

Further identification of Hippeastrum Mosaic Virus

D. HENDRINA BRANTS, N. J. FOKKEMA and R. DE BODE

Phytopathologisch Laboratorium "Willie Commelin Scholten", Baarn, The Netherlands

Accepted 8 September 1969

Abstract

Infectious virus was isolated from *Hippeastrum* plants by density gradient centrifugation and examined with the electron microscope. The average lengths of the flexuous particles were 643 ± 24 nm.

It has been reported that *Hippeastrum* plants (Amaryllidaceae) can be infected by at least three viruses: Cucumber Mosaic Virus (CMV), Tomato Spotted Wilt Virus (TSWV) and *Hippeastrum* Mosaic Virus (HMV) (Brants and van den Heuvel, 1965). These viruses sometimes produce rather similar mosaic symptoms in *Hippeastrum* but their host ranges differ. In this study special attention is paid to the last virus.

Gomphrena globosa L. proved to be a local lesion host for HMV. The lesions resemble those caused by Potato Virus X (PVX) on *Gomphrena*. However, lesions caused by PVX are visible 3–4 days after inoculation, while lesions caused by HMV are formed in 10 days at the least.

Two hundred *Hippeastrum* plants were checked on the presence of virus by inoculation of *Gomphrena globosa* with sap pressed from leaves of these plants. According to the symptoms in the plants tested and the results of inoculation the plants could be divided into 4 groups:

- a. Symptoms in *Hippeastrum*, local lesions on *Gomphrena globosa*
- b. Symptoms in *Hippeastrum*, no reaction on *Gomphrena globosa*
- c. No symptoms in *Hippeastrum*, local lesions on *Gomphrena globosa*
- d. No symptoms in *Hippeastrum*, no reaction on *Gomphrena globosa*

An attempt was made to isolate virus from plants of the four groups by using density gradient centrifugation. A saccharose gradient of 0–40% was prepared in a centrifuge tube and sap pressed from frozen *Hippeastrum* leaves was layered on top of the gradient. The tubes were then placed in the swinging-bucket rotor of a Spinco L2 ultracentrifuge and centrifuged for $1\frac{1}{2}$ h at 5°C at 24,500 rpm (rate zonal centrifugation). After centrifugation the zones formed in the tubes were tested for infectivity on *Gomphrena globosa* and examined with the electron microscope. A zone containing infectious material revealed flexuous virus particles. No particles could be demonstrated in zones with non-infectious material. Thus, infectivity was always associated with the presence of visible particles.

Iwaki (1967) also observed virus particles in preparations of *Hippeastrum* plants with mosaic symptoms. Using the dip method, long flexuous thread-like particles

Fig. 1. Particles of the *Hippeastrum* mosaic virus from *Hippeastrum hybridum* (magn. $\times 52,000$).

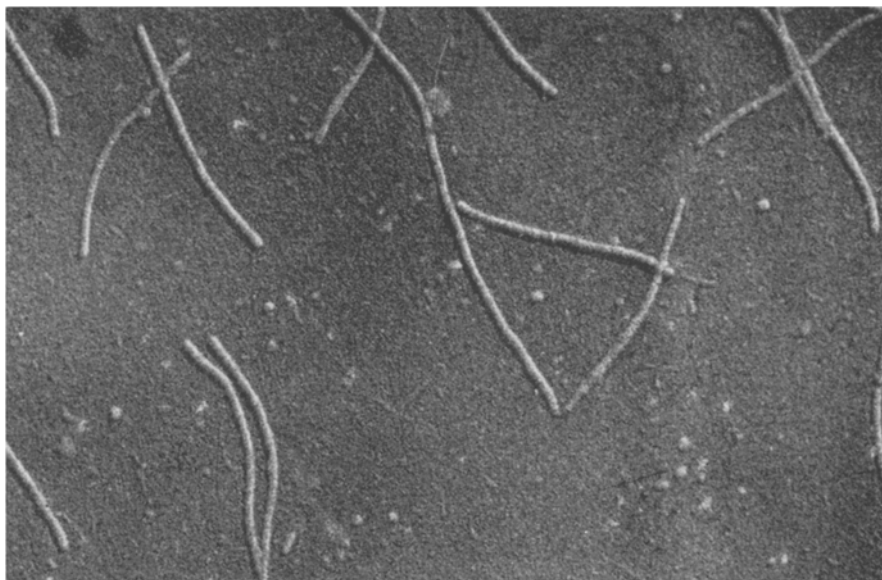


Fig. 1. Deeltjes van het *Hippeastrum*-mozaïekvirus uit *Hippeastrum hybridum* (vergr. 52.000 \times).

were found. The majority of the particles had a length of 600–800 nm, but there was no distinct mode.

We determined the average length of the virus particles in preparations obtained by density gradient centrifugation, using the method described by Brandes (1964). Specimens for the electron microscope were made from infectious zones derived from sap of plants belonging to group a. From the photographs of these preparations the lengths of about 600 flexuous particles were transferred to transparent paper. The virus particles were transferred to transparent paper. The virus particles were measured and classified by length intervals of 1 mm. From these data the average length of a particle could be calculated, being 643 ± 24 nm (Fig. 1).

Plants of group 2 were infected with HMV. It can be concluded that the length of the particles of the *Hippeastrum* mosaic virus is about 643 nm.

Preparations were also made from plants of group b and d, but no infectious material could be obtained. It appears that group d consists of healthy *Hippeastrum* plants. Further work on the isolation of infectious material from plants of group c is in progress.

Acknowledgments

The authors are greatly indebted to the Director and the Staff of the Laboratory for Electron Microscopy of the University of Amsterdam for the hospitality and co-operation and to Dr G. T. N. de Leeuw for taking the photograph.

Samenvatting

Verdere identificatie van het Hippeastrum-mozaïekvirus

Infectieus virus kon worden geïsoleerd uit *Hippeastrum*-planten die mozaïekverschijnselen vertoonden, door middel van centrifugeren in een dichtheidsgradiënt. Het materiaal werd bekeken in een elektronenmicroscop en gefotografeerd. De gemiddelde lengte van de buigzame staafjes van het *Hippeastrum*-mozaïekvirus, berekend uit 600 metingen, bedraagt 643 ± 24 nm.

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