Universal detection and identification of soybean-associated viruses, 61-2023 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

During the Year 1 of the project, we have focused on two major studies to understand diversity of viruses. First study focused on completing research on a virus discovered in a kudzu patch in Ackerman, MS (Figure 1) and investigating if the virus possibly could infect soybeans, taking in consideration that kudzu is a widespread invasive plant in the southern US and that the two plants (soybean and kudzu) are botanically related.

As a result, we thoroughly characterized the virus by mechanical and vector transmission, ultrastructural observation under transmission electron microscope, Sanger and High-Throughput genome sequencing and sequence analyses. We also conducted a study of the virus incidence and distribution in MS. The results revealed presence of a new virus in infected kudzu, closely related to wisteria vein mosaic virus (WVMV) and provisionally named kudzu chlorotic ring blotch virus (KuCRBV). KudCRBV genome features and detailed comparisons with three WVMV genomes currently available in the GenBank and three additional isolates sequenced in this work suggest that the virus may be a member of a new species in the genus *Potyvirus* (Figure 2). This genus already includes an important virus of soybean – soybean mosaic virus (SMV). Importantly, we proved that, under experimental conditions, KuCRBV can be transmitted from kudzu to kudzu, or from kudzu to soybeans with two species of aphids used in the

experiments (the cotton aphid, *Aphis gossypii* and the potato aphid *Macrosiphum euphorbiae*). and infected soybean and beans upon mechanical inoculation (Figure 3). The state-wide survey revealed several kudzu patches infected by the virus in northern MS (Figure 3).

For a second study, we executed several field trips during the 2023 production season to collect soybean samples of interest – those displaying virus-like symptoms (a couple of examples presented in Figure 4) . In addition, other samples were generously provided by Dr Tom Allen, collaborator on this project. After their transportation on ice, samples were labeled, aliquoted and preserved at -80C until processing. Total RNAs were extracted from each collected sample and their quality/quantity was assessed in agarose gel electrophoresis and reading at Qubit instrument. Good quality preparations were submitted to custom-based 150nt pair-end High-Throughput Sequencing in mid-October and early February. More than 5.3 billion reads (2.5 in October + 2.8 in February) were quality checked resulting in high-quality reads (Figure 5). These raw data composed of short reads were further processed by specific computer programs to assemble them into larger contiguous sequences ("contigs"), averaging 220,000 -320,000 contigs per sample. Each of these contigs were then compared with nucleotide sequence data available in GenBank and other public databases to identify those of viral origin.

As a result, we identified a dozen of viral genomes present in the samples collected in 2023. Some viruses were present in multiple samples therefore allowing to study their molecular diversity in Mississippi. The project has allowed to generate complete genome sequences of isolates from Mississippi for the very first time. This will make possible their comparison with genetic data of isolates of the same viruses reported from the US and/or worldwide and to understand/hypothesize about their origins. Curiously, besides viruses that were expected to find (bean pod mottle virus, soybean vein necrosis virus, soybean mottle viruses) this study revealed the presence of several other viruses. One of these is a soybean ilarvirus 1, reported in a 2023 published study from Iowa. Our data represent first report of this virus outside of Iowa and hint at possibly wider geographic distribution of this virus. We have examined/analyzed/annotated each of these viruses in a great detail and currently preparing a second manuscript reporting the diversity of viruses found in this study. An example is presented in Figure 6 showing genomic organization and the relationships of local peanut stunt virus (PSV) isolate infecting soybean sample collected in this study with other isolates reported worldwide and with members of the genus *Cucumovirus* where PSV belongs taxonomically.

Impacts and Benefits to Mississippi Soybean Producers

Primary benefit of the project is scientifically based knowledge about viruses associated with soybean production in Mississippi. This resulted in discovery of viruses not previously known to occur in the state. In addition, we generated first genome sequences for local isolates of known viruses which will allow comparison with those reported from other soybean-producing areas in the U.S. and worldwide, to design specific diagnostic methods for their early detection. Furthermore, in this study we identified a new virus that is relatively widespread in the kudzu patches in the norther part of Mississippi. Of particular significance is the fact that soybean, one of the major agricultural commodities in the state, was determined to be susceptible to KudCRBV infection under experimental conditions (transmission by the two aphid species and mechanical transmission). Our data were corroborated by a recent publication by the scientists from South Korea, who found natural infections of soybeans by the same virus in samples collected and tested in their study. Therefore, besides generating first data on its presence on the American continent, we set up an early warning about possible future virus problems for soybean producers.

