Neth. J. Pl. Path. 75 (1969) 137–143

Inclusion bodies of bean yellow mosaic virus, some less known closely related viruses and beet mosaic virus

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Accepted 25 July, 1968

Abstract

A modified simple staining technique readily demonstrated inclusion bodies in cytoplasm and nucleoli. Large amounts of cytoplasmic granular inclusions and abnormal nucleoli were observed with bean yellow mosaic virus. One isolate caused nucleoli to sprout like yeast. With a related pea virus nucleoli were much enlarged and usually covered with radiating crystalline needles. Inclusions produced by clover yellow vein virus were like those of bean yellow mosaic virus but nucleoli were very much enlarged. With beet mosaic virus, granular cytoplasmic inclusions and enlarged and sprouting nucleoli were observed.

Introduction

Several plant viruses have been found to evoke abnormal inclusions in the cells of their hosts. These inclusions are often of characteristic size and form irrespective of the host and may thus be of diagnostic value.
While working on the identification of some supposedly new viruses isolated from legumes and belonging to the "potato virus Y group", I found several of these produced large amounts of inclusions which were often of striking size and form. This paper reports some of the results obtained in stydying them with a light microscope by a slight modification of an extremely simple technique for staining inclusion bodies, and describes the amazing pathogenic effect viruses can have within the cell.

Some literature

For a long time tobacco etch virus (Kassanis, 1939) and bean yellow mosaic virus (McWhorter, 1941) were almost the only viruses of the potato virus Y group (with filamentous particles 730–860 mμ long) that were generally known for their inclusion bodies. They were the only known plant viruses to cause intranuclear inclusions. With etch virus, crystals occurred abundantly in the karyoplasm, whereas with bean yellow mosaic virus inclusions were found exclusively inside the nucleolus (see also McWhorter, 1965).
Especially through recent work we now know that the following other members of the potato virus Y group may produce cytoplasmic inclusions (mainly granular): anemone mosaic virus (Hollings, 1957), bean common mosaic virus (Christie, 1967), beet mosaic virus (Fujisawa et al., 1967), clover yellow vein virus (Hollings and Nariani, 1965), henbane mosaic virus (Sheffield, 1934), papaya ringspot virus (Zettler et al.,

1968), potato virus Y (Bawden and Sheffield, 1944), sugar-cane mosaic virus (Christie, 1967), tulip mosaic virus (McWhorter, 1940), turnip mosaic virus (Berkeley and Weintraub, 1952), watermelon mosaic virus (Schmelzer and Miličić, 1966), and western celery mosaic virus (Purcifull and Shepard, 1967).

Staining technique

Inclusion bodies were easily demonstrated by immersing epidermal strips from stems, petioles, or sometimes from the undersides of leaves for 15 min in a 1% solution of phloxine, either in water (Rubio-Huertos, 1950) or in a special staining solution. This solution consisted of a mixture of 2 volumes of cellosolve (ethylene glycol monomethyl ether), 1 volume of 95% ethanol, and 1 volume of distilled water, and allowed the dye to penetrate better (Christie, 1967). But often the phloxine solution was mixed 5:1 with a similar 1% solution of methylene blue for better contrast. Another difference from Christie was that I rinsed the stained strips for a few minutes in tap water and mounted them in water.

Results

Bean yellow mosaic virus always produced striking amounts of large granular inclusions in epidermal cells of pea (*Pisum sativum*), broad bean (*Vicia faba*) and *Nicotiana clevelandii* as reported in the literature. These inclusions were immediately visible through the microscope, even at low magnification. They were often compact and darkly stained. Sometimes they were partly crystalline. The nucleolus was sometimes slightly enlarged and angular, and was always clearly visible in contrast to nucleoli in normal plants.

Five isolates of bean yellow mosaic virus from pea (coded E 195 – E 199) produced inclusions similar to one another. Nucleoli usually looked normal except with E 196 and E 197 in broad bean, where the nucleolus contained some tiny crystals after infection with E 196 (Fig. 1A-C), or showed a yeast-like sprouting with E 197 (Fig. 1D-F).

