Universal detection and identification of soybean-associated viruses, 61-2024
Annual Report

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Background and Objectives:

Several new and/or emerging viruses have been recently reported in different crops in the USA (stone fruits, grapevines, cotton, etc.) because of introduction (importation) of exotic viruses, or "spill-over" from non-cultivated plants to agricultural fields. These new emerging viruses represent significant threat for agriculture and require specific control measures as they are spread by insect vectors.

Unlike fungal pathogens or insects, there is no chemical control against viruses. Accordingly, efficient control strategies in case of viruses rely on prevention and/or an early detection/identification. Delayed awareness about virus infections often results in serious economic damages (yield losses) and high costs associated with their managing.

During each of the past several years our lab received several symptomatic soybean samples from MSU extension experts or consultants/farmers which resulted negative when tested with traditional methods for common soybean viruses, therefore suggesting that unexpected (new) viruses may be present in those plants. Therefore, we propose to carry out surveillance activities aimed at: i) an early identification of any viruses (known and unknown) associated with soybean production by combining and applying highly sensitive and unbiased new technologies and approaches; ii) design and development of virus-specific diagnostics for all viruses identified in this work.

This project has a unique goal:

- To execute "cutting-edge" surveillance activities aimed at early and unbiased discovery of all viruses.

Report of Progress/Activity

Research activities on this project in 2024 focused on several topics to achieve a major goal of the project: unbiased discovery and characterization of viruses associated with soybean production in Mississippi. During the project we combined field survey and collection of the samples (targeted sampling), greenhouse-based studies, electron microscopy observations and molecular and biocomputational approaches to get a wholistic view on "virome" (community of all viruses in certain ecosystem) present in production and experimental soybean fields in Mississippi.

Because of space limits for the report, here we will list and comment **only on a subset of selected data** generated during the year 2024. Accordingly, we identified different virus-associated symptoms in surveyed fields, ranging from general stunting, leaf deformation, to vein necrosis, mosaics/mottle and chlorosis. In some cases, pod setting was compromised as well (see Annex: Figure 1). Those plants were processed in the lab by total RNA extraction. The quantity/quality of RNAs were ascertained by 1.5% tris-acetate-EDTA agarose gel electrophoreses (see Annex: Figure 2) and by spectrophotometric reading with Qubit instruments. Samples deemed of suitable quality/quantity were chosen for cDNA library preparation and custom based high-throughput sequencing at the sequencing facility of the University of Illinois at Urbana-Champaign. Quality control of > 7 billion of 150nt long raw sequences showed

excellent results (see Annex: Figure 3) and we proceeded with downstream biocomputational assembly and analyses of sequencing data.

As in previous year, some of symptomatic samples were infected by "expected" viruses such as bean pod mottle virus (BPMV), soybean vein necrosis virus (SVNV) or soybean mosaic virus (SMV). For these viruses we sequenced multiple isolates allowing us to better understand population structure and intraspecies genetic diversity (example for SVNV illustrated in the Annex: Figure 4). That prompted us to (re)design new/improved primer sets for their molecular detection based upon data generated in this project.

Indeed, we completely sequenced a total of 8 isolates of SVNV (Annex: Figure 5A) which represents 40% of all data generated in the U.S to date. Furthermore, we carried out an original electron microscope-based study on visualization of virus particles and their interaction with organelles within the cell of an infected soybean sample (Annex: Figure 5B). Similar analyses were performed for 4 isolates of bean yellow mosaic virus (BYMV) showing that isolates from MS belong to different clades of the phylogenetic tree (see Annex: Figure 6) indicating differences in the genetic makeup – which may result in differences in pathogenicity of distinct isolates.

We also found a second isolate of soybean ilarvirus 1 in Mississippi, a virus originally reported in 2023 from Iowa and found in Mississippi too (Annex: Figure 7). Discovery of this virus in both years of this project, along with the original report from Iowa, suggest a possible established foci of the virus in larger area of soybean production.

Finally, we discovered a subset of unexpected/novel viruses that need further studies. Here we limit the report **only to two** viruses. One of them is soybean thrips-associated tospovirus 1 (STaTV1), a virus originally reported from soybean thrips (*Neohyatothrips variabilis*) but never from soybean plants (Thekke-Veetil et al, 2020). Tospoviruses are important plant pathogenic viruses and include tomato spotted wilt virus (TSWV) which alone can cause billions in economic damages on different crops worldwide. A representative of tospoviruses is also soybean vein necrosis virus. These viruses are transmitted in nature by thrips. Our data "close the full circle" and prove that this virus is indeed capable of infecting soybean plants and very likely transmitted with soybean thrips.

