Multi-year Project Proposal to the National Watermelon Association

Final Report

Suppression of Fusarium Wilt of Watermelon by Mycoviruses

2025

Project Principal Investigator

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Research goals and objectives in 2024-2025

Over the past research period, substantial progress has been made in characterizing a naturally occurring mycovirus associated with reduced virulence in *Fusarium oxysporum* f. sp. *niveum* (FON), the causal agent of Fusarium wilt in watermelon. Work has focused on the follo0wig project objectives:

A. Collect and screen additional wild Fusarium isolates as well as known races of FON for mycoviruses.

We visited various watermelon fields in Oklahoma and collected tissues samples (root and stem) from infected watermelon that showed symptoms of wilting (Fig.1).

Fig.1 Infected watermelon in a grower field

All samples were brought to the laboratory and processed further for fungal isolation using the following steps (Fig. 2).

1. Surface sterilization of the tissues to avoid the growth of saprophytic fungi.
2. Cut the tissues into small pieces.
3. Place a piece of the tissues on the media in a petri dish.
4. Observe the growth of fungi.
5. Co-culture to obtain pure culture and avoid any contamination of mixed fungi.

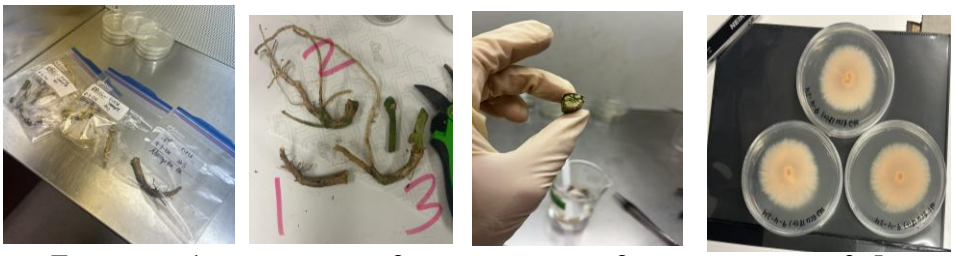


Fig.2 Steps for isolating fungi from field samples.

6. Pure culture was used for dsRNA extraction to determine the presence of a mycovirus (Fig. 3).

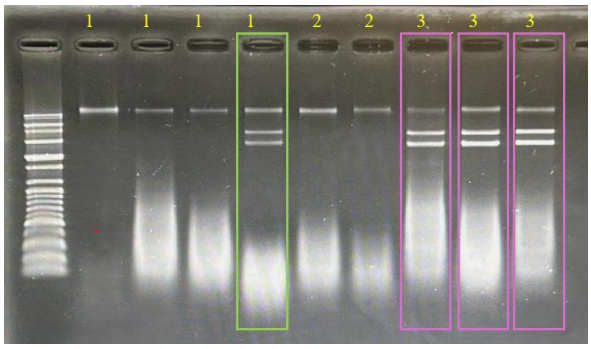


Fig. 3. Analysis of fungal dsRNA. The green panel (plant #1) and the pink panels (plant #3) each display two bands, indicating the presence of mycoviruses.

7. The dsRNA obtained from plants 1 and 3 was sequenced, and virus annotation is currently in progress. Initial findings suggest the presence of a potentially new virus.
8. Further confirmation through RT-PCR is planned. We have collected multiple *Fusarium* isolates from field-grown watermelon plants and processed them in the laboratory. To date, we have identified a new mycovirus associated with these isolates and are conducting additional characterization studies.

9. In the coming future, we will use these mycoviruses in pathogenicity assays on watermelon seedlings.

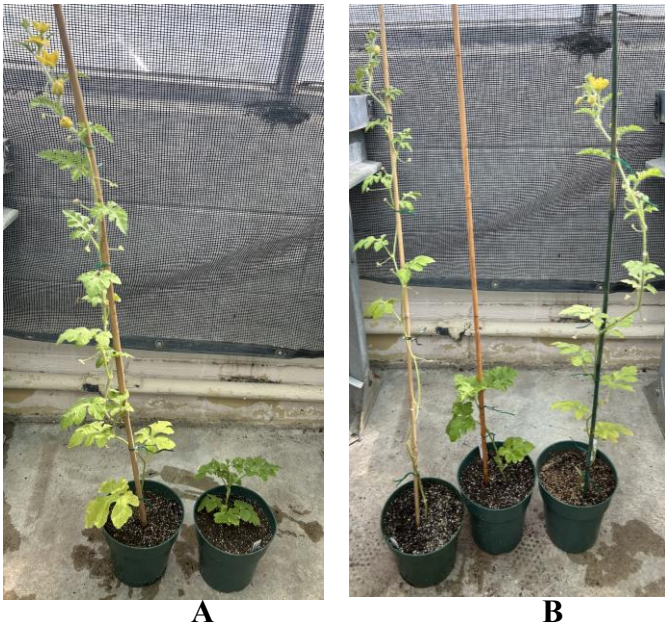
B. Characterize the collected mycoviruses.

