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A carlavirus serologically closely related to *Carnation latent virus* in Slovenian garlic

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ABSTRACT

Three carlaviruses have been reported in garlic: *Garlic common latent virus* (GCLV), *Shallot latent virus* (SLV) and a virus closely related to *Carnation latent virus* (CLV), this last reported in Argentina. We found a carlavirus related to CLV (abbreviation CG) in two Slovenian varieties of garlic that reacted to the homologous titre in EM decoration tests with antisera to CLV but reacted less strongly with antiserum to GCLV. A CLV antiserum absorbed with isolate of GCLV still clearly differentiated between CG, CLV and GCLV. The virus could not be separated from GCLV by mechanical inoculation to differential test plants, but the GCLV/CG mixture gave only local lesions on *Chenopodium quinoa* and *C. murale*, and did not infect *Nicotiana clevelandii* whereas CLV from carnation systemically infects both plants. CG particles were present at a level of about 2 % compared with particles of GCLV and may have been overlooked by other investigators, especially as they react quite strongly with GCLV antiserum.

Keywords*:* garlic, carlavirus, immunoelectron microscopy

IZVLEČEK

KARLAVIRUS, SEROLOŠKO SORODEN LATENTNEMU VIRUSU NAGELJA, V SLOVENSKEM ČESNU

Česen okužujejo trije karlavirusi, navadni latentni virus česna (GCLV), latentni virus šalotke (SLV) in virus soroden latentnemu virusu nagelja (CLV). Virus soroden CLV je bil na česnu prvič najden v Argentini. V prispevku poročamo o karlavirusu (v nadaljevanju kot CG) iz dveh slovenskih sort česna. Z imunsko elektronsko mikroskopijo smo ugotovili, da virus reagira do homolognega titra z antiserumom proti CLV in slabše s antiserumom proti GCLV. CLV antiserum, absorbiran z izolatom GCLV, jasno loči med CG, CLV in GCLV. Z mehansko inokulacijo testnih rastlin nismo uspeli ločiti CG od GCLV. Mešanico GCLV/CG smo našli v lokalnih poškodbah na *Chenopodium quinoa* in *C. murale*, nismo pa je našli na inokuliranih rastlinah *Nicotiana clevelandii*, za razliko od CLV iz nagelja, ki obe omenjeni rastlini okužuje sistemsko. Delež CG v mešanici CG/GCLV je bil le okrog 2 % in delci so se močno dekorirali z antiserumom proti GCLV, zato je možno, da je CG prisoten tudi drugod po svetu, vendar so ga zaradi omenjenih lastnosti spregledali.

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Ključne besede: česen, karlavirus, imunska elektronska mikroskopija

1 INTRODUCTION

Garlic is commonly infected with mixtures of different filamentous viruses from genera Allexivirus, Carlavirus and Potyvirus (Barg et al., 1994; Conci et al., 1992; Mavrič et al., 1999; Sumi et al., 1993; Sumi et al., 1999; Tsuneyoshi and Sumi, 1996; Van Dijk, 1991; Van Dijk, 1993a; Van Dijk, 1993b; Walkey, 1990). The most common garlic carlavirus is *Garlic common latent virus* (GCLV), first described in France as garlic latent virus (Delecolle and Lot, 1981). It was later proposed to use the name GCLV to avoid confusion with Japanese Garlic latent virus (GLV) (Van Dijk, 1993a). GCLV was reported on garlic from Asia, Europe, South America (Bellardi et al., 1995; Fajardo et al., 2001; Mavrič et al., 1999; Tsuneyoshi et al., 1998) and recently from North America (Pappu et al., 2005). SLV is another carlavirus infecting garlic (Van Dijk, 1993a). GLV was reported from Asia (Chen et al., 2002; Song et al., 2002; Takaichi et al., 2001; Tsuneyoshi et al., 1998). It differs from SLV by its biological properties (Van Dijk, 1993). It was later confirmed to be a strain of SLV as was already proposed by Van Dijk (Tsuneyoshi et al., 1998). Another carlavirus closely related to *Carnation latent virus* (CLV) (Conci et al., 1992) was reported from Argentina.

During a study of filamentous viruses in Slovenian garlic cultivars, we came across mixed infections of two carlaviruses, one of them was GCLV and the other a virus related to CLV, here referred to as CG. We report on the serological identification of CG.

