Final Report to the California Melon Research Board-2018

Project title: **Survey, characterization and biological properties of a California isolate of *Squash vein yellowing virus* (SqVYV) and associated cucurbit-infecting viruses**

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In 2014, the whitefly-transmitted potyvirus (ipomovirus) *Squash vein yellowing virus* (SqVYV), which causes the devastating watermelon vine decline disease, was detected associated with yellowing symptoms in pumpkins and melons in the Imperial Valley. This was the first report of this virus in California. In Florida, this virus is part of a complex of viruses infecting cucurbits and, importantly, it causes vine decline of watermelon, which causes substantial economic loss in this crop. More recently, SqVYV has been reported from Guatemala and Honduras, where it is causing economic losses on watermelon production. In addition. SqVYV has also been reported from Israel and Iran.

Therefore, because this is a virus with the potential to cause substantial economic losses in cucurbits, especially watermelon, we initiated this project to assess the potential for this new virus to impact production of melons and other cucurbits in California. The main goals of the project have been to: 1) characterization of SqVYV from California (SqVYV-CA), and 2) surveying of melon production in areas with *B. tabaci* to assess the relative incidence and severity of SqVYV-CA.

IV. Immediate objective

1. Conduct early and late season surveys of cucurbit plantings in the Imperial Valley/Blythe and other regions in California for SqVYV and other viruses in 2018

Based on information from growers and PCAs and our own field visits in Fresno County (Turini) the overall incidence of virus disease symptoms in melons was low in desert (southern) production areas as well as in the Central Valley. Therefore, formal virus surveys were not conducted in 2018. Even in commercial late-planted melon fields in Blythe and Yuma, whitefly populations remained (were kept) relatively low and virus incidence was low. Eventually, symptoms of infection by *Cucurbit yellow stunting disorder virus* (CYSDV) appeared in the late-planted melon fields in Blythe and Yuma (primarily at the edges of fields), but the economic loss was minimal.

In 2018, there were major outbreaks of *B. tabaci* whiteflies in late-planted melon fields in Fresno County. These outbreaks were characterized by very high adult whitefly populations (>50/leaf in some fields). Moreover, large numbers of nymphs and pupae were observed on many leaves indicating with was a reproducing population. These populations were far greater than the typical sporadic late season whitefly populations previously observed on melons, which tend to be substantially lower and show limited reproduction. Thus, the question arises as to whether this is an aberration, i.e., just a typical late season outbreak with unusually high populations, or does this reflect a change in the population.

Figure 1. Whitefly (*Bemisia tabaci*) damage in a late-planted melon field in Fresno Co. in 2018.

2. Perform host range assays with SqVYV-CA by agroinoculation

Here, we continued our host range studies of SqVYV-CA. One of the known biological properties of SqVYV isolates from Florida is that the virus is mechanically transmissible, i.e., by sap transmission. We previously showed that that SqVYV-CA is also mechanically transmissible. Thus, the goals of this objective were to 1) compared mechanical inoculation and agroinoculation for efficiency of infection and 2) determine the host range of SqVYV-CA in cucurbits and other plants.

In the first experiment, we compared agroinoculation and mechanical transmission. For mechanical transmission, we used symptomatic leaf tissues from watermelon plants previously infected after agroinoculation to make sap that was used in mechanical inoculations. As shown in Table 1/Experiment 1 and further discussed below, the SqVYV-CA agroinoculation method (done by stem puncture delivery) was very inefficient, even in susceptible squash plants, and failed to provide a reliable method for host range determination of SqVYV-CA as none of the inoculated plants developed symptoms and only two agroinoculated watermelon plants had symptomless infections as revealed by results of PCR tests for SqVYV-CA.

