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Use of deep sequencing to analyze replication Beet Curly Top virus replication fidelity

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Abstract

Beet curly top virus (BCTV) is a member of the geminivirus genus, a family of ssDNA plant infecting viruses, that has a wide host range and causes significant damage on a number of agronomically important crops. Populations of BCTV in agricultural fields are diverse and shift over time with different species dominating in different years. Prior studies examining geminivirus progeny produced from single infectious clones suggest that geminiviruses accumulate mutations at a rate similar to RNA viruses and suggest a potential mechanism for generation of BCTV diversity in the field. These prior studies were relatively small scale and utilized dideoxy based sequencing to produce ~100,000 base pairs of sequence. Here we report on work where plants were infected with a single infectious BCTV clone and progeny isolated from systemic tissue were characterized by deep sequencing. Several biological repeats were performed in *Nicotiana benthamiana* and additional treatments including analysis of viral progeny isolated from tomato, sugar beet, and cowpea. The goal of covering the entire genome at 1,000x coverage was met in many of the treatments. Our results show that the deep sequencing platforms have a high error rate and extensive error checking is required as part of data analysis. After careful validation of data we observed a high mutation rate for BCTV as expected and also noted that moderate sized deletions that generate defective interfering progeny genomes were common. The types of sequencing errors encountered, mutation frequencies and patterns, as well as applications for this approach will be discussed.