Neth. J. Pl. Path. 82 (1976) 95–102

Two potexviruses in Nerine

D. Z. MAAT

Institute of Phytopathological Research (IPO), Wageningen

Accepted 25 November 1975

Abstract

In *Nerine sarniensis* as well as in *N. manselli* PVX-type virus particles were detected. The virus of *N. sarniensis* could not be transmitted to *Gomphrena globosa*, *Chenopodium amaranticolor*, and *Nicotiana clevelandii*, and only incidentally to *C. quinoa*, in which it multiplied poorly. The virus had an average particle length of 541 nm and was serologically distantly related to clover yellow mosaic virus (ClYMV). It will be named *Nerine* virus X (NVX).

The virus of *N. manselli* could easily be transmitted to *G. globosa*, *C. amaranticolor*, *C. quinoa*, and *N. clevelandii*. The average particle length measured was 554 nm. It is serologically distantly related to PVX, and very closely to narcissus mosaic virus (NaMV). It will be considered as a strain of NaMV.

With their homologous antisera both viruses could be detected in clarified extracts of leaves and flowers of their respective *Nerine* hosts, applying the micro-precipitin test.

Introduction

The cultivation of various species of the bulbous plant genus *Nerine* for cut flower production is rapidly expanding in the Netherlands. The leaves, flower stalks and flowers may show disease symptoms, presumably caused by virus. The present study forms part of a research program to detect and identify possible causal agents of these symptoms and to establish the presence of other viruses in *Nerine*.

Koenig et al. (1973) reported narcissus mosaic virus to occur in *N. bowdenii*. Brunt et al. (1970) observed a ca 550 nm particle in 'Curiosity' and 'R.H.S. Vermilion'. Hakkaart et al. (1975) observed viruslike particles of about 545 nm in *N. sarniensis*. Later the present author detected similar particles in *N. manselli*. This paper deals with the identification of these viruses from *N. sarniensis* and *N. manselli*.

Materials and methods

Source plants. Plants of *N. sarniensis* and *N. manselli* showing various types of symptoms were collected from a nursery and maintained in the greenhouse of the Institute of Phytopathological Research, Wageningen. Of *N. sarniensis* also leaf material was collected and stored in the deep-freezer.

Transmission experiments. Mechanical transmission was studied using crude and partially purified preparations. The latter mainly correspond with fractions B, C, and in case of *N. sarniensis*, also D, mentioned below (virus purification). In addition,

inoculum was prepared by homogenizing leaf material in McIlvaine's phosphate-citric acid buffer at pH 7 (containing 0.1 % thioglycolic acid), diethyl ether and carbon tetrachloride, centrifuging the homogenate at low speed, and concentrating the virus by high-speed centrifuging. Moreover, for *N. sarniensis* clarification took place at pH 9 according to Huttinga (1973), whereafter centrifuging at high speed was applied.

As test plants *N. clevelandii*, *G. globosa*, *C. amaranticolor* and *C. quinoa* were used. They were grown in a temperature-controlled greenhouse at about 20°C. One-year-old seedlings of *Nerine bowdenii* were supplied by Ir F. A. Hakkaart, Research Station for Floriculture, Aalsmeer.

Virus purification. Virus was purified from naturally infected *N. sarniensis* or, as with the virus of *N. manselli*, from *N. clevelandii*. The latter host was usually inoculated twice at a 10 days' interval; the leaves were harvested 7 days after the second inoculation and stored frozen until use.

A buffer of 0.1 M tris(hydroxymethyl)aminomethane adjusted to pH 9 with citric acid and containing 0.1 % sodium thioglycolate and 0.02 M sodium diethyldithiocarbamate was used for homogenizing the leaf material and resuspending the sediment after precipitation with polyethylene glycol 6000 (PEG). In later steps of the procedure sodium thioglycolate and sodium diethyldithiocarbamate were omitted.

Leaf material was homogenized in a Waring blendor, using buffer and organic solvents chilled at 3°C. Unless otherwise stated, centrifuging at low speed was at 8000 rpm for 10 min in a Sorvall RC2-B centrifuge, the rotor used depending on the quantity of material. Centrifuging at high speed to sediment the virus was performed in a Beckman ultracentrifuge for 1 or 1.5 h at 25000 rpm using rotors 30 or 35, respectively, or for 3 h at 25000 rpm when the virus was in sucrose (see below).