In contrast, much higher rates of infection and symptom development were observed in plants mechanically inoculated with sap prepared from SqVYV-CA-infected plants. These included pumpkin and squash plants, which developed vein clearing, mosaic and yellowing (Figure 2), and watermelon, which developed light green flaccid leaves and eventually collapsed and died (vine decline) (Figure 3). In terms of melon, SqVYV-CA did not infect or cause symptoms in cantaloupe and honey dew melon, indicating a high degree of resistance or even immunity. Mechanically inoculated cucumber plants also did not develop symptoms, but symptomless infections were detected in 3/8 plants. Thus, these results indicated that the current SqVYV-CA agroinoculation system (in its current form) is not suitable for high throughput screening. However, the mechanical inoculation method allows for high rates of infection and can be used on a high throughput level, e.g., screening breeding materials for resistance.

We then conducted two host range experiments (Exp 2- and Exp 3-SAP) with the same plants and SqVYV delivered by mechanical inoculation. The results were similar to those of Experiment 1, with SqVYV-CA infecting and causing vein clearing and yellowing in pumpkin and squash (Figure 2) and vine decline in watermelon (Figure 3) (Table 1). No symptoms were observed in melon (honey dew and cantaloupe), but symptomless infections of some cucumber plants was detected by the PCR test. Together, the host range results indicated that melons are highly resistant or even immune to SqVYV-CA. This likely explains why SqVYV has not caused losses to melon production in southern California. In contrast, SqVYV does cause watermelon vine decline, which could cause economic losses to watermelon production, such as occurs in Florida. Despite being first detected in California in 2014, watermelon vine decline has not been detected in California. This may relate to the lower and more widely distributed acreages, and early planting dates for watermelon crops in the Imperial Valley and other regions, thereby avoiding high whitefly populations necessary for extensive infection and development of vine decline. Furthermore, the host range of SqVYV-CA is similar to that of isolates in Florida,

suggesting that the recombination event in SqVYV-CA did not dramatically change the host range of the virus.

Figure 2. Disease symptoms in a squash plant (left) and leaves of squash (lower right) and pumpkin (upper right) plants with vein clearing and yellowing symptoms.

Figure 3. Symptoms of vine decline in watermelon plants inoculated with SqVYV by mechanical inoculation.

In conclusion, our results indicated that SqVYV-CA causes strong yellowing and mosaic in pumpkin and summer squash, vine decline in watermelon, symptomless infection in cucumber and no symptoms or infection in melons (one variety each of honeydew and cantaloupe). Based on these findings, SqVYV does not pose a threat to melon production, although it could cause losses to watermelon production. Mechanical inoculation was considerably more efficient than direct agroinoculation for plant inoculation with SqVYV. This suggests that, for resistance screening, susceptible plants can be agroinoculated to build-up inoculum, followed by mechanical inoculation for the screening of materials for resistance. The finding that the melons tested appear to be immune to SqVYV-CA may explain why this virus has not become economically important in southern California (unlike in Florida or Central America).

Table 1. Results of three experiments to evaluate the response of various plant species to SqVYV-CA delivered by mechanical inoculation (SAP, mechanical inoculation of sap prepared from infected leaves) or agroinoculation (Agro) (Experiment 1 [Exp 1]) or with sap inoculation only (Experiment 2 [Exp 2-SAP] and Experiment 3 [Exp 3-SAP].

	SAP		Agro	
Exp 1	**Symptoms (~21DAI)**	**PCR**	**Symptoms (~21DAI)**	**PCR**
Watermelon Sugar Baby	0/6	4/6	0/5	2/5
Watermelon Charleston Gray	3/5	4/5	0/5	0/5
Watermelon Crimson Sweet	3/5	4/5	0/6	0/6
Melon Minnesota	0/7	0/7	0/6	0/6
Zucchini Jade	xx	xx	xx	xx
Squash Summer	2/6	6/6	0/6	0/6
Cucumber	0/8	3/8	0/8	0/8
Squash Heirloom	2/6	6/6	0/6	0/6
Melon honeydew	0/6	0/6	0/6	0/6
Pumpkin Big Max	3/6	3/6	0/6	0/6
Pumpkin Sugar Pie	4/6	4/6	0/6	0/6
Tomato cv. Glamour	xx	xx	xx	xx

