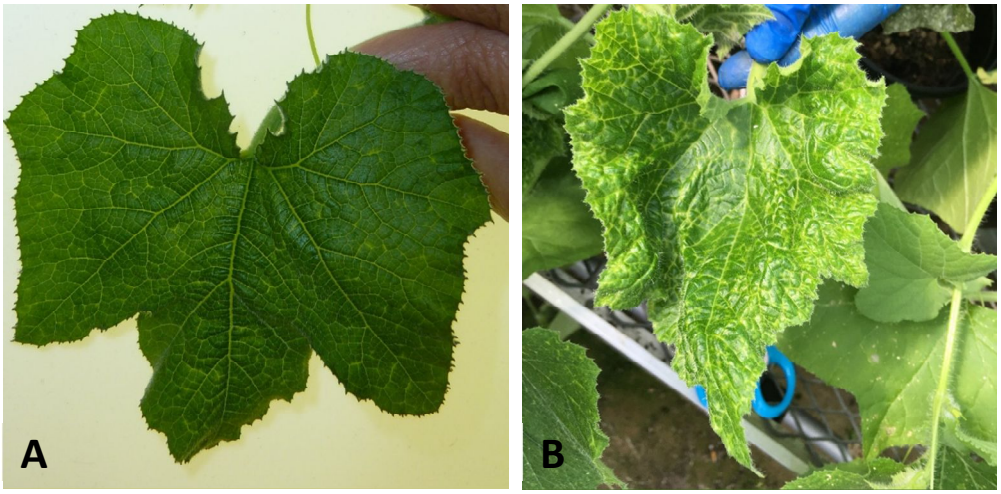

Figure 3. Symptoms induced by SqVYV at 21 days after agroinoculation. (A) Mild vein yellowing symptoms developed in pumpkin cv. Sugar Pie. (B) Strong symptoms of vein yellowing and leaf deformation in zucchini cv. Jade.

In conclusion, although SqVYV was still detected this year in the Imperial Valley, the incidence was very low. To date, this virus was not caused economic losses to cucurbits in California. The recombinant event detected in SqVYV-CA could be responsible for the low incidence of SqVYV in California, if it is less pathogenic than SqVYV-Florida and SqVYV-Israel. However, this hypothesis needs to be confirmed. In an attempt to understand what this recombination event means, in biological terms, we will generate a recombinant virus. We will exchange the P1a from SqVYV-CA with the P1a from Florida with the Gibson assembly strategy. We will test the infectivity of this recombinant virus and determine the host range of this recombinant. This will further reveal that relationship of the SqVYV-CA and the SqVYV from Florida and the pathogenicity of these viruses.