For sucrose-gradient centrifuging 10 ml of partially purified virus, obtained from 150 or 200 g of leaf material of *N. clevelandii* or *N. sarniensis*, respectively, was centrifuged for 1 h at 40000 rpm in a Ti-14 zonal rotor containing a sucrose gradient from 10–40 % (weight/volume). Gradients were prepared linear with volume using a Beckman gradient pump. Sucrose-containing virus zones were diluted 1:1 with buffer or distilled water before centrifuging at 25000 rpm for 3 h to concentrate the virus.

Every 100 g of leaf material was homogenized together with 200 ml (for *N. clevelandii*) or 300 ml (for *N. sarniensis*) of buffer, 25 ml diethyl ether and 25 ml carbon tetrachloride. After centrifuging at low speed to the supernatant (A) PEG was added to make a 5 % solution (w/v). The solution was stirred for about 1 h and then centrifuged at 10000 rpm during 15 min. Sedimented material was resuspended in about 175 ml of buffer, stirred during 1 h, and then centrifuged at low speed. The supernatant (B) was centrifuged at high speed and the sedimented virus resuspended in buffer, stirred overnight and centrifuged at low speed. The supernatant thus obtained (C) was subjected to sucrose-gradient centrifuging once (*N. sarniensis*) or (with reconcentration in between) twice (*N. clevelandii*). Final virus-containing zones (D) were ultracentrifuged and sediments resuspended to give about 1.5 ml per 100 g of starting leaf material. Mixed with an equal volume of glycerol the preparations were stored at about –20°C.

Serology. Rabbits were injected with purified preparations of the viruses of *N. sarniensis* and *N. manselli*. One ml was given intravenously in one or two portions (three

days' interval). A second ml was emulsified with an equal volume of Freund's incomplete adjuvant and given intramuscularly two weeks later. After another fortnight bleeding was started and antisera were collected for storage at −20°C until use.

The micro-precipitin test under paraffin oil was applied and, as an extra test for reactions with normal plant antigens, the Ouchterlony double-diffusion test. Ir D. H. M. van Slogteren, Laboratory of Flowerbulb Research at Lisse, kindly provided the antiserum to *Cymbidium* mosaic virus (CyMV), Dr R. Koenig, Abteilung für pflanzliche Virusforschung der BBA at Brunswick, Germany, to narcissus mosaic virus (NaMV), papaya mosaic virus (PaMV), cactus virus X (CVX), and clover yellow mosaic virus (ClYMV). Antisera to potato virus X (PVX), potato aucuba mosaic virus (PAMV) and white clover mosaic virus (WCMV) were from the author's collection.

To prevent reactions with normal plant antigens some of the antisera were absorbed with concentrated sap preparations from virus-free seedlings of *N. bowdenii*. The CyMV used as antigen in the serological tests was from *Lycaste* orchids. Dr R. Koenig supplied Brunt's isolate of NaMV.

To test serological relationships generally purified preparations were used, dilutions of which were made with tris-citric acid buffer at pH 9. Antisera were diluted with saline. As healthy controls concentrated preparations of virus-free *N. clevelandii* and *N. bowdenii* were used.

To test individual *Nerine* plants, clarified extracts were used. Sap was prepared with a power-driven crusher. Four drops of crude sap were collected in a small centrifuge tube containing 0.2 ml of a 1% solution of Na_2SO_3 in distilled water. The tubes were then centrifuged for 5–10 min at 6000 rpm and the supernatants used for serological testing. For these tests antisera were dialysed against tris-citric acid buffer at pH 8 containing 0.05% NaN_3 and diluted with the same buffer.

Electron microscopy. Clarified extracts of *Nerine* prepared according to A (see virus purification), and to the method used for serological testing of individual plants of *Nerine*, were examined with a Philips EM300 after negative staining with 2% phosphotungstic acid in distilled water adjusted with KOH to pH 6.5. Of *G. globosa*, *C. amaranticolor*, *C. quinoa*, and *N. clevelandii* also chop preparations were investigated. Chop preparations with TMV were photographed alternately with preparations containing the *Nerine* viruses. Particles were measured in photographic negatives using a low-power binocular microscope with objective lens × 1 and a micrometer eyepiece × 12.5. The ranges taken for particle length determination were restricted by micrometer classes in which no or only a very few particles were found.

To calculate particle lengths, for every series of photographs the average length expressed in micrometers was calculated and compared with the average length of TMV particles measured from photographs taken immediately before and after and also in between a series of photographs of a *Nerine* specimen. The average length of TMV was assumed to be 300 nm.