An unknown but related virus, isolated from necrotic pea plants (E 178), caused less striking granular inclusions in pea, broad bean, *N. clevelandii*, white clover (*Trifolium repens*) and French bean (*Phaseolus vulgaris*) than did bean yellow mosaic virus. However, the nuclei were always notably abnormal, containing massive inclusions of hitherto unreported shape and size (Fig. 2). The nucleoli were irregularly enlarged up to about two thirds of the diameter of the nucleus (Fig. 2A, B) and usually covered with many long radiating crystalline needles (Fig. 2C) or replaced by such needles, almost completely filling the nucleus (Fig. 2D).

Clover yellow vein virus, as well as producing amorphous cytoplasmic inclusions as reported for *N. clevelandii* by Hollings and Nariani (1965), was found to cause irregular enlargement of nucleoli of pea and broad bean (Fig. 3). Cytoplasmic inclusions were extensive and granular.

In beet, inoculated with beet mosaic virus, vague cytoplasmic inclusions were sometimes observed and nucleoli were occasionally slightly enlarged irregularly (Fig. 4A). In pea, however, nucleoli were greatly enlarged and sprouting (Fig. 4B, C).

Fig. 1. Granular cytoplasm inclusions (g) together with tiny nucleolar crystals (nc) (A–C) or with enlarged or yeast-like sprouting nucleoli (D–F) in epidermal cells of *Vicia faba* after infection with isolate E 196 (A–C) and E 197 (D–F) of bean yellow mosaic virus. nu = nucleus, no = nucleolus. Magn. × 500 (A–C) and × 770 (D–F).

