Particle Lengths of Whitefly-Transmitted Criniviruses

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ABSTRACT

Liu, H.-Y., Wisler, G. C., and Duffus, J. E. 2000. Particle lengths of whitefly-transmitted criniviruses. *Plant Dis.* 84:803-805.

An improved method for particle length measurement was used for six members of the genus *Crinivirus*. Particle measurements were conducted with a CCD-72S solid state camera, which was interfaced with a Zeiss EM 109 electron microscope, and analyzed using the analysis 2.1 Image Analysis Software. In comparisons of specimen preparation methods, the leaf dip method is more representative and reproducible than the antibody capture method or preparation from purified virions. Particle length (nm) ranges of whitefly-transmitted criniviruses are: *Abutilon yellows virus* (AYV), 800 to 850; *Cucurbit yellow stunting disorder virus* (CYSDV), 750 to 800; *Lettuce chlorosis virus* (LCV), 800 to 850; *Lettuce infectious yellows virus* (LIYV), 700 to 750; *Tomato chlorosis virus* (ToCV), 800 to 850; and *Tomato infectious chlorosis virus* (TICV), 850 to 900.

Additional keywords: *Closteroviridae*, histogram

A number of whitefly-transmitted viruses with bipartite ssRNA genomes are members of the recently recognized genus *Crinivirus* in the family *Closteroviridae* (15). Particle length is an important criterion for classifying closteroviruses into genera. Early classifications of closteroviruses were based on particle lengths: long types with particles from 1,200 to 2,000 nm and short types with particles from 700 to 800 nm (6,12). The measurements were, however, made by a number of different individuals utilizing different techniques and/or criteria for measurement, i.e., modal lengths, average lengths, or even the longest length observed. *Lettuce infectious yellows virus* (LIYV), for instance, was reported to have particles ranging from 400 to 500 nm to particles 1,800 to 2,000 nm (8). Particles of *Tomato infectious chlorosis virus* (TICV) have been reported with an average length of 645 nm, a modal length of 850 nm, and longest particle of 1,600 nm (9). Other whitefly-transmitted closteroviruses have similar inconsisten-

cies in particle length. Published particle measurement data must be regarded with caution because different procedures for virus isolation were used. Apparent differ-

ences may actually be the result of different techniques.

We used the same techniques and criteria to measure the length of several whitefly-transmitted criniviruses (3,8–11,14,18–20). We also examined *Beet yellows virus* (BYV) and *Citrus tristeza virus* (CTV), two known aphid-transmitted closteroviruses, and compared the lengths of all by parallel preparation and measurements to ensure consistency in particle measurements to provide information for closterovirus taxonomy.

MATERIALS AND METHODS

Viruses. The viruses were whitefly-transmitted criniviruses including *Abutilon yellows virus* (AYV), *Cucurbit yellow stunting disorder virus* (CYSDV), *Lettuce chlorosis virus* (LCV), *Lettuce infectious yellows virus* (LIYV), *Tomato chlorosis virus* (ToCV), and *Tomato infectious chlorosis virus* (TICV). All viruses were

Fig. 1. Electron micrograph of *Lettuce infectious yellows virus* particles stained in 2% uranyl acetate. (A) From leaf-dip preparations. (B) From purified virions. Bars represent 100 nm.

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Accepted for publication 14 April 2000.

Publication no. D-2000-0517-01R

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maintained in *Nicotiana clelandii* plants in the greenhouse except CYSDV, which was maintained in cucumber (*Cucumis sativus*) plants and transferred from plant to plant via inoculation with the appropriate whitefly vector (18). Aphid-transmitted BYV was maintained in sugar beet (*Beta vulgaris*) and transferred from plant to plant via inoculation with the green peach aphid, *Myzus persicae*. CTV leaf dip grids were kindly prepared by V. J. Febres, University of Florida.

Table 1. Particle lengths of whitefly-transmitted criniviruses and aphid-transmitted closteroviruses measured from leaf dips

Virus	Length (nm) ^a
Whitefly transmitted	
<i>Abutilon yellows</i>	800-850
<i>Cucurbit yellow stunting disorder</i>	750-800
<i>Lettuce chlorosis</i>	800-850
<i>Lettuce infectious yellows</i>	700-750
<i>Tomato chlorosis</i>	800-850
<i>Tomato infectious chlorosis</i>	850-900
Aphid transmitted	
<i>Beet yellows</i>	1,200-1,300
<i>Citrus tristeza</i>	1,900-2,000

^a Particle lengths represent the highest peak range of particles measured on histogram.

Virus purification. Virions were purified using modifications of methods used for TICV (9). Two homogenization methods compared were (i) a VirTis 60 homogenizer for 3 min and (ii) a ball mill for 2 h at 4°C. LIYV and TICV virions were purified from *N. clelandii* plants. CYSDV virions were purified from melon (*Cucumis melo* cv. Dublon) plants.

