Potyvirus Cylindrical Inclusions—Subdivision-IV

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ABSTRACT

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Subdivision-IV cytoplasmic cylindrical inclusions consisting of pinwheels, scrolls, and short curved laminated aggregates are induced by 17 potyviruses. Five of these viruses infect primarily monocots; 12 infect primarily dicots. Although most isolates of potato virus Y induce subdivision-IV cylindrical inclusions, at least one isolate induces subdivision-I cylindrical inclusions, pinwheels, and scrolls. Viruses inducing scrolls and short curved laminated aggregate inclusions are assigned to subdivision-IV.

Additional key words: subdivisions -II and -III.

Cytoplasmic cylindrical inclusions are one of the main characteristics of the potyvirus group (16,18,34). That portions of potyvirus genomes control the formation of cylindrical inclusions is supported by the following: only potyviruses induce cylindrical inclusions (15,16,34), the same potyvirus induces its characteristic type of cylindrical inclusion in a wide range of host cells including cells of species in different families (16), different potyviruses induce their characteristic types of cylindrical inclusions in the same host (16), cylindrical inclusion proteins are serologically unrelated to virus coat proteins and host proteins (41), and potyvirus RNAs have been translated in vitro in the rabbit-reticulocyte system; among the translation products are cylindrical inclusion proteins (13).

Differences in morphology of cylindrical inclusions have been used to separate the potyviruses into three subdivisions (16). These differences have been established by examination of the inclusions in cross sections in which the pinwheel configuration is evident. Viruses inducing cylindrical inclusions containing scrolls and pinwheels were assigned to subdivision-I, those inducing cylindrical inclusions containing laminated aggregates and pinwheels were assigned to subdivision-II, and those inducing cylindrical inclusions containing pinwheels and both scrolls and laminated aggregates were assigned to subdivision-III. Examination of electron micrographs of cylindrical inclusions in the literature as well as those described in this study indicate there are viruses that induce a fourth distinctive type of cylindrical inclusion that can form the basis for a new subdivision-IV.

MATERIALS AND METHODS

The differences in inclusion morphology that form the basis of the established subdivisions are demonstrated in Fig. 1A–C: subdivision-I, typical inclusions induced by papaya ringspot virus (PRSV) in *Cucurbita pepo* L. (Fig. 1A); subdivision-II, typical inclusions induced by bean yellow mosaic virus (BYMV) in *Pisum sativum* L. (Fig. 1B); and subdivision-III, typical inclusions induced by turnip mosaic virus (TuMV) (Florida isolate) in *Brassica perviridis* Bailey.

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The following virus-infected leaf tissues fixed in 3% glutaraldehyde were sent to the Plant Virus Laboratory, Gainesville, FL, for cytological studies: Datura 437 (D 437V) in *Datura* sp. (V. D. Damsteegt), groundnut eyespot virus (GEV) in *Arachis hypogaea* L. (J. C. Thouvenel), tomato (Peru) mosaic virus (TPMV) in *Nicotiana occidentale* Wheeler (C. D. Fribourg), and wisteria vein mosaic virus (WVMV) in *Wisteria floribunda* DC. (L. Bos). Antigens of potato virus Y (PVY) from Australia (R. I. B. Francki) and Canada (H. W. J. Ragetli) were transmitted to *Nicotiana clevelandii* Gray and *Nicotiana tabacum* L., respectively. These infected leaf tissues were first examined by light microscopy (epidermal strips stained with Luxol brilliant green-calcomine orange [8]) to ensure that the samples contained abundant inclusions. Material from regions containing abundant inclusions was dehydrated in an ethanol series, embedded in Spurr's plastic, and sectioned with a diamond knife. Thin sections were stained in uranyl acetate and Reynold's lead-citrate, and studied in a Hitachi EM 600 electron microscope. Micrographs obtained from these studies were compared with micrographs of cylindrical inclusions on file at our laboratory and with micrographs in the literature.