Experiments and results

Transmission experiments. Of *N. sarniensis* seven (partially) purified preparations were tested on *G. globosa*, *C. amaranticolor*, *C. quinoa*, and *N. clevelandii*. No symp-

Table 1. Results of micro-precipitin tests, performed to identify two viruses of *Nerine*.

Antigens	Titres of antisera to										
	virus from *Nerine sarniensis*	virus from *Nerine manselli*	narcissus mosaic virus	potato virus X	potato aucuba mosaic virus	white clover mosaic virus	clover yellow mosaic virus	papaya mosaic virus	cactus virus X	*Cymbidium* mosaic virus	normal serum
Virus from *Nerine sarniensis*	1024	4	–	1	1	1	4	–	1	–	1
Virus from *Nerine manselli*	–	4096	1024	16	–	1	–	–	–	–	1
Narcissus mosaic virus-Brunt	–	1024	1024	16	–	1	–	–	1	–	–
Cymbidium mosaic virus	–			–	–	–	–	–	–	16	–
Healthy *Nerine bowdenii*	–	–	–	–	–	–	–	–	–	–	–
Healthy *Nicotiana clevelandii*	4	–	–	4	–	–	1	–	–	–	1
Homologous antigens[1]				4096	4096	4096	1024	512	1024		

[1] Titres determined in separate tests.

Tabel 1. Resultaten van serologische proeven, uitgevoerd ter identificatie van twee virussen van Nerine.

toms were induced. Incidentally only in extracts of *C. quinoa* some PVX-type virus particles were observed. With one exception, however, from these plants no virus could be further transmitted. Only once in extracts of *C. quinoa* inoculated with sap of *C. quinoa* a few virus particles were detected. Virus particles were also observed in preparations of two out of ten seedlings of *N. bowdenii* inoculated with a partially purified preparation. In all cases abundant virus particles were present in the inoculum.

Of *N. manselli* two partially purified preparations at pH 9 and one at pH 7 were tested for infectivity. *G. globosa*, *C. amaranticolor* and *C. quinoa* showed local lesions ten-twelve days after inoculation. Symptoms on *N. clevelandii* were not clear. *G. globosa* and *N. clevelandii* became systemically infected by the virus. In these plants numerous virus particles were detected.

Using crude preparations of *Nerine* no virus could be transmitted.

Serology. The results of the serological experiments performed to identify the two *Nerine* viruses are summarized in Table 1. They suggest the viruses of *N. sarniensis* and *N. manselli* to be distantly related. The virus of *N. sarniensis* also reacts with the antiserum to ClYMV. A close relationship exists between the virus of *N. manselli* and NaMV. The latter two viruses are distantly related to PVX.

With homologous antisera the two *Nerine* viruses could be detected in clarified extracts of leaves as well as of flowers of their respective *Nerine* hosts.

Electron microscopy. Results of particle measurements are given in Table 2. Between the ten samples of *N. sarniensis* average particle lengths varied from 530–554 nm and between the seven samples containing the virus of *N. manselli* from 539–578 nm. Taking the 817 particles of *N. sarniensis* together their average length is about 541 nm and of the 870 particles of the virus of *N. manselli* about 554 nm. Incidentally in extracts of *N. sarniensis*, and more frequently in those of *N. manselli*, some longer particles were also found, indicating a mixture of viruses to be present in these plants. Fig. 1 represents an electron micrograph of a clarified extract of *N. sarniensis*.

Discussion

Average particle lengths varied much among the different samples. Causes of this may have been the use of TMV as an external standard, rather than using it as an internal standard as recommended by Bos (1975). Moreover not all samples were prepared in the same manner and for the virus of *N. manselli* different hosts were used. As a consequence not too much value can be attached to the difference between the average lengths calculated for the two viruses, although a rather large number of particles was measured. Of all samples, however, the average length was within the limits of the potexvirus group (Harrison et al., 1971).

Based on host plant reactions and on serological experiments the virus of *N. sarniensis* differs from other well-known potexviruses. According to its particle length and to its distant serological relationship to ClYMV, however, it can be assumed to belong to this group. The lack of a suitable propagation host as well as of a suitable test plant greatly hampers the determination of other properties.

Unfortunately the symptoms caused by this virus are not yet known, partly because

Table 2. Results of particle length measurements of two *Nerine* viruses, using TMV as an external standard (300 nm).