2 MATERIALS AND METHODS

Garlic material

Naturally infected garlic plants cv. Ptujski jesenski (Pj) and Ptujski spomladanski (Ps) were studied. They came from selected stock grown in the eastern region of Slovenia by the seed company Semenarna Ljubljana. Plants showed light and dark green or yellow leaf stripes.

Test plants

The following plants were mechanically inoculated: *Allium cepa* cv. Belokranjka, *A. porrum* cv. Carentan, *Celosia argentea* var. plumosa "Miss Nippon Mix", *Chenopodium amaranticolor*, *C. murale*, *C. quinoa*, *Nicotiana clevelandii* and *N. occidentalis*.

Virus isolates

A CLV isolate was provided by E. Luisoni (Istituto di Fitovirologia Applicata, CNR, Torino, Italy) and a GCLV isolate was obtained from D.-E. Lesemann (Institut für Pflanzenvirologie, Mikrobiologie und biologische Sicherheit, BBA, Braunschweig, Germany).

Antisera

For immunoelectron microscopy following antisera were used. Antisera to *Onion yellow dwarf virus* (OYDV; As 8639), *Leek yellow stripe virus* (LYSV; As 8262), SLV (As 7482) and CLV (As 7665) were provided by D.Z. Maat (Plant Research International, Research Institute for Plant Protection, Wageningen) and antisera to GCLV (As 892), SLV (As 944) and LYSV (As 1035) were provided by D.-E. Lesemann (Institut für Pflanzenvirologie, Mikrobiologie und biologische Sicherheit, BBA, Braunschweig, Germany). A second antiserum to CLV (A 178) came from E. Luisoni (Istituto di Fitovirologia Applicata, CNR, Torino, Italy).

The antisera to LYSV and OYDV used for ELISA were commercial products from Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France.

Mechanical inoculation
Leaves of infected garlic samples were extracted in 0.1 M phosphate buffer pH 7 or in 0.05 M phosphate buffer pH 7 plus 0.1% (w/v) cysteine hydrochloride, and inoculated to leaves of at least 36 individuals of each test plant species. After inoculation the plants were placed in growth chambers at 23-25°C, 16 h illumination at 50 $\mu Em^{-2}s^{-1}$ and 70 % humidity and observed for symptoms.

Electron Microscopy
For negative staining, parts of leaves or cloves were homogenized in a small amount of 0.1 M phosphate buffer pH 7 and the extracts were applied to carbon-coated Formvar filmed grids. They were rinsed with distilled water, negatively stained with 1 % uranyl acetate, and viewed in a JEOL 1200 EX II transmission electron microscope.
For decoration, plant extracts were incubated on grids for five minutes followed by antiserum incubation for 15 minutes. Antiserum titres were obtained for different virus-antiserum combinations. For ISEM, antiserum diluted 1/1000 was incubated on grids for 5 minutes and plant sap for another 5 minutes. Grids were then rinsed and stained, or decorated before staining. Inoculated test plants were checked for the presence of different viruses by decoration, or ISEM followed by decoration (ISEM-D).

Antiserum absorption
CLV antiserum from Torino was absorbed with GCLV as follows. 8 mg of dry GCLV-infected *C. quinoa* leaf was homogenized in 900 µl of 0.1 M phosphate buffer pH 7 and 50 µl of antiserum were added. The mixture was incubated overnight at room temperature, and after centrifugation for 13 minutes at 10500 g, the supernatant was used for ISEM and decoration. In decoration tests this dilution of antiserum was labelled as 1/1.

ELISA
Double antibody-sandwich (DAS) ELISA (Clark & Adams, 1977) was used for detecting OYDV and LYSV in infected garlic plants, using Sanofi reagents.

3 RESULTS

Mechanical inoculation

The two buffers used for mechanical inoculation gave the same results.

All *C. quinoa* plants reacted with chlorotic local lessions 7 - 11 days after inoculation. Decoration tests on homogenized single lesions showed the presence of GCLV alone or mixed with CG. Potyviruses were thus eliminated from the mixed infection by mechanical inoculation.

Local lesions on *C. amaranticolor* developed 15 - 20 days after inoculation and contained GCLV and allexiviruses. Allexiviruses were identified by their typical morphology. In local lesions which appeared 5 - 10 days after inoculation on *C. murale* we detected GCLV, GCLV plus CG or allexiviruses.