The second virus has one of the largest RNA genomes (>20,000 nt) and contains four major ORFs (genes). This virus appears related to some of known insect-infecting viruses belonging to the family *Mesoniviridae* (Annex: Figure 9). However, the virus discovered in this study represent a new species in the family (and possibly belonging to a new genus in this family). The importance of this virus is yet to be fully understood.

In addition to molecular results in part presented here, we continued biological studies on some of these viruses.

Impacts and Benefits to Mississippi Soybean Producers

Primary benefit of the project is factual, scientifically based, and comprehensive knowledge about viruses associated with soybean production in Mississippi. Research activities within this project resulted in discovery of viruses not previously known to occur in the state. In addition, in this project first genome sequences for local isolates of known viruses (i.e. bean pod mottle virus, soybean mosaic virus, soybean vein necrosis virus) were generated. This will allow comparison with those reported from other soybean-producing areas in the U.S. and worldwide. Furthermore, we designed specific diagnostic methods for their early detection. Finally, in this project we identified several new viruses associated with soybean production, either directly from plant tissue or from insect pests. Therefore, our results represent a comprehensive survey and an early warning about possible future virus problems for soybean producers.

End products – Completed and Forthcoming

Project has already produced one peer-reviewed publication in 2023, while another is submitted to the journal and is currently under peer review. In addition, an abstract has been published (work presented at the "Plant Health 2024" - 2024 Annual Meeting of the American Phytopathological Society held in Memphis, TN) and second one has been written and submitted for presentation at the "Plant Health 2025" – the Annual Meeting of the American Phytopathological Society (APS) to be held in Honolulu, HI.

Submitted paper

Aboughanem-Sabanadzovic N, Stephenson RC, Allen T, Sabanadzovic S, 2025. *Biological, ultrastructural and molecular characterization of soybean vein necrosis virus in Mississippi*. Journal of Plant Pathology (in review)

Manuscript under drafting

Aboughanem-Sabanadzovic N, Allen T, Sabanadzovic S, 2025. *Soybean-associated virome in Mississippi*. Viruses (submission planned for September 2025)

Published Conference Abstracts

Aboughanem-Sabanadzovic N, Stephenson RC, Allen T, Henn A, Sabanadzovic S, 2024. Characterization of a new potyvirus from kudzu. (Abstr.) Phytopathology 114: S1.80 https://doi.org/10.1094/PHYTO-114-11-S1.1

Submitted Conference Abstracts

Sabanadzovic S, Aboughanem-Sabanadzovic N, TW Allen, 2025. *Viruses associated with soybean production in Mississippi*. Annual Meeting of the APS "Plant Health 2025", August 2-5 Honolulu, HI

Conference presentations with no published abstract

Aboughanem-Sabanadzovic N, Stephenson RC, Allen T, Henn A, Sabanadzovic S, 2024. *Isolation and characterization of a novel potyvirus infecting kudzu in Mississippi*. 13th Annual Meeting of the Mississippi Association of Entomologists, Nematologists and Plant Pathologists, October 28-29, 2024 MAFES

Annex: Figures

Figure 1. Examples of symptomatic plants (vein necrosis, severe foliar deformations and chlorotic mosaic) collected during surveys of soybean production and experimental fields in Mississippi**.**

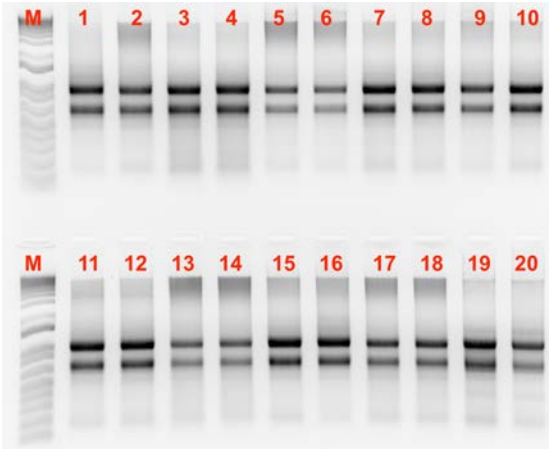

Figure 2. A representative agarose gel containing total RNAs extracted from 20 soybean samples and sent for high throughput sequencing along with other samples collected during 2024. M: DNA ladder (marker).

