

**Project 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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**SIGNIFICANCE OF THIS PROJECT**

Cucurbits, especially watermelon, are infected by numerous different viruses. Some of the known and recently identified viruses have been reported in various Southern states. However, little information is available about the large-scale frequency of these viruses that occur regularly in our crops. There is no information available as to the extent of unreported or new viruses affecting the US watermelon industry. Most of the work on watermelon viruses has been in response to outbreaks such as the *Squash vein yellowing virus* (SqVYV) that has been particularly damaging to watermelon crops in Florida. It is our goal to be ahead of these viruses and identify them before they reach epidemic levels that may threaten the crop. With such knowledge, it may be possible for research scientists to develop management strategies prior to severe economic impact.

**PROJECT OBJECTIVES**

1. Survey for old, new and unknown viruses in watermelon production fields in major Southern States (Texas, Georgia, South Carolina, Florida and Alabama)
2. Screening of greenhouse watermelon transplant if virus like symptoms exist.

**Collection of samples**

During the growing season of 2010 (June-October), a total of 654 symptomatic leaf samples (545 from watermelon, and 109 samples from other cucurbits nearby and weeds in watermelon fields) were collected from 9 different Southern States. Other cucurbits like squash, pumpkin, cucumber and melon are sometime grown nearby to watermelon fields. Any virus that infects other cucurbits could potentially infect watermelon. For example, SqVYV, the cause of watermelon vine decline was originally isolated from squash in Florida but have drastic effects on watermelon. Samples from Alabama, Georgia, Kentucky, Louisiana, Mississippi and Missouri were collected by our research collaborators and shipped overnight (FedEx) to the University of Tulsa. Samples from Arkansas, Oklahoma and Texas were collected by the PI and Drs. Bruton and Fish at USDA Lane, OK. Samples from Florida were received on Dec 01, 2010 and will be processed soon.

**Sample preparation and processing**

Tissues from each sample were used for dot-immunobinding assay (DIBA) and total nucleic acid extraction. Depending on the amount of tissue and the expression of unique symptoms, samples were also aliquoted for virus-like particles preparation, dsRNA extraction and DNA isolation. Available antisera of 10 known cucurbit viruses belong to 8 different genera in 7 different families (Table 1) were obtained commercially. PCR positive controls of the *Cucurbit yellow stunting disorder virus* (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.

Table 1 The following 10 different viruses were tested by DIBA using their polyclonal antibodies

Family	Genus	Virus	Acronym
<i>Bunyaviridae</i>	<i>Tospovirus</i>	<i>Watermelon silver mottle virus</i>	WSMoV*****
<i>Closteroviridae</i>	<i>Crinivirus</i>	<i>Cucurbit yellow stunting disorder virus</i>	CYSDV** <sup>A</sup>
<i>Comoviridae</i>	<i>Comovirus</i>	<i>Squash mosaic virus</i>	SqMV****
	<i>Nepovirus</i>	<i>Tobacco ringspot virus</i>	TRSV*****
<i>Geminiviridae</i>	<i>Begomovirus</i>	<i>Squash leaf curl virus</i>	SLCV**
<i>Luteoviridae</i>	<i>Polerovirus</i>	<i>Cucurbit aphid-borne yellows virus</i>	CABYV*
<i>Potyviridae</i>	<i>Potyvirus</i>	<i>Papaya ringspot virus</i>	PRSV*
		<i>Watermelon mosaic virus-2</i>	WMV-2*
		<i>Zucchini yellow mosaic virus</i>	ZYMV*
		<i>Squash vein yellowing virus</i>	SqVYV** <sup>A</sup>
<i>Togaviridae</i>	<i>Tobamovirus</i>	<i>Cucumber green mottle mosaic virus</i>	CGMMV***
<i>Tombusviridae</i>	<i>Carmovirus</i>	<i>Melon necrotic spot virus</i>	MNSV***

<sup>A</sup> Not tested by DIBA due to non-availability of antisera. However, PCR testing is in progress

\*Aphid transmitted \*\*White fly transmitted \*\*\*Seed/fungus transmitted \*\*\*\*Beetle transmitted \*\*\*\*\* Thrips transmitted  
 \*\*\*\*\*Nematode transmitted

### Symptom expression of samples

Most common symptoms observed on collected samples were mosaic, mottling, leaf rolling, interveinal chlorosis, and leaf curling. All samples were photographed individually to establish a record of various symptoms that may be helpful in future for preparing a symptom manual to assist growers and extension personnel in identifying virus disease in their field.

