MICROARRAY-BASED DETECTION AND IDENTIFICATION OF VIRUSES IN PLANTS

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Globalization has increased the risk that pernicious plant pathogens will be introduced or reintroduced into susceptible plant populations. Because imported plants and germplasm harboring infections may not present obvious disease symptoms, a rapid assay that can detect known and even uncharacterized viruses at low titer is required. To address this concern we are developing a microarray-based assay for the identification and categorization of plant viruses to at least the genus level. Many plant species produce a variety of metabolites that impair timely isolation of pure nucleic acids. Isolation methods often are designed for a limited subset of species and are not widely tested. We have incorporated potassium acetate (KOAc) and spin columns into a CTAB (cetyl trimethylammonium bromide)-based protocol (CKC protocol; short for CTAB, KOAc, Column) for isolating an acceptable yield of amplifiable total nucleic acid (TNA) from a wide variety of plants in about two hours. To probe isolated nucleic acids for the presence of viral genomes, we have designed and produced a 10,000 element Universal Plant Virus Microarray (UVPM) composed of 60-mer oligos covering every taxon/node of the taxonomic tree for all plant viruses available in GenBank. Currently, we are building a database of array signatures from known viruses. Because some viruses may be present at very low titer in plant tissue, we are developing amplification methods to avoid misdiagnoses and false-negative assay results.