Specimen preparation. *Leaf dips.* One square centimeter of virus-infected plant tissue was ground in 100 µl of 0.1 M Tris-HCl, pH 7.4, with 10% sucrose with a sterilized mortar and pestle. The leaf extracts were transferred to carbon-coated Formvar grids and held for 1 min. After washing the grids with 30 drops of distilled water, grids were stained with 2% uranyl acetate containing 250 µg of bacitracin per ml (4).

Antibody capture of LIYV particles. Polyclonal antibody, anti-LIYV, was diluted in phosphate buffered saline (0.02 M phosphate buffer, pH 7.0, with 0.8% NaCl) at 1:1,000 and allowed to bind to Formvar-carbon coated grids for 10 min at room temperature. After washing three times with distilled water, the specimen preparations were same as leaf dips.

Purified virions. One drop of purified virions was transferred to carbon-coated

Formvar grids, held for 1 min, and then stained as above.

Particle measurement. Negatively stained specimens were viewed with a Zeiss EM 109 electron microscope. Particle measurements were conducted with a CCD-72S series solid state camera (Dage-MTI, Inc., Michigan City, IN), which was interfaced with the microscope, and were analyzed using the analysis 2.1 Image Analysis Software (Soft-Imaging Software Corp., Golden, CO). More than 200 particles were measured from each sample.

RESULTS

The six whitefly-transmitted crinivirus particle lengths ranged from 700 to 900 nm (Table 1). Particles of the aphid-transmitted BYV and CTV were 1,200 to 1,300 nm and 1,900 to 2,000 nm, respectively.

Particle length distributions from leaf dips and purified virions are shown in Figures 1, 2, and 3. The modal lengths from leaf dips were similar to those from purified preparations when samples were homogenized with the ball mill (Figs. 2A and C, and 3A and B). When the VirTis 60 homogenizer was used, the particle lengths were widely distributed, with no distinct peak (Fig. 2B).

Fig. 2. Particle length distributions of *Tomato infectious chlorosis virus* (A) in leaf dip preparations. Purified virions extracted with (B) a VirTis 60 homogenizer or (C) a ball mill.

Fig. 3. Particle length distributions of *Lettuce infectious yellows virus* (A) in leaf dip preparations. (B) Purified virions using ball mill for extraction. (C) Antibody captured method.

Particle length distributions of polyclonal antibody captured LIYV virions from leaf dips were evenly distributed and apparently captured more shorter particles than those of leaf dips alone (Figs. 3A and C).

DISCUSSION

Classification of closteroviruses has changed significantly in recent years and continues to change as more members are discovered. Since members of the *Closteroviridae* are divided into two genera based on particle length and a bipartite ssRNA genome (6,12), consistency in particle measurements is essential for characterization.

Our results confirm that whitefly-transmitted bipartite crinivirus particles are shorter than particles of aphid-transmitted monopartite closteroviruses, as would be expected from a divided genome (17). Our results on particle lengths of BYV and CTV confirm those of previous reports (1,2).

Because the long, flexuous rod particles break during purification due to stresses such as mechanical chopping, osmotic and pH changes, the modal lengths of particles from leaf dips are longer than those from purified virions. Where the VirTis homogenizer was used for extraction, the virus particles were mechanically broken, resulting in evenly distributed particle lengths (Fig. 2B).

A method for the detection and identification of viruses that uses electron microscope grids coated with an adsorbed film of antiserum specific for a given virus has been used (5). This method is very useful for identifying viruses from leaf dips, especially for those viruses of low concentration in plant sap. However, for particle length measurements, this antibody trapping method absorbed the shorter particles on the grids (Fig. 3C) that were not representative of the range or mode of particle lengths for the virus found in our assays.

Although criniviruses are bipartite (15), two peaks were not observed to represent the particle lengths for RNA 1 and 2. This is likely because particle lengths corresponding to RNA 1 and 2 are very close in size.

AYV (14) is transmitted by the banded-wing whitefly (*Trialeurodes abutilonea*),

and its particle length ranges from 800 to 850 nm. AYV was recently shown to be bipartite (H.-Y. Liu, *unpublished data*). LCV (10) has not been shown to be bipartite (16), although it is whitefly-transmitted, and its particle length ranges from 800 to 850 nm. *Beet pseudo-yellows virus* (BPYV) is transmitted by the greenhouse whitefly (*T. vaporariorum*) (7,13). Because of its low titer in the infected plant, it is difficult to obtain enough particles for a good histogram. However, more than 20 BPYV particle length measurements were between 600 and 850 nm. Whitefly-transmissibility, particle length, and other closterovirus characteristics suggest that AYV, LCV, and BPYV might be members of the new genus *Crinivirus*.

ACKNOWLEDGMENTS

We thank B. W. Falk and A. V. Karasev for their critical review of the manuscript and V. J. Febres for providing *Citrus tristeza virus* leaf dip grids. We thank Ling Ling Jia, A. A. Cortez, J. L. Sears, and K. O. Schlueter for their excellent technical assistance.

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