RESULTS

The D437V, GEV, TPMV, and WVMV viruses were found to induce cytoplasmic cylindrical inclusions consisting of pinwheels, scrolls, and short curved laminated aggregates. The short curved laminated aggregates (Fig. 2A–D) are unlike the long straight laminated aggregates of subdivision-II and subdivision-III inclusions. The Canadian isolate of PVY (47) (Fig. 2D) that was reexamined in this study was also found to induce scrolls and short curved laminated aggregates. However, the Australian isolate of PVY (36) reexamined in this study induced only subdivision-I cylindrical inclusions. Cytological studies of cylindrical inclusions reported in the literature indicate that several potyviruses induce inclusions similar to those shown in Fig. 2A–D (Table 1).

DISCUSSION

The inclusions consisting of pinwheels, scrolls, and short curved laminated aggregates constitute a distinctive type (Fig. 2A–D) that can be distinguished from subdivision-I inclusions (Fig. 1A), subdivision-II inclusions (Fig. 1B), and subdivision-III inclusions (Fig. 1C). Subdivision-IV inclusions are induced by 13 members and four possible members of the potyvirus group (Table 1). Five of these viruses infect primarily monocots and 12 primarily dicots. These viruses are assigned to subdivision-IV.

Differences in morphologies of cylindrical inclusions are readily apparent. Information on the morphology of cylindrical inclusions available at this time is sufficient to place 26 viruses in subdivision-I, 33 in subdivision-II, 16 in subdivision-III, and 17 in subdivision-IV. However, in interpreting the structures of cylindrical inclusions, it should be kept in mind that insufficient sampling of thin sections with the electron microscope can lead to misassignments of viruses to subdivisions. Many cytological studies of potyviruses have described the presence of only pinwheels and bundles. Such investigations support the proposition that cylindrical inclusions are diagnostic for infections by potyviruses. Published reports usually contain only a few micrographs. Some involve only brief written descriptions of the inclusions. Although a single micrograph may contain all of the components of cylindrical inclusions, permitting assignment of the inducing virus to a subdivision, the micrographs in many studies do not.