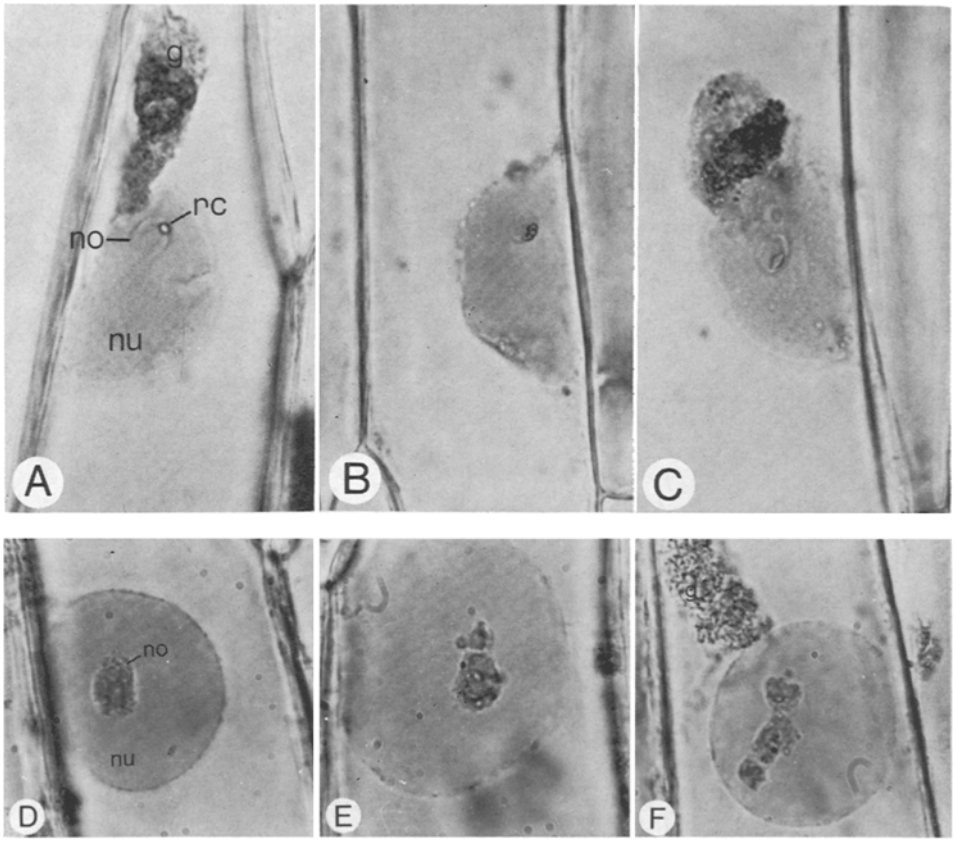

Fig. 1. Granulaire cytoplasma insluitsels (g) met kleine nucleolaire kristallen (nc) (A–C) of met vergrote of gistachtig spruitende nucleoli (D–F) in epidermiscellen van Vicia faba na infectie met isolaat E 196 (A–C) en E 197 (D–F) van scherpmozaïekvirus van boon. nu = nucleus, no = nocleolus. Vergr. 500 × (A–C) en 770 × (D–F).

Discussion

The results point to striking similarities in inclusion bodies produced by different members of the potato virus Y group. Thus, viruses known to be related serologically and in particle size and form, have other features in common as well. As early as 1941 McWhorter concluded that bean yellow mosaic virus and pea mosaic virus, long considered to be a distinct entity, were closely related because inclusions caused by them, were basically similar.

However, there are slight differences also. Mueller and Koenig (1965) observed that different strains of bean yellow mosaic virus could be distinguished by their nucleolar

Fig. 2. Massive nuclear inclusions, often with radiating crystalline needles (cn) in leaf petiole epidermis of *Pisum sativum* after infection with a necrosis inducing virus isolated from pea.
Magn. × 770 (A, B) and × 1500 (C, D).