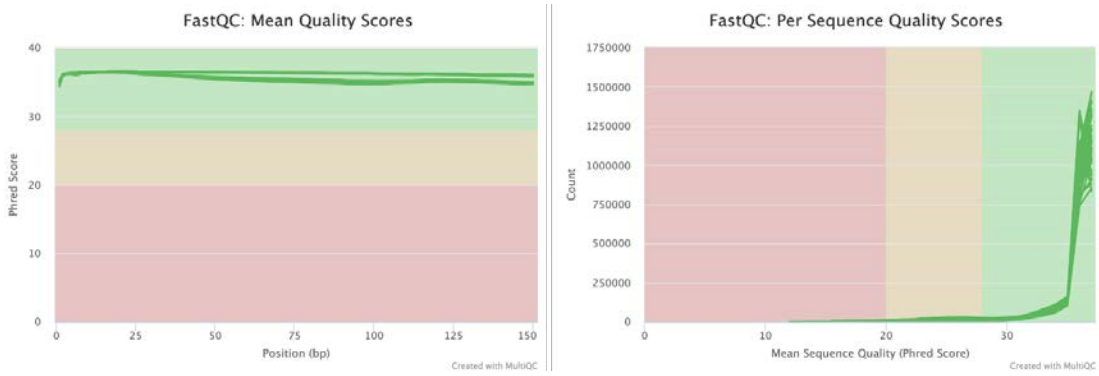

Figure 3. Representative results of raw sequence data quality control (mean quality score & per sequence quality score) prior to biocomputational analyses and search for virus genome data. Green fields indicate excellent quality of high-throughput sequence data.

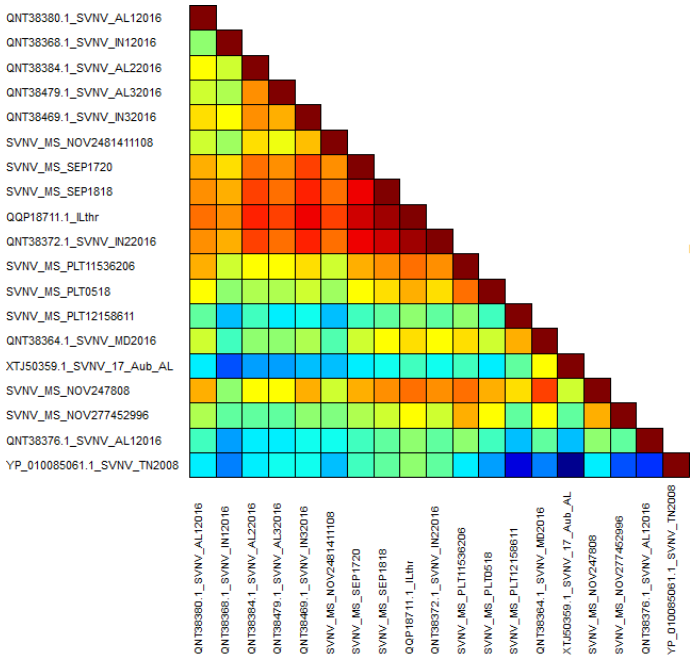

Figure 4. Pair-wise genomic identity levels among different isolates of soybean vein necrosis virus (SVNV). The same analyses were performed for bean pod mottle virus (BPMV) as well as for soybean mosaic virus (SMV).