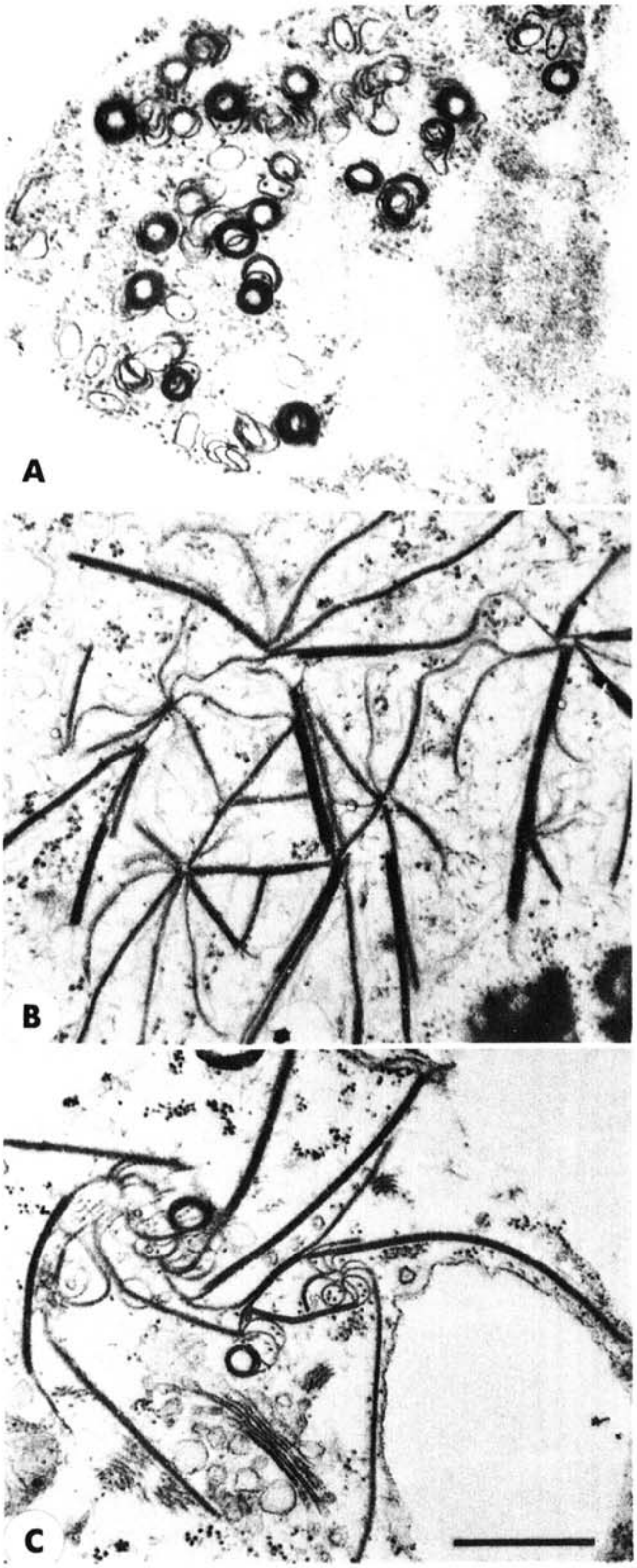

Fig. 1. Ultrastructure of leaf tissue infected with: **A,** papaya ringspot virus infected *Cucurbita pepo* containing subdivision-I cylindrical inclusions, pinwheels and scrolls. Bar = ~500 nm; **B,** bean yellow mosaic virus infected *Pisum sativum* containing subdivision-II cylindrical inclusions, pinwheels, and laminated aggregates; **C,** turnip mosaic virus (Florida isolate) infected *Brassica perviridis* containing subdivision-III cylindrical inclusions, pinwheels, scrolls, and laminated aggregates.

The preceding cautionary remarks about sampling are reinforced when additional cytological studies are conducted. For instance, sugarcane mosaic virus (SCMV) (strain A) was first reported to induce subdivision-II inclusions in corn leaf tissues (15). Further examination of this material revealed the presence of pinwheels, scrolls, and laminated aggregates, which are subdivision-III inclusions (16). Bearded iris mosaic virus (BIMV) was first reported to induce pinwheels and bundles (1); however, further cytological studies of BIMV revealed laminated aggregates (2).

PVY cylindrical inclusions have been the subject of 24 cytological studies. Subdivision-IV cylindrical inclusions are evident in the results of eight of these investigations (5,9,16,35,42,44,45) and in the Canadian isolate in the present study. The depictions of only pinwheels and bundles in several studies (3,4,15,19,20,33,40) do not provide sufficient information to determine what type of cylindrical inclusions these PVY isolates induce. In some cases, insufficient sampling or micrograph selection is apparently responsible for this situation. For instance, the PVY isolate used in two studies (3,4) described only pinwheels and bundles, although two additional studies of the same isolate contain micrographs with subdivision-IV inclusions (5,9). In three other reports (15,16,40), although the same PVY isolate was studied, only one (16) showed subdivision-IV inclusions.

Some reports (8,11,12,47) do not contain sufficient information on inclusion morphology to permit assignment of the inducing

TABLE 1. Subdivision-IV potyviruses inducing cylindrical inclusions containing scrolls and short curved laminated aggregates

Virus	Group assignment[a]	Citation
Primarily on monocots		
Cocksfoot streak	Member	7
Iris mild mosaic	Member	22
Leek yellow stripe[b]	Member	25,26,38,46
Sugarcane mosaic[b] strain E	Member	42
Wheat spindle streak	Possible member	24
Primarily on dicots		
Carnation vein mottle	Member	39
Pepper mottle	Member	8,16
Pepper veinal mottle	Member	16
Potato virus A	Member	17
Potato virus Y[b]	Member	5,9,16,35,43–45, and the present study
Sweet potato russet crack	Member	29,37
Turnip mosaic[b]	Member	6,8,10,17,31,32
Watermelon mosaic virus-2[b]	Member	15,16
Wisteria vein mosaic	Member	Present study
Datura 437	Possible member	Present study
Groundnut eyespot	Possible member	14, and present study
Tomato (Peru) mosaic	Possible member	Present study

[a]Viruses assigned as members or possible members to the potyvirus group (34).