Exp 2-SAP	10 DAI	20 DAI	30 DAI
Watermelon Sugar Baby	0/6	4/6	4/6
Watermelon Charleston Gray	0/6	4/6	4/6
Watermelon Crimson Sweet	0/6	3/6	4/6
Melon Minnesota	0/6	0/6	0/6*
Zucchini Jade	4/6	6/6	6/6
Squash Summer	5/5	5/5	5/5
Cucumber	0/6	0/6	0/6
Squash Heirloom	4/6	4/6	4/6
Melon honeydew	0/6	0/6	0/6*
Pumpkin Big Max	0/5	3/5	3/5
Pumpkin Sugar Pie	5/7	5/7	5/7
Tomato cv. Glamour	0/6	0/6	0/6*

Exp 3-SAP	10 DAI	20 DAI	30 DAI
Watermelon Sugar Baby	0/6	4/6	4/6
Watermelon Charleston Gray	0/6	3/6	3/6
Watermelon Crimson Sweet	0/6	4/6	4/6
Melon Minnesota	0/6	0/6	0/6*
Zucchini Jade	4/5	5/5	5/5
Squash Summer	4/6	6/6	6/6
Cucumber	0/6	0/6	0/6*
Squash Heirloom	4/6	5/6	5/6
Melon honeydew	0/6	0/6	0/6*
Pumpkin Big Max	0/6	3/6	3/6
Pumpkin Sugar Pie	0/4	1/4	1/4
Tomato cv. Glamour	0/6	0/6	0/6*

*** PCR negative**
DAI=days after inoculation

Our results indicated that SqVYV-CA causes severe yellowing and mosaic in pumpkin and summer squash, vine decline in watermelon, symptomless infection in cucumber and no symptoms or infection in melons (one variety each of honeydew and muskmelon). Furthermore, mechanical inoculation was considerably more efficient than direct agroinoculation. This suggests that, for resistance screening, susceptible plants can be agroinoculated to build-up inoculum, followed by mechanical inoculation for the screening of materials for resistance.

3. Assessment of the importance of the recombination event in the biology of SqVYV-CA

The finding that the melons inoculated with SqVYV by agroinoculation or mechanical inoculation were highly resistant or immune to SqVYV-CA may explain why this virus has not become economically important in southern California (unlike in Florida or Central America). Furthermore, because melons are susceptible to SqVYV-FL (although symptoms are relatively mild) we wanted to determine if the recombinant part of SqVYV-CA is responsible for this difference in virulence in melons. Therefore, to investigate the role of the ~1000 nucleotide (nt) recombinant region in the SqVYV-CA genome (P1a gene), which appears to have been derived from a yet to be characterized member of the genus *Potyvirus*, we obtained a cloned fragment of a SqVYV-FL isolate that contains the equivalent SqVYV region from our colleague in Florida, Dr. Scott Adkins (Figure 4). This fragment was exchanged with the recombinant region in the infectious clone of SqVYV-CA, thereby restoring the complete SqVYV genomic sequence (Figure 4). This newly reconstituted SqVYV-CA genome (SqVYV-CA-R) with all SqVYV sequence was generated with the Gibson Assembly strategy and the integrity of the construct was confirmed by sequencing.