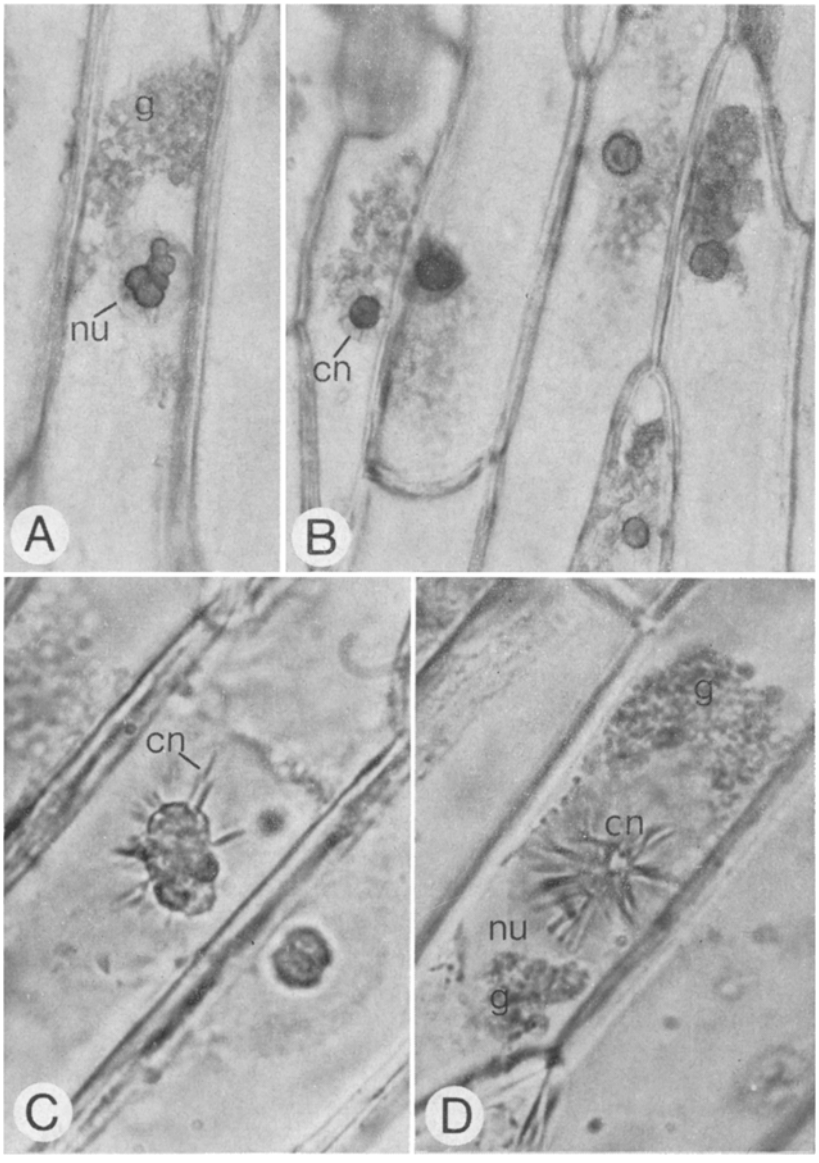

Fig. 2. Omvangrijke kerninsluitsels, vaak voorzien van uitstralende kristalnaalden (cn), in bladsteelepidermis van Pisum sativum na infectie met een necroseverwekkend virus uit erwt.
Vergr. 770 × (A, B) en 1500 × (C, D).

Fig. 3. Inclusions in cytoplasm and nuclei of epidermal cells of leaf petioles of *Pisum sativum* after infection with clover yellow vein virus. (Magn. × 770)

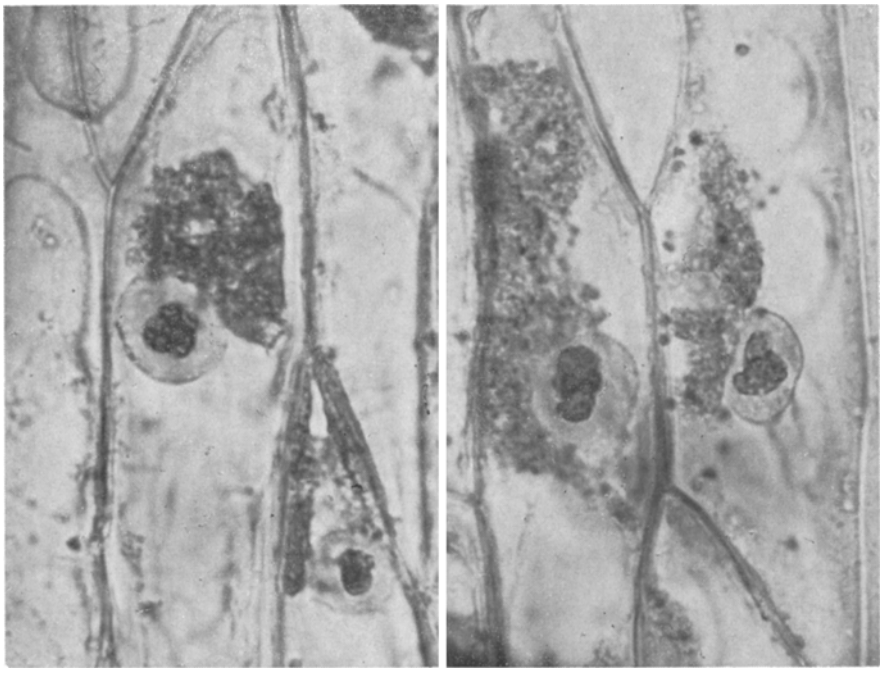

Fig. 3. Insluitsels in cytoplasma en kernen van bladsteelepidermiscellen van Pisum sativum na infectie met het "clover yellow vein virus". (Vergr. 770 ×)

Fig. 4. Inclusions in cytoplasm and nucleus of *Beta vulgaris* (A) and enlargement and "sprouting" of nucleolus of *Pisum sativum* (B, C) after infection with beet mosaic virus (Magn. × 650)