End products – Completed and Forthcoming

Project has already produced one peer-reviewed publication, while the other is currently in preparation. In addition, an abstract has been prepared and submitted for presentation at the "Plant Health 2024" – the Annual Meeting of the American Phytopathological Society (APS)

Published paper

Aboughanem-Sabanadzovic N, Stephenson RC, Allen T, Henn A, Moore WF, Lawrence A, Sabanadzovic S, 2023. Characterization of a new member of the genus *Potyvirus* from kudzu (*Pueraria montana* var. lobata) in Mississippi. Viruses 15(11), 2145. https://doi.org/10.3390/v15112145

Manuscript under drafting

Aboughanem-Sabanadzovic N, Allen T, Sabanadzovic S, 2024. Soybean-associated virome in Mississippi. Viruses (submission planned for June 2024)

Abstract submitted for Plant Health 2024 Conference

Aboughanem-Sabanadzovic N, Stephenson RC, Allen T, Henn A, Sabanadzovic S, 2024. Characterization of a new potyvirus from kudzu. Plant Health 2024 to be held on July 27-30, 2024, Memphis, TN.

ADDENDUM - FIGURES

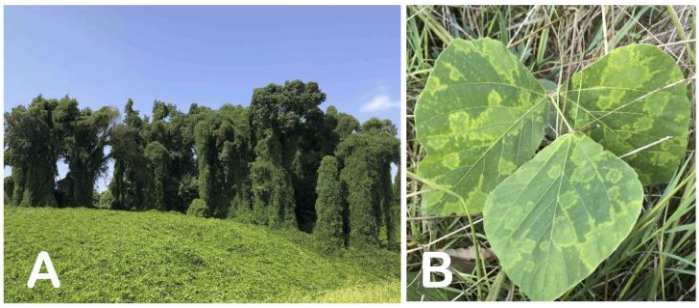

Figure 1. A: A kudzu patch frequently found Mississippi as a part of the landscape. **B:** Systemic chlorotic ring blotch symptoms observed on kudzu sample from Ackerman - a source for the virus characterization**.**

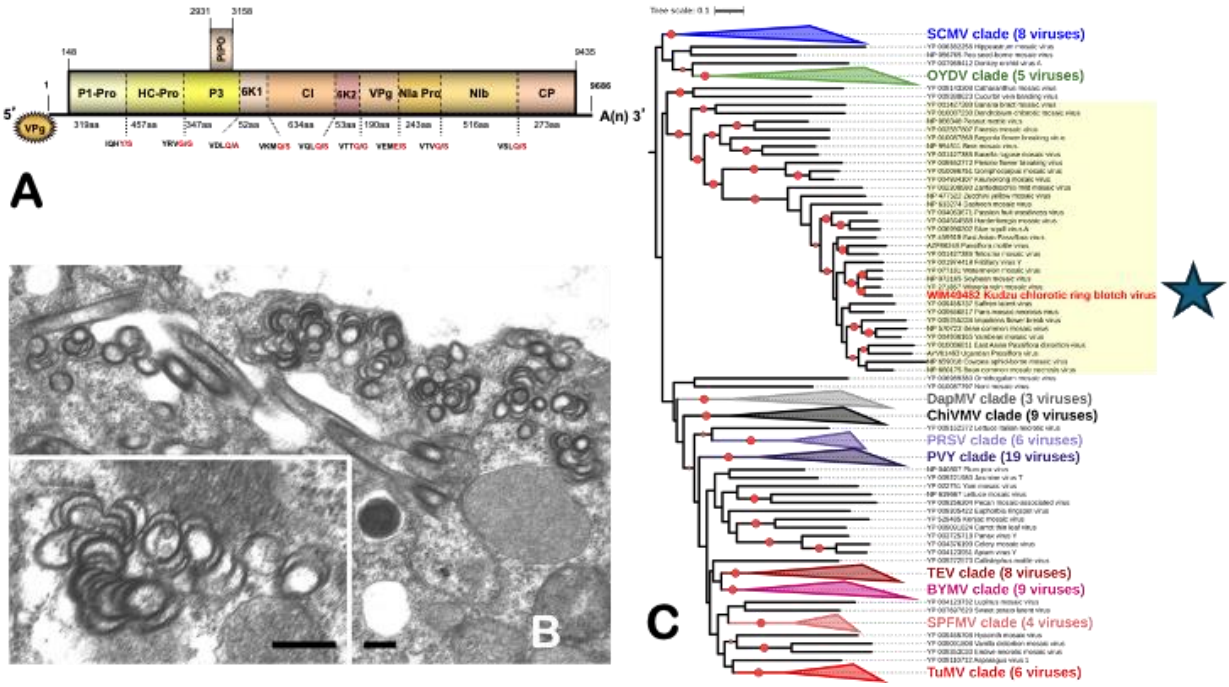

Figure 2. A: Schematic representation of a genome organization of kudzu chlorotic red blotch virus. **B:** Pinwheels and laminar inclusions, ultrastructural modifications of infected cells indicative of potyvirus infections, observed in tissue collected from symptomatic kudzu. An enlarged view of a pinwheel structure (inset). C: Phylogenetic tree showing relationships of kudzu chlorotic ring blotch virus (KudCRBV) to members of the genus *Potyvirus.*