Virus isolate	Host	Range measured (nm)	Total number of particles measured	Average particle length (nm)	Top at (nm)	Number of particles at top
Nerine sarniensis 110	*Nerine sarniensis*	507–590	54	531	542	23
110		506–586	36	540	533	19
110		537–582	17	554	552	9
110		512–600	161	550		
310		525–580	75	544	545	56
310		535–609	136	552	550	54
110 +310		518–558	92	536	531	52
110 +310		511–550	45	531	524	24
110 +310		504–558	171	530	531	108
110 +310		513–553	30	530	526	18
	total		817	541		
Nerine manselli	*Nerine manselli*	530–584	57	554	540	20
		512–600	142	562	556	49
		525–630	80	578	570	27
	Chenopodium quinoa	502–582	203	543	542	94
	Gomphrena globosa	500–579	102	539	539	59
		507–597	86	552	552	23
	Nicotiana clevelandii	507–621	200	557	552	77
	total		870	554		

Tabel 2. Resultaten van de deeltjeslengtemetingen van twee virussen van Nerine, bij gebruik van TMV als externe standaard (300 nm).

Fig. 1. Electron micrograph of a clarified extract of *N. sarniensis*. Bar represents 500 nm.

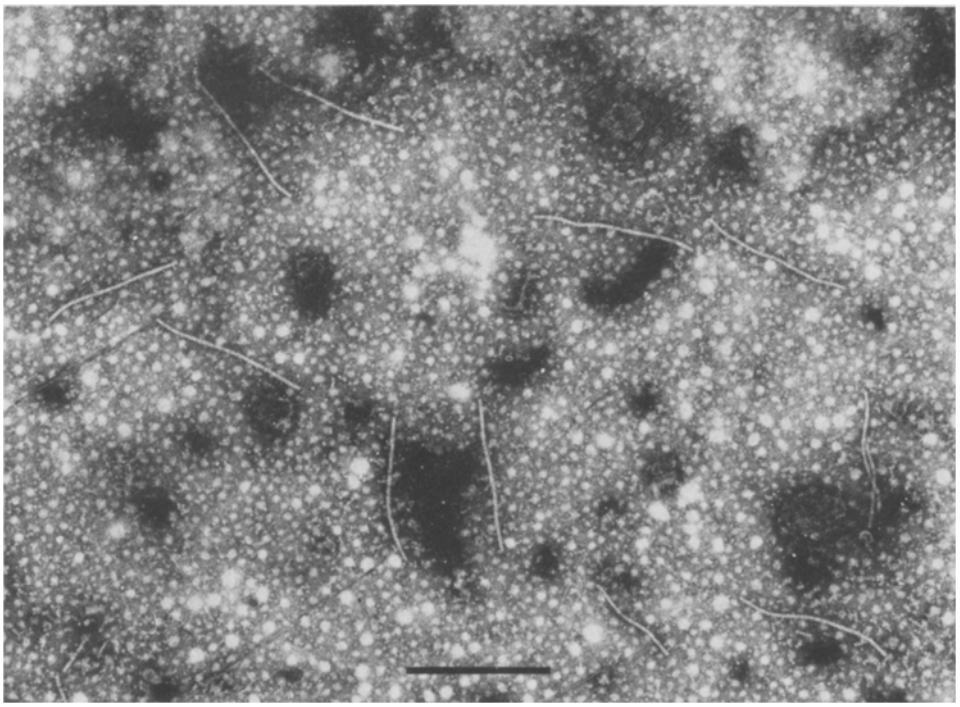

Fig. 1. Elektronenmicroscopische opname van een geklaard sappreparaat van N. sarniensis. De vergrotingsstreep geeft 500 nm weer.

of the fact that more viruses were present in the plants investigated. Therefore, analogous to PVX and CVX, this hitherto undescribed virus is named *Nerine* virus X (NVX).

The results of the serological experiments prove that the virus of *N. manselli* is closely related to NaMV. This is further supported by test plant reactions observed and the average particle length (554 nm) as compared with the literature on NaMV (Brunt, 1966; Mowat, 1971; Koenig et al., 1973). Therefore, the virus of *N. manselli* will be considered a strain of NaMV.

Trials to transmit the NaMV of *N. manselli* to narcissus have not yet been made. According to Brunt (1966) it may take 17 months before NaMV can be detected in mechanically inoculated narcissus seedlings. In experiments to transmit NaMV as well as NVX to *N. bowdenii* so far only NVX could be detected in two out of ten inoculated seedlings, 4 months after inoculation.

