European Journal of Plant Pathology **100**: 269–277, 1994.

Short communication

New mite-borne virus isolates from rakkyo, shallot and wild leek species

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Accepted 15 June 1994

Key words: *Aceria tulipae*, *Allium ampeloprasum*, *Allium cepa* var. *ascalonicum*, *Allium chinense*, *Allium commutatum*, onion mite-borne latent virus, onion mosaic, shallot mite-borne latent virus, shallot virus X

Abstract. Flexuous viruses were transmitted from rakkyo (*Allium chinense*) and wild leek species (especially *A. commutatum*) to plants of crow garlic (*A. vineale*), by transfer of dry bulb mites. By electron microscope decoration tests using three antisera and by inoculations onto test plants, it was concluded that from each of the two natural host species at least two viruses were isolated. The viruses from wild leeks are both pathogenic on *Allium* spp. and may be of economic importance. Decoration tests on a virus mixture from shallot obtained earlier, revealed another new mite-borne virus in this species. The mite-borne viruses of *Allium* spp. appear to be very common; they are largely diverse and their identification remains difficult.

The eryophyid mite *Aceria tulipae* (syn. *Eriophyes tulipae*; dry bulb mite or wheat curl mite) is a wide-spread pest of *Gramineae* and *Liliaceae*, including several *Allium* species [Conijn and Van Aartrijk, 1994]. During a recent virus survey at IPO-DLO, bulbs of garlic (*A. sativum*) and shallot (*A. cepa* var. *ascalonicum*) were often found infested by *A. tulipae*. The mite was shown to be the vector of two new viruses, viz. onion mite-borne latent virus (OMbLV) of onion (*A. cepa* var. *cepa*) and shallot, including a garlic strain of the virus (OMbLV-G) of garlic, and shallot mite-borne latent virus (SMbLV) of shallot [Van Dijk et al., 1991]. Based on the then available information on *Potyviridae*, both viruses were classified into the genus *Rymovirus* of the family *Potyviridae*. 'Shallot virus X', more recently reported in Russia [Kanyuka et al., 1992; Vishnichenko et al., 1993] could be re-identified as OMbLV, SMbLV, or a complex of the two viruses by decoration electron microscopy with antiserum to recombinant coat protein of 'shallot virus X' [Van Dijk, 1993a]. These experiments further revealed the existence of a third mite-borne virus of shallot [Van Dijk, 1994]. Research in Germany on mite-borne virus isolates from *Allium* spp. led to a distinction of at least five serologically different types. These types, including OMbLV and SMbLV, could not be classified as rymoviruses because no cytoplasmatic pinwheel inclusions were found there in ultrathin sections [Barg et al., 1994], and 'shallot virus X' had in Russia been shown

to contain nucleotide sequence homology to the carla- and potexviruses but not to the *Potyviridae* [Kanyuka et al., 1992]. Independent Japanese molecular biological investigations on unidentified but probably similar viruses from mosaic-diseased garlic have provided further evidence that those viruses constitute a new virus group closely related to the carlaviruses [Sumi et al., 1993].

Decoration tests with antiserum to shallot latent virus (SLV) during a survey for carlaviruses in rakkyo (*A. chinense*), revealed decorated particles of SLV and Sint-Jan's-onion latent virus, and non-decorated highly flexuous particles that in further experiments appeared to be of a complex of mite-borne viruses [Van Dijk, 1993b, 1994]. A collection of wild leeks (wild species of the *Allium ampeloprasum* complex), maintained by vegetative propagation at the DLO Centre for Plant Breeding and Reproduction Research (CPRO-DLO), Wageningen, the Netherlands, also contained a mite-borne virus complex [Van Dijk, 1993a, 1994].

This paper now reports on the biological isolation of these new virus isolates from rakkyo and wild leeks by mite transfer. It tentatively characterizes these isolates and the new virus from shallot by host reactions and reactions with three antisera in comparison with some earlier described isolates of OMbLV and SMbLV (Table 1). Details of the decoration method and the cultivars and accessions of test plants used, have been mentioned earlier [Van Dijk et al., 1991]. Plants of rakkyo and *A. commutatum* were naturally infected, and other *Allium* spp. were usually inoculated by transfer of mites. Antisera used were the 'garlic latent virus' antiserum from Dr K. Graichen (Institut für Phytopathologie, Aschersleben, Germany), also containing antibodies to OMbLV-G [Van Dijk et al., 1991], and two antisera from Dr S.K. Zavriev (Institute of Agricultural Biotechnology, Moscow, Russia) to 'shallot virus X, one to a partially purified virus preparation and one to recombinant coat protein (Table 1).