### Detection by DIBA

Sap extracted from all 654 samples was dotted on a nitrocellulose membrane and were replicated 10 times. All 10 membranes were tested individually against the antisera of 10 known cucurbit viruses. The results showed (Table 2) that 7 out of 10 known viruses were detected in these samples but none of the samples were positive to CABYV, CGMMV and SLCV. All seven viruses were detected in Texas and only six in Oklahoma. Out of 654 total samples, 31.2% tested positive for PRSV, followed by WMV (23.9%), ZYMV (13.6%), TRSV (6.7%), SqMV (2.9%), MNSV (2.0%) and WSMoV (0.6%). Mixed infection of the three potyviruses (PRSV, WMV and ZYMV) were commonly observed in some samples. Both MNSV and WSMoV DIBA positive samples are in the process for further confirmation by reverse transcription-polymerase chain reaction (RT-PCR) using virus specific primers. Similarly, samples obtained from Alabama, Florida, Louisiana and Missouri are also under process to be tested by RT-PCR. So far PCR results have shown that one samples each from Alabama and Louisiana was positive to WMV. These are the initial results and further work is continuing to identify potential new viruses by extracting nucleic acids (see below) from samples that were negative by DIBA.

Virus detection in commercial companies is routinely done by serology. If a virus is not reacting to available antisera, then it might go undetected which is the main disadvantage of serology. As shown in Table 2, more than 200 samples did not react to the antisera of 10 viruses used in this study.

### Detection by virus purification and electron microscopy

Samples showing different symptoms were selected from each location for partial virus purification and were considered as unique representative sample of that particular location. A total of 160 samples were partially

purified and some were already examined by electron microscopy and SDS-polyacrylamide gel electrophoresis (SDS-PAGE). So far, flexuous rod shaped and spherical particles have been observed. This work is still in progress.

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

State	County/ Region	Samples tested	MNSV	PRSV	SqMV	TRSV	WMV	WSMoV	ZYMV	Samples negative <sup>*</sup>
Alabama		13	NT	NT	NT	NT	NT	NT	NT	
Arkansas		55	0	34.5	0	1.8	54.5	0	12.7	17
Georgia		1	0	100	0	0	0	0	0	
Kentucky		1	0	0	0	0	100	0	0	
Louisiana		3	NT	NT	NT	NT	NT	NT	NT	
Mississippi		17	0	0	0	0	35.2	11.7	0	7
Missouri		1	NT	NT	NT	NT	NT	NT	NT	
Oklahoma	Atoka	49	0	44.8	4.0	2.0	10.2	2.0	12.2	21
	Blain	78	0	44.8	0	2.5	8.9	0	0	38
	Bixby	98	0	10.2	9.1	12.2	3.0	0	23.4	25
	Jefferson	24	0	66.6	12.5	16.6	0	0	16.6	6
Texas	Rio Grand Valley	174	4.6	49.4	2.8	5.1	24.7	0.5	9.1	63
	East	20	40.0	10	0	0	20	0	0	15
	West- Central	50	2.0	20	0	10	44	0	28	16
	North-Central	70	5.7	4.2	0	14.2	50	0	28.5	15
Total		654	2.0	31.2	2.9	6.7	23.9	0.6	13.6	34.0

• None of the samples were positive to CABYV , CGMMV , SLCV

\* Number of symptomatic samples negative by DIBA

NT Not tested by DIBA but testing by PCR is in progress

### Detection by reverse transcription-polymerase chain reaction (RT-PCR)

Total nucleic acid was extracted from all 654 samples which was a great achievement to do it in such a short time. Samples that are negative by DIBA against the antisera of above 10 viruses are in the process to be tested by RT-PCR using specific primers to CYSDV and SqVYV. Positive control for PCR is available for these two viruses. Samples that are negative by RT-PCR against these two viruses will be tested below by dsRNA for other potential viruses.

### Detection of unknown viruses by dsRNA

Currently there are more than 200 samples which include 109 from Texas that did not react to any viruses tested by DIBA (Table 2). These samples exhibited typical virus-like symptoms and may harbor potentially new or reemerging viruses. Therefore, we need to process the remaining tissues of those samples by dsRNA isolation. Purified dsRNA will be used in random RT-PCR and the amplified product will be cloned and sequenced. The sequence obtained will be blasted against the GenBank database. The resulting information will allow the identification of viruses that were not detected by the above techniques and may represent potentially new viruses that have not been reported previously.

### Detection of DNA viruses

Total DNA was also extracted from some samples and kept at -20°C. So far, 8 samples from Alabama and one sample from Missouri were tested by PCR using degenerate primers of geminiviruses but none were positive.