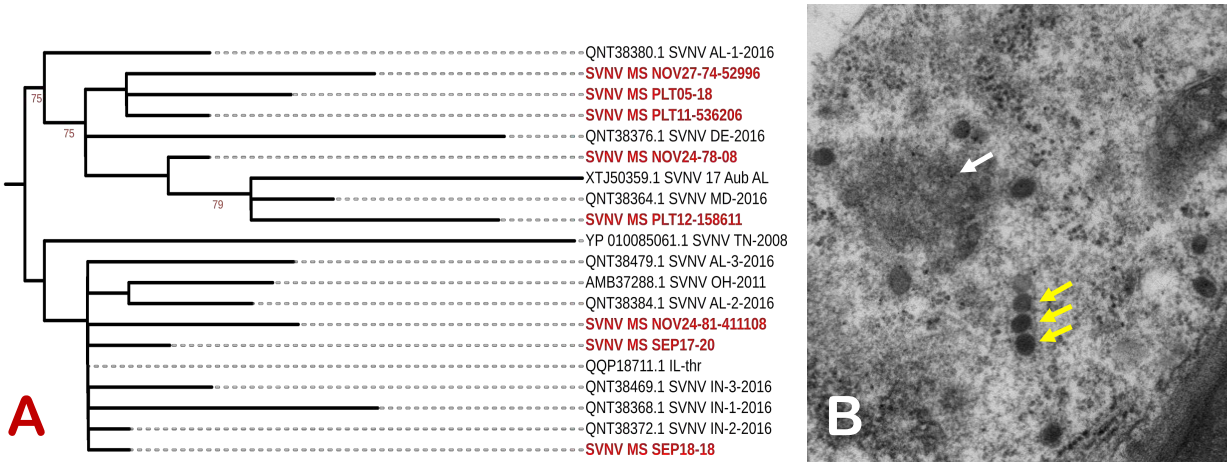

Figure 5. A. Phylogenetic tree showing relationships of all sequenced soybean vein necrosis virus (SVNV) isolates. Isolates reported in red font are those characterized in this study. **B.** Transmission electron microscope observation of a "viroplam" (darker area in the cell cytoplasm indicated by a white arrowhead) and mature virus particles (yellow arrowhead) in the ultrathin sections of SVNV-infected soybean plant cells.

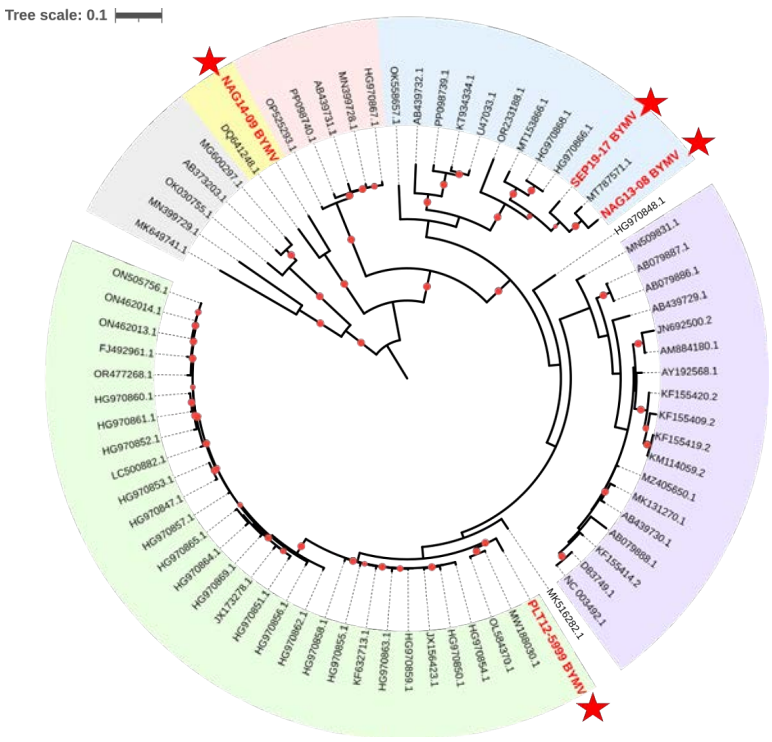

Figure 6. Phylogenetic tree showing relationships of four isolates of bean yellow mosaic virus (BYMV) from MS with isolates characterized from different crops worldwide. The four BYMV isolates from MS are indicated in red font (and star). Note that the four MS isolates belong to different subgroups (as indicated by difference in colors), indicating possible differences in biological traits (i.e. pathogenicity, host range, etc)

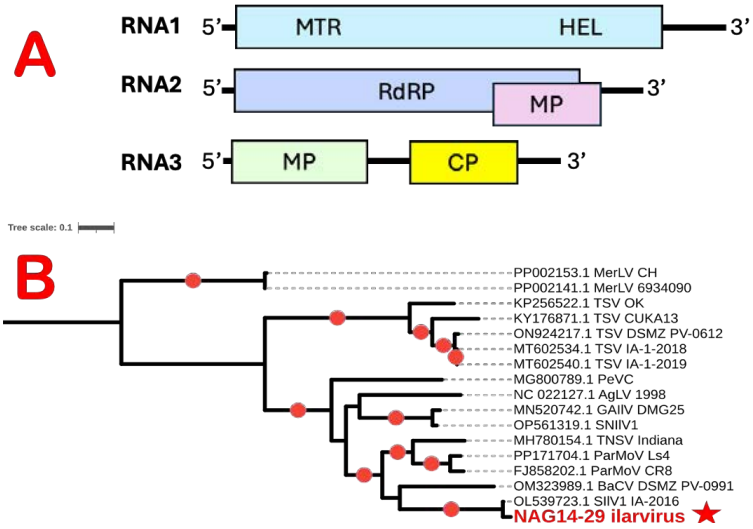

Figure 7. A. Genomic organization of soybean ilarvirus 1 genome. The genome is composed of three RNA segments. Functional genes are represented as differently colored boxes. **B.** Phylogenetic tree showing relationships of isolates from MS and Iowa with recognized members of the genus *Ilarvirus* (family *Bromoviridae*). Isolate from MS is indicated in red font (and star).