According to host plant reactions and serological properties, NVX and NaMV are different viruses. The results of the serological tests (Table 1) suggest a very distant relationship. As in the electron microscope sometimes particles longer than NVX or NaMV have been observed in preparations of *N. sarniensis* and *N. manselli*, it is not excluded that contamination with another common virus might be responsible for this so-called relationship.

Samenvatting

Twee virussen uit de aardappelvirus-X-groep in Nerine

De teelt van *Nerine* als snijbloem maakt in Nederland snel opgang. Dit bolgewas vertoont dikwijls symptomen, die aan virusbesmetting doen denken. In twee soorten, *Nerine sarniensis* en *Nerine manselli*, werden met behulp van de elektronenmicroscoop virusachtige deeltjes aangetroffen, die wat hun lengte betreft overeenkomen met die van virussen behorend tot de aardappelvirus-X-groep.

Het virus van *N. sarniensis* kon niet mechanisch worden overgebracht naar *Gomphrena globosa*, *Chenopodium amaranticolor* en *Nicotiana clevelandii* en slechts met moeite naar *C. quinoa*, waarin het zich echter slecht vermeerderde. Ook kon het worden aangetoond in enkele zaailingen van *Nerine bowdenii*, die vier maanden tevoren met een gezuiverd preparaat van het virus waren geïnoculeerd. Serologisch verschilde dit virus duidelijk van het aardappelvirus X (PVX), het aardappelaucubamozaïekvirus, het witte-klavermozaïekvirus, het papajamozaïekvirus, het cactusvirus X, het *Cymbidium*-mozaïekvirus en het narcissemozaïekvirus (NaMV), terwijl het slechts een geringe verwantschap vertoont met het klaverscherpmozaïekvirus (Tabel 1). Voor het virus werd een gemiddelde deeltjeslengte van 541 nm gemeten (Tabel 2; Fig. 1.) Waarschijnlijk is het een nieuw virus, behorend tot de aardappelvirus-X-groep. Het wordt nu *Nerine*-virus X genoemd.

Het virus uit *N. manselli* bleek vrij gemakkelijk (met behulp van gezuiverde preparaten) over te brengen naar *G. globosa*, *C. amaranticolor*, *C. quinoa* en *N. clevelandii*.

Serologisch bleek dit virus nauw verwant aan het NaMV, terwijl ook een geringe verwantschap met het PVX werd gevonden (Tabel 1). De gemiddelde deeltjeslengte was 554 nm (Tabel 2). Dit virus wordt beschouwd als een stam van het NaMV.

Beide virussen konden met hun eigen antisera worden aangetoond in geklaarde blad- en bloemextracten van *Nerine* met behulp van de micro-precipitatietoets.

References

Bos, L., 1975. The application of TMV particles as an internal magnification standard for determining virus particle sizes with the electron microscope. Neth. J. Pl. Path. 81: 168–175.

Brunt, A. A., 1966. Narcissus mosaic virus. Ann. appl. Biol. 58: 13–23.

Brunt, A. A., Hollings, M. & Stone, O. M., 1970. Rep. Glass house Crops Res. Inst. 1969: 138.

Hakkaart, F. A., Maat, D. Z. & Quak, F., 1975. Viruses and meristem culture of nerine. Acta Hort. 47: 51–53.

Harrison, B. D., Finch, J. T., Gibbs, A. J., Hollings, M., Shepherd, R. J., Valenta, V. & Wetter, C., 1971. Sixteen groups of plant viruses. Virology 45: 356–363.

Huttinga, H., 1973. Properties of viruses of the potyvirus group. 1. A simple method to purify bean yellow mosaic virus, pea mosaic virus, lettuce mosaic virus and potato virus Y^N. Neth. J. Pl. Path. 79: 125–129.

Koenig, R., Lesemann, D., Brunt, A. A. & Kühne, H., 1973. Narcissus mosaic virus found in *Nerine bowdenii*. Identification aided by anomalies in SDS PAGE. Intervirology 1: 348–353.

Mowat, W. P., 1971. Narcissus mosaic virus. CMI/AAB, Descriptions of Plant Viruses. No. 45.

Address

Instituut voor Plantenziektenkundig Onderzoek, Binnenhaven 12, Wageningen, the Netherlands.