We successfully isolated dsRNA from cultures of the available FON isolates (labelled as FON1 and FON2) in the lab that harbors mycoviruses. We have completed the molecular characterization of these mycoviruses, both of which belong to the newly proposed *Alternaviridae* family. Members of this family, identified in other fungal hosts, have been associated with hypovirulence in their respective fungi.

C. Compare the pathogenicity of FON1 isolate with and without mycovirus on watermelon cultivars and determine the effects on virulence of Fusarium isolates.

We performed pathogenicity assays on the Sugar Baby watermelon cultivar using a FON1 isolate harboring a mycovirus and a virus-free FON1 isolate. Two-week-old watermelon seedlings were inoculated in pots with mycelial plugs of the respective isolates.

The results were promising (Fig. 4). The presence of the mycovirus appeared to confer hypovirulence to the fungus. Seedlings inoculated with the virus-free FON isolate developed typical Fusarium wilt symptoms within 10 days, whereas those inoculated with the mycovirus-infected FON1 isolate remained symptom-free.



A **B**

2-weeks post-inoculation 4-week post-inoculation

Fig. 4 Comparison of pathogenicity test of FON1 isolate with and without mycovirus on watermelon seedling (*Sugar baby*) 4 weeks post-inoculation.

(A) Plant 1 (left) was inoculated with the FON1 isolate containing a mycovirus, whereas Plant 2 (right) was inoculated with a mycovirus-free FON isolate.

(B) Plant 1 (left) was inoculated with the FON1 isolate containing a mycovirus. Plant 2 (center) was inoculated with a mycovirus-free FON isolate. Plant 3 (right) served as healthy control and was inoculated with buffer only, without fungus.

Recovery of fungi from inoculated watermelon plants in Fig.1

After the experiment was completed. All the plants were uprooted, and roots were washed gently.

The following steps were performed for each plant tissues.

a. Surface sterilization of the tissues to avoid the growth of saprophytic fungi.
b. Cut the tissues into small pieces.
c. Place a piece of the tissues on the media in a petri dish.
d. Observe the growth of fungi.
e. Co-culture to obtain pure culture and avoid any contamination of mixed fungi.

FON isolates were recovered successfully from the roots of watermelon seedlings and were sued below for dsRNA confirmation.

Confirmation of mycovirus

Mycelia from both FON isolates, one harboring a mycovirus and one free of mycoviruses were analyzed using the dsRNA extraction procedure. The results confirmed the presence of the same mycovirus in the FON1 isolate, while no mycovirus was detected in the virus-free isolate. These findings further demonstrate that the hypovirulence observed in the inoculated watermelon seedlings was due to the presence of the mycovirus in the FON1 isolate.

Addendum to proposal

Most people think of viruses as harmful, but some viruses that infect fungi, called **mycoviruses**, can actually be helpful. These viruses do **not** infect plants. Instead, they weaken certain plant-disease-causing fungi.

In this project, we are studying mycoviruses that infect **Fusarium oxysporum f. sp. niveum (FON)**, the fungus that causes **Fusarium wilt in watermelon**. Some mycoviruses can reduce the fungus' ability to cause disease, a condition called **hypovirulence**. When the fungus carries these "good" viruses, it becomes much less aggressive.

Our goal is to identify FON isolates that naturally carry beneficial mycoviruses and are less harmful to watermelon. These weakened fungal isolates could eventually be used as a **biological control**, like how beneficial microbes are used in other crops. This approach could reduce the need for expensive and potentially harmful fumigants and fungicides. In addition, this project could lead to a safe, cost-effective, and environmentally friendly method to manage Fusarium wilt in watermelon by using the fungus's own viruses against it.

In short: **not all viruses are bad; some fungal viruses may become valuable tools to help growers control Fusarium wilt.**