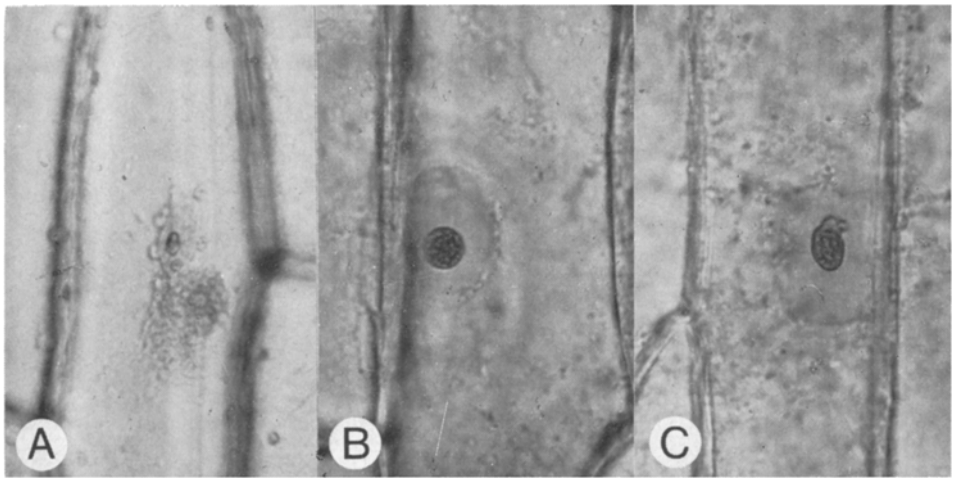

Fig. 4. Insluitsels in cytoplasma en kern van Beta vulgaris (A) en vergroting en "spruiting" van nucleolus van Pisum sativum (B, C) na infectie met bietemozaïekvirus. (Vergr. 650 ×)

inclusions. Nucleolar inclusions caused by my pea necrosis virus were found to be specific and very useful in recognizing the disease.
The nucleolar abnormalities produced by most of the viruses tested were striking and much larger than any hitherto reported. The staining technique used, allowed rapid easy detection of inclusions in cytoplasm and nucleus and I consider it valuable for diagnosis. These results strongly support McWhorter's conclusion (1965) that more virologists should learn to recognize viruses from their signatures in the plant cells.

Acknowledgment

I am greatly indebted to Mr R. E. Labruyère for his kind help in making the photomicrographs.

Samenvatting

Insluitsels van scherpmozaïekvirus van boon, enige minder bekende nauw verwante virussen en bietemozaïekvirus

Uit de literatuur blijkt van een toenemend aantal virussen uit de aardappel-Y-virusgroep dat ze in staat zijn insluitsels in het cytoplasma te veroorzaken. Tot dusver waren het tabaksetsvirus en het scherpmozaïekvirus van boon de enige plantevirussen waarvan bekend was dat ze microscopisch zichtbare kerninsluitsels doen ontstaan.
Met een iets gewijzigde, zeer eenvoudige kleurmethode werden bij aantasting door scherpmozaïekvirus gemakkelijk en snel de grote hoeveelheden, uit de literatuur bekende, meestal korrelige, onregelmatig gevormde celinsluitsels in het cytoplasma van enkele plantesoorten gevonden. Bovendien waren de nucleoli meestal hoekig vergroot. Bij inoculatie met enkele isolaten van dit virus uit erwt leken de kernen echter normaal. Met twee andere werden soms kleine kristalletjes in de nucleolus aangetroffen of sproten de nucleoli gistachtig (Fig. 1).
Een nog niet beschreven, maar verwant virus uit necrotische erwten deed slechts zelden granulaire insluitsels ontstaan, terwijl daarentegen de nucleoli sterk vergroot waren en dan meestal voorzien van merkwaardige, nog niet eerder in de literatuur vermelde uitstralende kristalnaalden (Fig. 2).
De celinsluitsels veroorzaakt door "clover yellow vein virus" leken sterk op die ontstaan door infectie met het scherpmozaïekvirus van boon, maar de nucleoli waren zeer sterk vergroot (Fig. 3). Ook door het bietemozaïekvirus ontstonden granulaire insluitsels en sterk vergrote en vaak spruitende nucleoli (Fig. 4).

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