

**Progress Report to the National Watermelon Association  
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**Project title: Frequency and Distribution of Old and Possible New Viruses in Watermelon**

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**SUMMARY OF THIS PROJECT**

The purpose of this project was to determine the frequency and distribution of viruses infecting watermelon crops in various southern states. We achieved these goals during the last two years by analyzing more than 700 samples and determining the type and prevalence of viruses infecting watermelon across the 10 southern states. Among these viruses, the three Potyviruses (*Papaya ringspot virus-W*, *Watermelon mosaic virus* and *Zucchini yellow mosaic virus*) had the highest frequency in virus-infected watermelon crops. In addition, possible new viruses have been detected from watermelon, squash and/or weeds growing in watermelon fields. This study illustrates that various viruses do exist in the field and only need an insect vector and a conducive environment to create an epidemic infection.

**1. Characterization of unknown samples from 2010**

A total of 163 virus-like particle (VLP) preparations were made from samples (collected during 2010 and 2011) showing an array of different symptoms. All the VLP preparations were tested serologically using dot-immunobinding assay (DIBA) against the antisera of 10 known cucurbits viruses used before (see last year report). In addition, antisera of 5 more viruses were also used against the VLP preparations (Table 1). VLP were also tested by RT-PCR using primers against PRSV-W and WMV-2.

Table 1 Detection of VLP preparations extracted from samples in 2010 and 2011 by DIBA

States	No. VLP samples	AMV	BPMV	CABV	CMV	MNSV	PRSV	SLCV	SMV	SqMV	TRSV	WMV	ZYMV
Arkansas	12	0	0	0	1	0	3	0	0	0	1	9	3
Florida	1	0	0	0	0	0	0	0	0	0	0	0	0
Mississippi	4	0	0	0	0	0	0	0	0	0	0	0	0
Missouri	1	0	0	0	0	0	0	0	0	0	0	0	0
Oklahoma	81	8	5	0	8	4	20	0	2	4	5	4	11
Texas	64	0	6	1	2	0	10	4	0	0	5	14	11
Total	163	8	11	1	11	4	33	4	2	4	11	27	25
% infection		4.9	6.7	0.6	6.7	2.4	20.2	2.4	1.2	2.4	6.7	16.5	14.1

*Alfalfa mosaic virus (AMV), Bean pod mottle virus (BPMV), Cucurbit aphid born virus (CABV), Cucumber mosaic virus (CMV), Melon necrotic spot virus (MNSV), Papaya ringspot virus-W (PRSV-W), Squash leaf curl virus (SLCV), Soybean mosaic virus (SMV), Squash mosaic virus (SqMV), Tobacco ringspot virus (TRSV), Watermelon mosaic virus-2 (WMV-2), and Zucchini yellow mosaic virus (ZYMV).*

None of the samples were positive to: *Cucumber green mottle mosaic virus (CGMMV), Watermelon silver mottle virus (WSMoV), and Zucchini green mottle mosaic virus (ZGMMV)*

### Identification of potential new viruses from Florida, Oklahoma and Texas

The remaining DIBA negative VLP samples (or tissues from those samples) were used to extract RNA or dsRNA for random hexamer RT-PCR. Amplified PCR products were cleaned and cloned in pGEM-T-vector. At least 3-5 recombinant clones were sequenced from each sample. The sequences were compared against the sequences available in the GenBank database.

Sequences from a Florida sample (squash) had 73% identity to PRSV-W and lower than 70% similarities to WMV and SqVYV. The virus was mechanically transmittable to squash and showed similar symptoms to the one produced in original tissues. Virus was also detected in VLP sample and inoculated plants and was also negative by serology and RT-PCR to PRSV-W and WMV. This is a potential new potyvirus, and we are testing and evaluating the effect of this virus on watermelon since SqVYV was also originally isolated from squash.

Sequences from two other samples obtained from weeds in watermelon fields in Oklahoma showed some similarities to *Cestrum yellow leaf curling virus* and *Dahlia mosaic virus* indicating possible new virus sequences. Sequences from two other samples obtained from watermelon did not match anything in the database indicating viruses that have not previously been reported.

Ten samples from watermelon plants obtained in Texas were inoculated to *Nicotiana benthamiana* and squash; and three of those samples produced mosaic symptoms on both hosts. Sequences obtained from these samples as well as from other three samples did not match to any previously reported sequences in the GenBank database indicating that these are new viral sequences infecting watermelon in Texas. Further characterization will determine the biological and molecular properties of these new viruses. The remaining DIBA negative samples were again mechanically inoculated to squash seedlings but did not produce any symptoms indicating that these viruses are not mechanically transmissible.

### 2. Analysis of samples in 2011

During the 2011 growing season, a total of 81 leaf samples obtained from six different states (Table 2) were analyzed by DIBA for the presence of viruses listed in Table 2. The results showed that the three main Potyviruses were detected in these samples.

Table 2 Frequency of known viruses in watermelon and other cucurbits/weeds as illustrated by percent infection

State	Samples tested	CABYV	PRSV-W	SLCV	WMV	ZYMV
Alabama	13	15.3	23.0	30.7	46.1	23.07
Florida	9	0	88.8		77.7	0
Georgia	2	0	0	0	100	0
Louisiana	6	0	0	0	50	0
Missouri	1	0	0	0	0	0
Oklahoma						
Atoka	14	0	42.8	0	71.4	0
Blaine	22	0	27.2	0	0	0
Bixby	15	0	93.3	0	0	0
Total	81	2.4	49.3	4.9	34.56	3.7

None of the samples were positive to: CGMMV, MNSV, SqMV, TRSV, WSMoV and ZGMMV

**Detection of *Cucurbit yellow stunting disorder virus (CYSDV)* and *Squash vein yellowing virus (SqVYV)***

Total RNA of the negative samples by DIBA against the above viruses obtained in Texas and Oklahoma were tested by RT-PCR against CYSDV and SqVYV. PCR positive controls of the (CYSDV) and SqVYV were kindly supplied by Dr. Scott Adkins, Research Plant Pathologist, USDA ARS, Florida and Dr. Wintermantel, Research Plant Pathologist, USDA-ARS, California, respectively. None of the samples were positive for these two viruses.

**Detection of DNA viruses**

Total DNA was extracted from some DIBA negative samples and tested by PCR using degenerate primers of geminiviruses but none of the samples were positive.

**3. Preparing symptoms manual**

A number of symptoms on watermelon plants in various fields from different states are illustrated in the attached manual. These symptoms will assist growers to differentiate a disease caused by viruses, fungi, or bacteria, but it will not be able to identify a particular virus. The reason is that most viruses produce similar mosaic symptoms on watermelon either in individual or mixed infection and need further tests to confirm the identity of a particular virus. We are willing to test suspect samples free of charge if growers find virus-like symptoms and send samples to us for identification. It is important that you contact Dr. Ali by email or telephone for guidance on how to collect and ship the samples.

**4. Testing of greenhouse seedlings**

No seedlings were submitted to be tested for viruses.