[b]Different virus strains and isolates reported to induce different types of cylindrical inclusions.

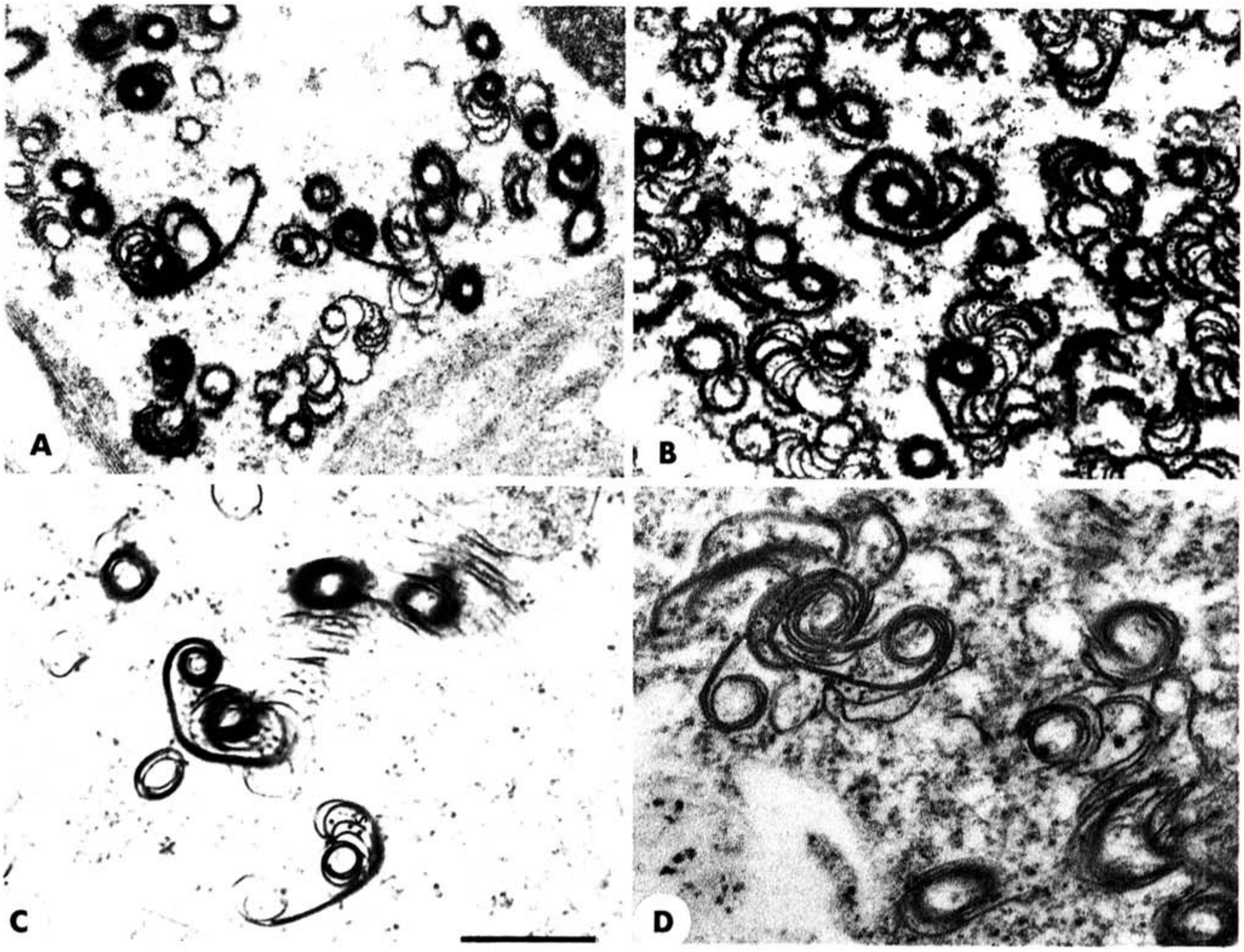

Fig. 2. Ultrastructure of leaf tissue infected with viruses inducing subdivision-IV cylindrical inclusions, pinwheels, scrolls and short curved laminated aggregates: **A,** groundnut eyespot virus infected *Arachis hypogaea*. Bar = ~500 nm; **B,** pepper mottle virus infected *Capsicum annuum*; **C,** turnip mosaic virus (R isolate) infected *Brassica perviridis*; **D,** potato virus Y (Canadian isolate) infected *Nicotiana tabacum*.

virus to a subdivision. However, the PVY isolate employed in two of these (8,11) is the same as that used in another study (16) in which subdivision-IV inclusions were induced. The Canadian isolate of PVY (47) reexamined in this study (Fig. 2D) induced subdivision-IV inclusions.

In two additional studies (23,31), the presence of pinwheels and scrolls suggests assignment of the inducing PVY isolate to subdivision-I; however, the PVY isolate employed (23,31) is also the same as that used in the studies (16) in which subdivision-IV inclusions were induced.

Two Brazilian (30) and an Australian (36) isolate of PVY have been reported to induce only pinwheels and scrolls. In our reexamination of the Australian isolate, only pinwheels and scrolls were observed, indicating assignment of this isolate to subdivision-I. There are other cases in which strains or isolates of certain potyviruses induce inclusion types that cause them to be assigned to different subdivisions. For instance, SCMV strain E induces subdivision-IV cylindrical inclusions (42), while SCMV strains A, D, and H have been reported to induce subdivision-III inclusions (16). The Johnsongrass strain of SCMV induces subdivision-I inclusions (16,36). Matthews (34) classified these strains together with strains of maize dwarf mosaic virus as SCMV. Another