Local lessions on *Celosia argentea*, appearing as large chlorotic spots, started to develop 14 days after inoculation. They contained only GCLV. One *N. occidentalis* plant was infected with GCLV and showed systemic symptoms. All other inoculated *N. occidentalis* and *N. clevelandii* plants showed no symptoms.

GCLV was found in 9 of 53 inoculated leek plants and in 6 of 33 inoculated onion plants, while no CG was observed. All inoculated plants were checked for the presence of OYDV and LYSV by ELISA, but no infections were found.

Electron microscopy

EM decoration and titration experiments showed that garlic cultivar Ps was infected mostly with OYDV, LYSV, GCLV and small amounts of CG. Cultivar Pj was infected with OYDV, GCLV and small amounts of CG. Occasionally plants of both cultivars were found infected with SLV or allexiviruses.

CG reacted up to the homologous titres with both CLV antisera. It was present in all tested garlic plants at very low levels. It also reacted with GCLV antiserum (Table 1). Using CLV antisera diluted 1/500 for decoration we observed a few strongly decorated particles among a great majority of weakly or not decorated particles, indicating that the strongly decorated particles were those of a virus serologically close to CLV and more distantly related to GCLV. To test this hypothesis the titres of the CLV and GCLV antisera with their respective viruses and with particles from the two Slovenian garlic cvs. (Table 1) were determined.

Table 1. Reciprocal EM decoration titres of an antiserum to GCLV and two antisera to CLV, in tests with the respective viruses and a virus population in the garlic cv. Ptujski spomladanski. Data for the cv Ptujski jesenski were closely similar.

	viruses from garlic		virus isolates	
As	98% (GCLV)	2% (CG)	CLV	GCLV
GCLV (Lesemann)	3200	8	64	2048
CLV (Luisoni)	512	4096	4096	256
CLV (Maat)	1024	4096	4096	512

In sap of infected garlic two titres for each of the CLV antisera were obtained. The lower titre was given by the particles forming the majority of the virus population and the higher titre was given by particles estimated to represent about 2% of the population. On the basis of these results we assumed that CLV antiserum cross-reacted with GCLV which is usually present in garlic, while about 2% of particles belonged to another carlavirus, CG.

Isolation of CG by inoculation of test plants did not succeed, so we tried to differentiate between the two carlaviruses using CLV antiserum absorbed with GCLV isolate. Absorbed antiserum no longer reacted with GCLV particles but still reacted with CLV up to the dilution 1/128 and with CG up to the dilution 1/16.

4 DISCUSSION

All garlic plants tested contained OYDV, thought to be the main cause of the symptoms. In addition, two carlaviruses were always present in diseased garlic plants, CLV-related virus here referred to as CG, and GCLV. CG could not be separated from GCLV by mechanical inoculation to differential test plants. The GCLV/CG mixture gave only local lesions on *Chenopodium quinoa* and *C. murale*, and did not infect *Nicotiana clevelandii* whereas CLV from carnation systemically infects both plants. We could isolate GCLV in some local lesions on test plants but that was not possible for CG. This could indicate that for infection, CG depends on the presence of GCLV. However infection of test plants may be difficult due to its low concentration in garlic.
The results of EM study confirm that three serologically different carlaviruses are present in the garlic studied in Slovenia SLV, GCLV and CG. GCLV and CG were present in all tested plants. Decoration with GCLV-absorbed CLV antiserum clearly differentiated between GCLV, CLV and CG.

A carlavirus from garlic with a similar reaction to CLV antiserum was reported from Argentina (Conci et al., 1992) but the virus was not present in such low concentration in that case. Also the reactivity with GCLV antiserum was not checked so we can not conclude that the virus from Argentina and CG are the same viruses. Further molecular analyses would be needed to establish the relationship between CG, CLV and CLV-related virus from Argentina.

Since CG was widely distributed in Slovenian garlic we suppose it could also be present together with GCLV in garlic from other parts of the world. Because it is present at such low levels and also serologically related to GCLV, it could easily have been overlooked.

The separation of CG from GCLV on test plants was not possible, so we have no direct evidence that CG is responsible for the development of local lesions on inoculated *Chenopodium* plants. Because both viruses were always present together it is possible that CG could be somehow dependent on GCLV for its multiplication or movement within and between plants.

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