

Exploring Emerging Viruses From a Protected Reserve

Authors : Nemat Sokhandan Bashir

Abstract : Threats from viruses to agricultural crops could be even larger than the losses caused by the other pathogens because, in many cases, the viral infection is latent but crucial from an epidemic point of view. Wild vegetation can be a source of many viruses that eventually find their destiny in crop plants. Although often asymptomatic in wild plants due to adaptation, they can potentially cause serious losses in crops. Therefore, exploring viruses in wild vegetation is very important. Recently, omics have been quite useful for exploring plant viruses from various plant sources, especially wild vegetation. For instance, we have discovered viruses such as Ambrossia asymptomatic virus I (AAV-1) through the application of metagenomics from Oklahoma Prairie Reserve. Accordingly, extracts from randomly-sampled plants are subjected to high speed and ultracentrifugation to separated virus-like particles (VLP), then nucleic acids in the form of DNA or RNA are extracted from such VLPs by treatment with phenol–chloroform and subsequent precipitation by ethanol. The nucleic acid preparations are separately treated with RNase or DNase in order to determine the genome component of VLPs. In the case of RNAs, the complementary cDNAs are synthesized before submitting to DNA sequencing. However, for VLPs with DNA contents, the procedure would be relatively straightforward without making cDNA. Because the length of the nucleic acid content of VLPs can be different, various strategies are employed to achieve sequencing. Techniques similar to so-called "chromosome walking" may be used to achieve sequences of long segments. When the nucleotide sequence data were obtained, they were subjected to BLAST analysis to determine the most related previously reported virus sequences. In one case, we determined that the novel virus was AAV-1 because the sequence comparison and analysis revealed that the reads were the closest to the Indian citrus ringspot virus (ICRSV). AAV-1 had an RNA genome with 7408 nucleotides in length and contained six open reading frames (ORFs). Based on phylogenies inferred from the replicase and coat protein ORFs of the virus, it was placed in the genus Mandarivirus.

Keywords : wild, plant, novel, metagenomics

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