example involves TuMV. Subdivision-III inclusions are present in 15 cytological studies of TuMV-infected tissues. However, in six studies of different TuMV isolates (6,8,10,27,31,32) subdivision-IV inclusions are evident (Fig. 2C). Finally, subdivision-IV inclusions appear in tissues infected with watermelon mosaic virus-2 (WMV-2) isolates (15,16), while subdivision-III inclusions have been reported for other WMV-2 isolates (8,21,28). Perhaps, some of these isolates and strains will be demonstrated to be distinct viruses. However, cylindrical inclusions are controlled by portions of the virus genome (13,15,16,41). Therefore, variations in the genome can be expected to produce differences in inclusion morphology.

At present, 113 viruses are assigned to the potyvirus group (34). Because there are only four recognized subdivisions, it is obvious that alone they cannot be used to identify specific viruses. However, differences in cylindrical inclusion morphology are useful in separating certain potyviruses as well as some of their isolates and strains. Viruses within the subdivision assemblages may be further separated by the presence of other types of inclusions induced by many potyviruses (8,16). Such information, when considered with additional potyvirus properties, significantly reduces the number of comparisons required for specific virus identification.

To facilitate comparisons of cylindrical inclusions, to expand the potyvirus subdivisions, and to increase their usefulness, the following viruses that induce typical cylindrical inclusion types are available to interested investigators from J. R. Edwardson or R. G. Christie, Agronomy Department, PVL, HS/PP Bldg., University of Florida, Gainesville 32611: PRSV (subdivision-I), BYMV (subdivision-II), TuMV (Florida isolate) (subdivision-III), and pepper mottle virus (subdivision-IV).

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