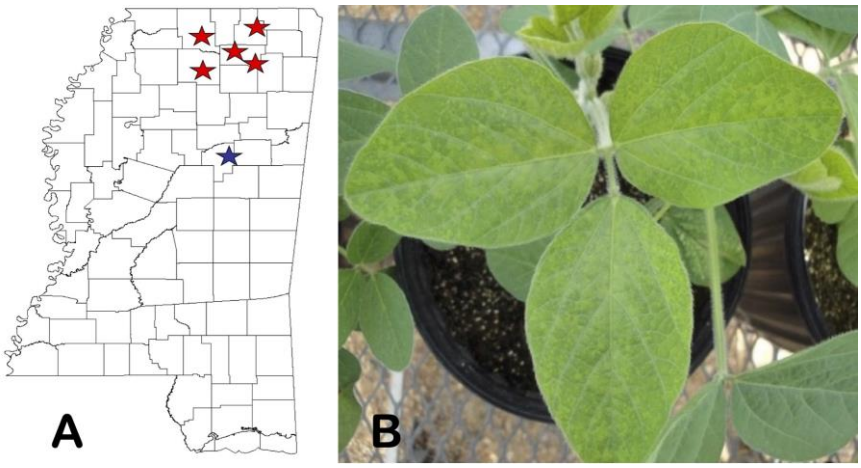

Figure 3. (**A**) Approximate locations of kudzu chlorotic ring blotch virus (KudCRBV)-infected kudzu patches. A blue star represents the original source of the virus, while the red ones indicate additional infections revealed during the survey. **B:** Chlorosis and systemic mosaic/vein discoloration symptoms on the trifoliate of a soybean plant mechanically inoculated with KudCRBV.

Figure 4. Some symptoms observed on collected soybean samples: foliar distortion (left) and mosaic/mottling (right)

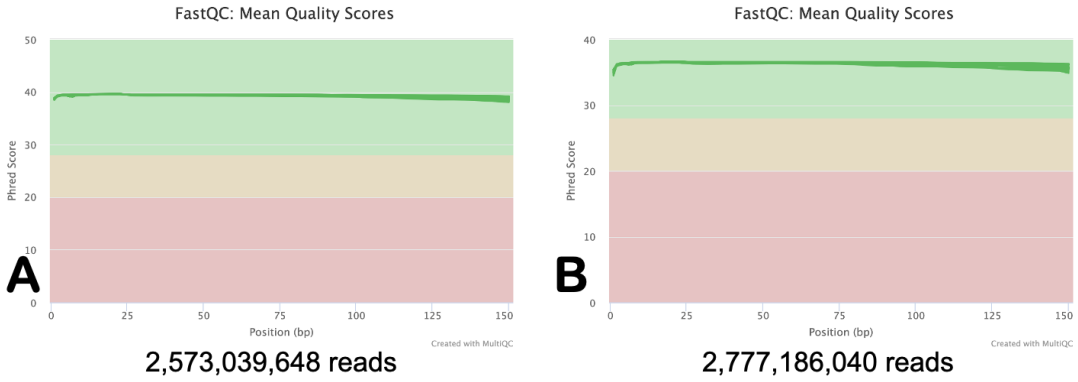

Figure 5. Graphs showing high quality of raw sequence data generated by high-throughput sequencing.

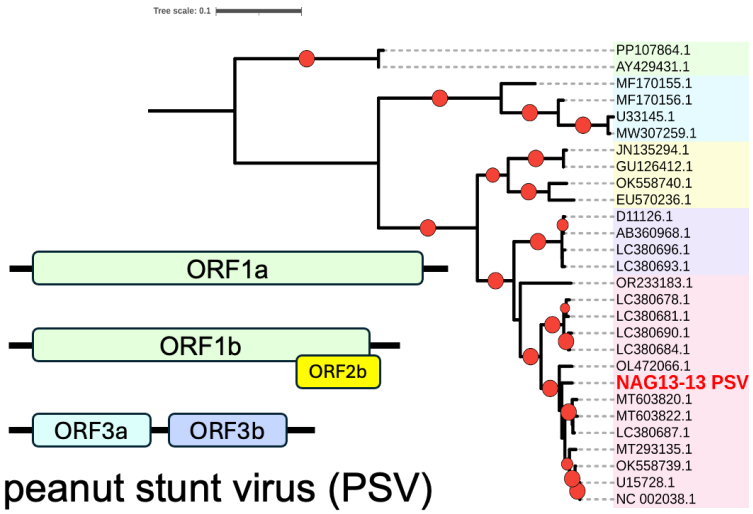

Figure 6. Genome organization of local isolate of peanut stunt virus (PSV) and phylogenetic tree showing its relationships with other isolates reported from various hosts and geographic locations worldwide. The isolate from Mississippi is denoted with red font.