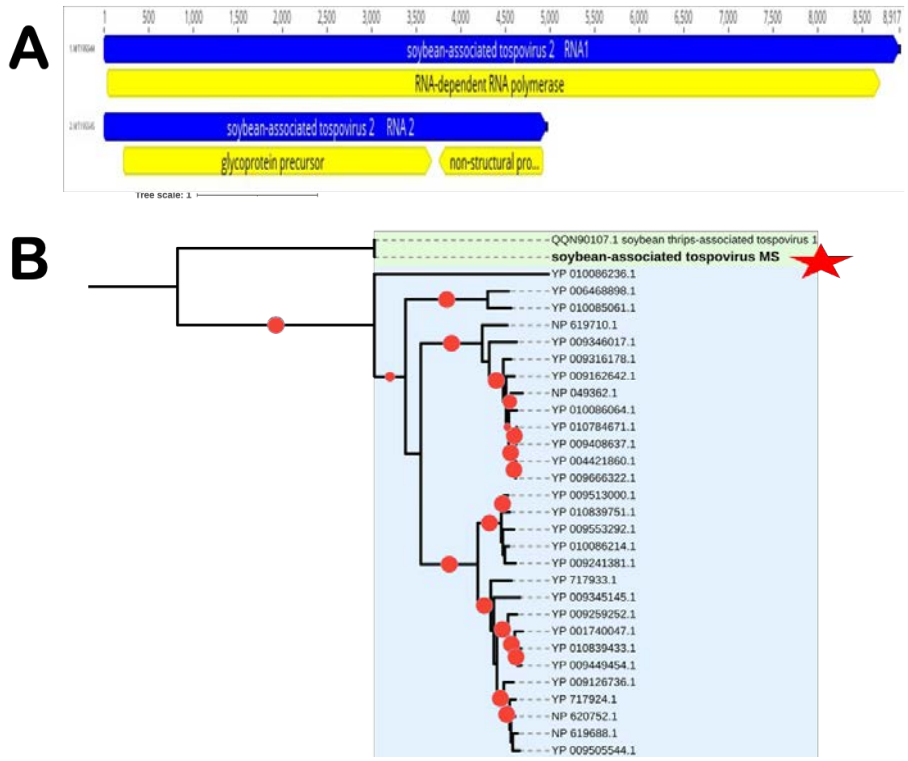

Figure 8. A. Genomic organization of soybean thrips-associated tospovirus 1 genome. The genome is composed of two RNA segments of negative orientation. Functional genes are represented as yellow-colored boxes. **B.** Phylogenetic tree showing relationships of isolates reported from soybean in MS (indicated by a star) and the one from thrips (green-shaded clade) with a plant-infecting tospoviruses (a clade shaded blue).

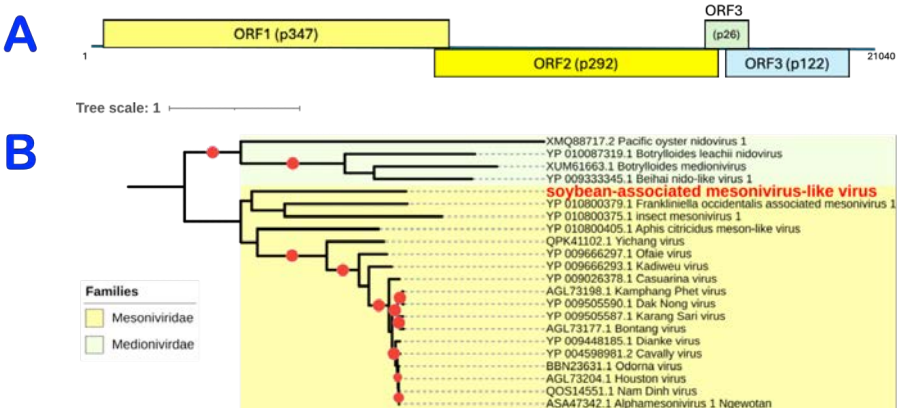

Figure 9. A. Genomic organization of soybean-associated mesonivirus-like genome of 21,040 nt. Functional genes are represented as differently colored boxes. **B.** Phylogenetic tree showing relationships of soybean-associated mesonivirus-like virus (red font) with recognized members of the *Mesoniviridae* family. Distance from the known mesoniviruses suggest that the virus may represent a new genus in the family.