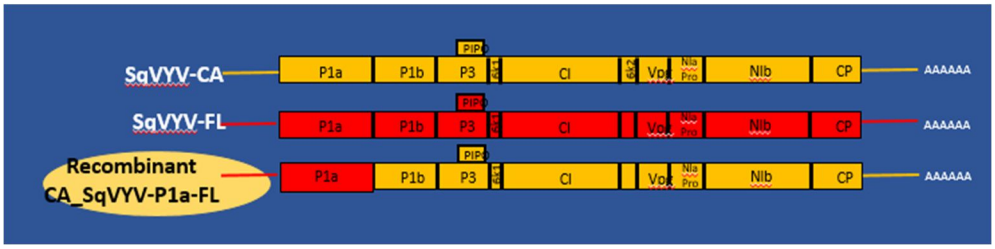

Figure 4. Genetic maps of SqVYV isolates from California (SqVYV-CA), Florida (SqVYV-FL) and the recombinant isolate formed by replacing the potyvirus-like P1 gene of SqVYV-CA with the equivalent SqVYV gene from SqVYV-FL).

To date our attempts to demonstrate the infectivity of the full-length clone of SqVYV-CA-R have been unsuccessful. This has been due to the low efficiency of the SqVYV-CA agroinoculation system. To determine if it is a function of the stem puncture method, we tested additional inoculation methods in which the *A. tumefaciens* cells or the purified binary plasmid DNA were rub-inoculated onto leaves of a susceptible squash and watermelon (Table 2 and 3). However, these plants did not develop symptoms typical of SqVYV and were negative for infection based upon PCR tests.

Table 2. Inoculation of California (CA) and Recombinant (REC) strains of *Squash vein yellowing virus* (SqVYV) in watermelon var. Sugar Baby.

Virus clones	Method of inoculation		
	Agroinoculation	**Rub-inoculation of agrobacterium cells[1]**	**Rub-inoculation of viral-DNA extracted from *E. coli***
SqVYV-CA	0/10	0/8	0/8
SqVYV-REC	0/10	0/8	0/8
Mock	0/1	0/1	0/1

[a]These *A. tumefaciens* cells (strain C58) were grown in two types of media: LB + Kanamycin (50 ug/ml) and LB + Kanamycin (50 ug/ml) + Tetracycline (2.5 ug/ml).

[b]Number of plants with symptom or PCR-positive for SqVYV infection

Table 3. Inoculation of California (CA) and recombinant (CA-R) strains of *Squash vein yellowing virus* (SqVYV) in pumpkin var. Big Max.

Virus clones	Method of inoculation[a]		
	Agro	**Rub-inoculation of agrobacterium cells**	**Rub-inoculation of plasmid-DNA extracted from *E. coli***
SqVYV-CA	0/10[b]	0/8	0/4
SqVYV-CA-R	0/10	0/8	0/4
Mock	0/1	0/1	0/1

[a]These *A. tumefaciens* cells (strain C58) were grown in two types of media: LB + Kanamycin (50 ug/ml) and LB + Kanamycin (50 ug/ml) + Tetracycline (2.5 ug/ml).

[b]Number of plants with symptom or PCR-positive

The presence of the plasmids (infectious clones) in Agrobacterium cells (C58 strain) were checked by mini-purification and restriction enzyme digestion. Furthermore, the presence of the correct plasmid in the agro strain was checked by using SqVYV CP primers from a DNA extraction of the plasmid as template. Interestingly, for the recombinant strain of SqVYV a reduction on the size of the CP gene was obtained. This indicates that a potential recombinantion from the large size of the plasmid is occurring in the Agro strain.

Conclusions. The incidence of virus diseases of melons was low in 2018. In southern production areas, this was due to below normal whitefly populations. However, unusually heavy whitefly outbreaks occurred in late-planted melon fields in Freson County. An agroinoculation system for SqVYV was not suitable for high throughput inoculation. Therefore, mechanical inoculation was used to conduct a host range study of SqVYV. In these experiments, SqVYV induced vein clearing and yellowing of pumpkin and squash, and vine decline in watermelon. No symptoms were induced in cucumber or melon, and symptomless infections were detected in some cucumber plants. Finally, the SqVYV-FL clone of the 5' end of the virus was obtained and exchanged with the potyvirus-like 5' end of SqVYV-CA. However, efforts to show the infectivity of this construct via agroinoculation have not been successful.