Molecular characterization and coat protein serology of watermelon leaf mottle virus (Potyvirus)*

Brief Report

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Summary. A cDNA library was generated from purified RNA of watermelon leaf mottle virus (WLMV) (Genus Potyvirus). Two overlapping clones totaling 2,316 nucleotides at the 3’ terminus of the virus were identified by immunoscreening with coat protein antiserum. The sequence analyses of the clones indicated an open reading frame (ORF) of 2,050 nucleotides which encoded part of the replicase and the coat protein, a 243-nucleotide non-coding region (3’UTR), and 23 adenine residues of the poly (A) tail. The taxonomic status of WLMV was determined by comparisons of the sequence of the cloned coat protein gene and 3’UTR with potyvirus sequences obtained from GenBank. The nucleotide sequence identities of WLMV compared with 17 other potyviruses ranged from 55.6 to 63.5% for the coat protein, and from 37.2 to 48.3% for the 3’UTR. Phylogenetic analyses of the coat protein region and the 3’UTR indicated that WLMV did not cluster with other potyviruses in a clade with high bootstrap support. The coat protein gene was expressed in Escherichia coli and a polyclonal antiserum was prepared to the expressed coat protein. In immunodiffusion tests, WLMV was found to be serologically distinct from papaya ringspot virus type W, watermelon mosaic virus 2, zucchini yellow mosaic virus, and Moroccan watermelon mosaic virus. In Western blots and ELISA, serological cross-reactivity with other cucurbit potyviruses was observed. Serological and sequence comparisons indicated that watermelon leaf mottle virus is a distinct member of the Potyvirus genus.

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(WMV-2), have been found infecting cucurbits in the state of Florida, USA [9, 14]. In 1991, a watermelon sample collected in Florida was found to be infected by a Potyvirus isolate with distinct biological and serological properties. This isolate was initially referred to as field collection 2932 (FC 2932) and was tentatively named watermelon leaf mottle virus (WLMV) [15]. WLMV is nonpersistently transmissible by the aphid Myzus persicae and induces mottle or mosaic in watermelon (Citrullus lanatus) and mosaic in squash (Cucurbita pepo). It systemically infects several cultivars of cucumber (Cucumis sativus) and cantaloupe (Cucumis melo) without inducing symptoms, and has been found to cause mosaic in ‘Prelude II’ [15], which has coat protein gene mediated resistance to WMV-2 and ZYMV. WLMV induces the production of conspicuous inclusion bodies in infected plant cells: cylindrical cytoplasmic inclusions, amorphous inclusions in the cytoplasm and nucleus, and elongated or globular inclusions in the nucleus [12, 15]. In reciprocal immunodiffusion tests, WLMV was found to be serologically distinct from PRSV-W, WMV-2, ZYMV, zucchini yellow fleck virus (ZYFV) and Moroccan watermelon mosaic virus (MWMV) [15].

Here we report the nucleotide (nt) and the deduced amino acid (aa) sequence of the 3' terminus of this novel member of Potyvirus, and its relationship to 17 other viruses within the genus, using comparative sequence analyses [8]. Preliminary accounts of this study have been published [5, 6]. We also report on the characterization of a polyclonal antiserum, produced to coat protein expressed in Escherichia coli, by immunodiffusion, indirect ELISA, and Western blotting.

WLMV was maintained in squash ‘Early Prolific Straightneck’ (EPSN), watermelon ‘Charleston Gray’ and ‘Crimson Sweet’, and cantaloupe ‘Hale’s Best Jumbo’ by mechanical inoculation. Isolates of PRSV-W, WMV-2, ZYMV and MWMV were also maintained in EPSN squash and were transmitted by mechanical inoculation.

A cDNA library of purified WLMV RNA was generated using the “Time Saver” cDNA synthesis kit (Pharmacia Biotech Inc.) according to manufacturer’s instructions with random and oligo (dT) primers. Blunt-ended cDNA fragments were ligated to Eco RI/Not I adapters and inserted into the Eco RI sites of Lambda Zap II (Stratagene). The ligated Lambda DNA was then packaged using the “Packagene” Lambda DNA Packaging system (Promega). Amplification of the cDNA library and immunoscreening for clones expressing the coat protein was done according to the “Lambda Zap II Instructional Manual” (Stratagene), and positive clones were plaque purified by three rounds of immunoscreening, using a rabbit polyclonal antiserum (AS 1163) prepared to the coat protein obtained from dissociated, purified virus [15].

Clones found to be positive in immunoscreening were sequenced by the dideoxy chain termination method [17] using the “Sequenase Version 2.0 DNA T7 Polymerase” sequencing kit (USB) and by automated sequencing at the DNA Sequencing Core Facility of the Interdisciplinary Center for Biotechnology Research (ICBFR) at the University of Florida, Gainesville, FL. Two overlapping clones (pCPA and pCPB) and two Eco RI restriction site-directed subclones of clone pCPB, were sequenced using M13 forward and reverse primers. Two