# Project proposal to the National Watermelon Association

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## Project title:

## Frequency and distribution of old and possible new viruses in watermelon

**Project Principal Investigator:**

Dr. Akhtar Ali
Assistant Professor of Plant Virology
Department of Biological Science
The University of Tulsa
Tulsa, Oklahoma 74104
Tel: 918-631-2018
Fax: 918-631-2762
Email: Akhtar-ali@utulsa.edu

**Research Collaborators:**

Dr. Benny D. Bruton (USDA-ARS) Lane, Oklahoma
Dr. David B. Langston (University of Georgia)
Dr. Pamela D. Roberts (University of Florida
Dr. Shouan Zhang (University of Florida)
Dr. Shaker Kousik (USDA-ARS), Charleston, South Carolina
Dr. Edward Sikora (University of Auburn), Alabama

## A. BACKGROUND AND SIGNIFICANE

Watermelon is a major cash crop of vegetable growers in the southern United States. In 2008, 3,955,100,000 pounds of watermelon valued in excess of $490 million were produced on 133,700 acres nationwide (NASS, 2008). Watermelon is attacked by a variety of plant pathogens. Plant viruses are widespread and are economically important plant pathogens that cause significant losses in yield and quality of cultivated plants worldwide including the U.S. More than 15 viruses have been reported to infect watermelon worldwide and majority of them are present in the U.S. Plant viruses are a constant threat to watermelon production through the U.S. Severe outbreaks of viral disease tend to occur on a sporadic basis and can result in significant losses to watermelon growers.

For the last three decades, several aphid transmitted potyviruses have been reported to infect watermelon in the U.S. For example, the most common viruses that infect watermelon are *Papaya ringspot virus* (PRSV), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV), *Squash mosaic virus* (SqMV), and *Cucumber mosaic virus* (CMV).

Recently, new viruses have been reported to infect watermelon and other cucurbits crops in the U.S. For example, watermelon vine decline (WVD) which is caused by *Squash vein yellowing virus* (SqVYV). WVD was first found in Florida in 2003 on squash. Later, it caused serious economic losses in watermelon industry. It is transmitted by whiteflies and has caused great losses for Florida's watermelon growers in 2005. The disease so far has been observed in Florida and Indiana. Two additional whitefly-transmitted viruses of cucurbit are (*Cucurbit leaf crumple virus* and *Cucurbit yellow stunting disorder virus*) which have been reported from Florida. Both are potential pathogens of watermelon.

We continue to find new viruses attacking watermelon on a regular basis. At the movement, the only way to detect watermelon viruses is through serology using antisera. Agdia (commercial company) has ELISA kits for 7-8 viruses when running a test sample for a watermelon virus. They do not have kits for recently identified new viruses of watermelon (mentioned above). Similarly, if there is an unknown virus present in the field, it would go undetected. At this stage we do not know how many new or unknown viruses are present in the US watermelons which can potentially impact growers in the future. A small amount of work has been done in isolated areas in some states but nothing comprehensive.

So far nothing is known about the occurrence of these new viruses or other unknown viruses in the major watermelon growing states. It is risky to wait for these viruses to cause devastating losses in watermelon crops before any work is done. Therefore, further research is needed to find the incidence and possible vectors of these viruses in watermelon crops before these viruses become established in a locality and reach epidemic conditions. At that time economic losses to watermelon growers and

industry may be greater and the disease will be more difficult to manage. This research can have a big impact on greenhouse operations as well. Almost yearly, some viruses are suspected of originating from the greenhouse production. Having the tools for quick and accurate identification are critical.

This project will lead to a better understanding of the emerging viruses and an enhanced ability to detect new virus diseases. Knowledge of the temporal distribution of plant viruses is necessary to develop effective management strategies against the disease. This information will be crucial for growers to be aware that the virus present in their fields is possibly waiting for favorable environment to develop at epidemic level. This will also lead to an understanding of the frequency of old, new and unknown viruses within a given area. There is a great potential for this project to lead to our long term goals to combat and reduce the effects of viruses on both the quality and yield of watermelon.

## B. OBJECTIVES OF THE PROJECT

The goal of this work is to gain a comprehensive understanding of the incidence and distribution of viruses affecting watermelons in the major Southern States (Texas, Georgia, South Carolina, Florida, and Alabama). This information is crucial in understanding the emergence of new viral diseases as well as the distribution of old virus diseases and their impact on watermelon production. With this information, we will ultimately be able to develop very precise methods for accurate detection.

**1. Survey of old, new and unknown viruses in watermelon field in major Southern States (Texas, Georgia, South Carolina, Florida and Alabama)**

**2. Screening of greenhouse watermelon transplant if virus-like symptoms exist**

## C. EXPERIMENTAL PROCEDURES

**Survey**

Symptomatic leaf samples will be collected from watermelon fields in all Southern States (mentioned above). We will conduct several surveys during the growing season targeting, Texas, while samples in Georgia, South Carolina, Florida, and Alabama will be collected by our collaborators (Research and Extension faculties) and sent to the University of Tulsa on ice overnight. Samples from other states will be accepted after adequate instruction on sample preparation and shipment are given.

**Detection of viruses in leaf samples by reverse transcription polymerase chain reaction (RT-PCR)**

Total nucleic acid will be extracted from leaf samples according to the Tri-reagent method used routinely in the PI lab. We will also use our previously developed PCR method against the above old viruses and use them to test all collected samples. For the

detection of newly identified viruses (mentioned above) including Watermelon Vine Decline (WMV) caused by SqVYV, we will design primers from published viral sequences and develop a PCR assay. For unknown viruses we will use double stranded RNA (dsRNA) method which is already established in the PI Lab. Analysis of the PCR products will be confirmed by agarose gel electrophoresis, cloned and sequenced. The sequence will be blasted against the GenBank data base to see that it match with the any previously reported virus or classified as a new virus.

**Detection of viruses in watermelon transplant**

In cases where a virus disease is suspected of originating from greenhouse transplant, we will assist in virus identification and its origin. We will also test seed sources for possible seed transmission of the virus depending on the availability of the seed lot. This information will be very helpful to predict the possible source of virus and its spread in the field.

## D. OUTCOMES FOR THE GROWERS

In order to manage a viral disease, it is important to correctly identify the disease and determine the origin, incidence and distribution of old (known) viruses as well as the new emerging viruses and potentially unknown viruses. If the growers have perceived a virus problem, we will accept samples from growers anywhere in the US to test for possible virus presence. The growers will need to contact us for sample preparation and shipment procedure. The procedures developed in this research will allow for quicker and more accurate identification of viruses than the present system. The information obtained in this research will be disseminated through various County Extensions, Watermelon Growers Meetings or regional and national meetings.

## E. ESTIMATED BUDGET

The following funds are requested for part-time stipend for a student, necessary supplies enzymes for PCR assays and travel cost for surveys.

| **Itemized budget** | **Estimated cost** |
|---|---|
| Personal- Part-time salary for student | $ 1,080 |
| Equipment | - |
| Supplies-disposable plastic ware | $ 2,000 |
| Molecular reagents-PCR (enzyme, primers, nucleotides etc) | $ 2,500 |
| Travel | $ 4,000 |
| **Total from NWA** | **$ 9, 580** |
| Tulsa University contribution (Indirect costs) | $ 556 |
| Total | $10,136 |