INTRODUCTION:
The International Committee on Taxonomy of Viruses has estimated the total number of viruses to be around 200,000, which we believe is a gross underestimate. Our study is focused on plant viruses in the Tallgrass Prairie Preserve. The plants are collected regardless of their appearance diseased or healthy with a close-up shot of the plant along with an identification sheet, which includes GPS location information, date of collection, tentative identification and aerial number. An intact plant is sent to the Oklahoma State University (OSU) Herbarium where experts confirm its identification. Specimens are sent to OSU, where possible viromes are isolated and the viral nucleic acid is extracted, amplified using degenerate primers, cloned into a vector and sequenced. Another specimen is sent to the Noble Foundation, Ardmore, where the total nucleic acid (TNA) is extracted. The TNA is processed for the identification and purification of double stranded RNA, which serves as an indicator of viral infection. A part of the extracted TNA is sent to Advanced Center for Genome Technology, Norman, for bar-coding using ITS and psbA primers which aids in the identification of plant species. Another part of the extracted TNA is sent to OSU for microarray analysis. Fluorescently tagged targets derived from TNA will be hybridized to microarray slides, which contain more than a thousand probes. The probes were designed by using conserved sequences in NCBI reference genomes of viruses infecting plants and fungi. The resulting intensity pattern will be analyzed using E-Predict software, which identifies known viruses most similar to the virus in the sample. The next phase of the project will deal with ecological aspects, like distance decay, burn effects and co-occurrence. The significant impacts of the project include discovering novel viruses, homeland security, and predicting outbreaks of agricultural diseases.

ISOLATION OF VIRIONS: CLONING AND SEQUENCING:

- Plant Specimen
- Virus Isolation
- Isolation of Viral Nucleic acid
- PCR using degenerate primers
- Cloning of PCR product to TOPO TA cloning vector
- Sequencing

Identification of the Virus based on sequence homology
Application of this method to a plant experimentally infected with Maize necrotic streak virus led to the successful recovery of the viral sequence.

DOUBLE STRANDED RNA PURIFICATION:

IMPACTS
- Discovering novel viruses
- Homeland security
- Outbreak predictions

REFERENCES
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