

Enhancing geminivirus detection in plants: unveiling hidden threats with a cost-efficient RCA-RFLP method

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Monitoring diseases is crucial for effective plant health management. Choosing the right analysis method is key to obtaining maximum information with minimal effort and cost. In Virology, traditional diagnostics based on symptom analysis and specific tests might overlook viruses causing mild symptoms or asymptomatic infections, especially in cases of unknown agents or mixed infections. High Throughput Sequencing (HTS) technology has emerged as an efficient tool for detecting unknown or unreported viruses. However, its high per-sample cost limits widespread use. This work describes a highly efficient method for detection of ssDNA viruses, the RCA-RFLP, for universal detection of geminiviruses. RCA, a unique DNA amplification technique, coupled with RFLP, generates species-specific band profiles, allowing preliminary identification of circular ssDNA viruses. Until recently, begomoviruses were the only geminivirus members identified in Brazil. Their detection relied on a widely adopted PCR method using universal primers. Since 2018, however, two other geminivirus members, mastreviruses and topileviruses, were also identified in the country; these viruses cannot be detected by begomovirus universal PCR. An analysis of composite tomato leaf samples exhibiting typical begomovirus infection symptoms over different years, G1 (2003-05), G2 (2009-11), and G3 (2014-16), revealed a decreasing diversity of begomoviruses in the tomato production area of Taquara, in the Federal District. This shift from high diversity and mixed infections to few viruses in simpler infections was identified using PCR, HTS and RCA-RFLP methods (Souza et al., FPS 2020). The study uncovered the presence of a topilevirus in G1 tomato samples, initially undetected by begomovirus-specific PCR, but later confirmed by HTS and RCA. This indicated the limitations of using PCR for detecting a broad range of geminiviruses. Recent visits to Brazilian tomato fields consistently identified two topileviruses, which were spread in the major growing areas. Symptoms induced by topileviruses resembled those caused by begomoviruses, leading to failures in topilevirus detection if using begomovirus-specific PCR. Therefore, our laboratory now adopts RCA-RFLP as the standard test for suspected geminivirus infections. Ongoing research focuses on understanding the importance and distribution of topileviruses in tomatoes and associated plants.

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