Onion mite-borne latent virus (OMbLV) and shallot mite-borne latent virus (SMbLV)

SMbLV differs from OMbLV by not infecting leek (*A. ampeloprasum* var. *porrum*), not inducing local lesions in *Chenopodium* spp., and not reacting to the 'garlic latent virus' antiserum (OMbLV-G antibodies) [Van Dijk et al., 1991]. Decoration studies showed only weak decoration of OMbLV isolates Ac111 and Ac123II with the 'garlic latent virus' antiserum. Isolate Ac257II reacted only very weakly with this antiserum, if at all, in contrast to the medium rate of decoration reported earlier [Van Dijk et al., 1991]. All isolates were strongly decorated with the two 'shallot virus X' antisera (Table 1). Isolates Ac204II and Ac268 of SMbLV did not react to the 'garlic latent virus' antiserum, but could not be differentiated from OMbLV when the 'shallot virus X' antisera were used (Table 1). Using a dilution series of the antiserum raised against the coat protein expressed in

Escherichia coli, in decoration experiments, isolate Ac268 (SMbLV) was still clearly decorated with 1/16 to 1/32 dilutions of the antiserum while isolate Ac257II (OMbLV) showed only weak decoration with 1/8 dilution and no decoration with 1/16 and 1/32 dilutions. These, and previously reported results [Van Dijk, 1993a], suggest that OMbLV and SMbLV are distinct viruses. However, they are serologically related and 'shallot virus X' is most likely a complex of OMbLV and SMbLV [see also Van Dijk, 1993a]. The decoration titre studies suggest that the coat protein expressed in *E. coli* is probably that of SMbLV but most likely contains epitopes also shared by OMbLV.

The new shallot virus

'Shallot virus X' was isolated in Russia from the Mongolian shallot cv. Tagar [Vishnichenko et al., 1993]. A mixture of Ac183I and Ac183III had in the Netherlands been isolated by mite transfer from a Russian shallot sample [Van Dijk et al., 1991], which likely was the same accession. Ac183I was identified as OMbLV because it induced local lesions in *C. murale* and reacted to a medium degree with the German antiserum (OMbLV-G antibodies). Presence of non-decorated particles in the culture of Ac183I had led to the conclusion that SMbLV (Ac183III) was also present [Van Dijk et al., 1991]. However, Ac183III now appears to differ from SMbLV. When the mixture of Ac183I and Ac183III was tested with the two 'shallot virus X' antisera, both isolates strongly reacted with the antiserum to the virus preparation, and Ac183I strongly reacted with the antiserum to recombinant coat protein, but Ac183III did not react at all with the latter. Particles of Ac183I were not decorated and those of Ac183III to a medium degree only, by the German antiserum. They were decorated strongly and to a medium degree, respectively, when the German antiserum and the Russian antiserum to coat protein were mixed. This leads to the conclusion that Ac183I is OMbLV or SMbLV (earlier identified as OMbLV), and that Ac183III is a new virus reacting to a higher degree to the German antiserum than OMbLV, and not reacting to the Russian antiserum to recombinant coat protein (Table 1). The reaction of Ac183III with the Russian antiserum to the partially purified virus preparation may indicate that the preparation also contained the new virus. Other decoration studies with the antiserum had shown the presence of additional antibodies to the carlavirus SLV [P. van Dijk, unpubl.]. The reaction of Ac183III with the German antiserum suggests that the new shallot virus, or a serologically related virus, also occurs in garlic.

The virus complex from wild leeks

Most of the collection of wild leeks at CPRO-DLO had been collected by Von Bothmer [1974] in the Mediterranean region (IPO-DLO samples

Table 1. Tentative characterization of five groups of mite-borne virus isolates from rakkyo (*Allium chinense*), shallot (*A. cepa* var. *ascalonicum*), and wild leeks (*A. ampeloprasum* and *A. commutatum*), in comparison with onion mite-borne latent virus (OMbLV) and shallot mite-borne latent virus (SMbLV), by their reactions in test plants and with three antisera

Differentiation by	Virus Isolates from						
	Shallot			Wild Leeks		Rakkyo	
	OMbLV: Ac111, Ac123II, Ac257II	SMbLV: Ac204II, Ac268	Ac183III	Aa147I, Aa179, Aa181	Aa147II, Aa149, Aa164	Ach14III, Ach15II	Ach15III
Test plants[1]							
Allium ampeloprasum var. *porrum* (leek)	•[2] s	• –	• –*	• –	• –	• •	• •
A. cepa var. *cepa* (onion)	• (S)	• (S)	• s	• –*	• •	• •	• •
A. chinense (rakkyo)	• •[3]	• •[3]	• •	• •	• •	• s	• s
A. commutatum (wild leek)	• •	• •	• •	• S	• S	• •	• •
A. vineale (crow garlic)	• s	• s	• •	• s	• s	• s	• s
Atriplex hortensis	(L) –*	• •	• •	L –*	–* –*	• •	–* –*
Celosia argentea var. *plumosa*	–* –*	–* –*	–* –*	–* –*	–* –*	• •	–* –*
Chenopodium amaranticolor	(L) –*	–* –*	–* –*	L –*	–* –*	• •	–* –*
C. murale	L –*	–* –*	• –*	L –*	–* –*	• •	L –*
C. quinoa	–* –*	–* –*	–* –*	L –*	–* –*	–* –*	–* –*
Nicotiana benthamiana	–* –*	–* –	–* –*	–* –*	• •	• •	• •
N. hesperis	–* –*	–* –	–* –*	–* –*	• •	• •	• •
N. occidentalis	l –*	–* –*	–* –*	–* –*	• •	• •	• •
Vicia faba (broad bean)	–* –*	–* –*	• •	–* –*	• •	• •	• •

Table 1. Continued

Differentiation by	Virus Isolates from						
	Shallot			Wild Leeks		Rakkyo	
	OMbLV: Ac111, Ac123II, Ac257II	SMbLV: Ac204II, Ac268	Ac183III	Aa147I, Aa179, Aa181	Aa147II, Aa149, Aa164	Ach14III, Ach15II	Ach15III
Antisera[4]							
'Garlic latent virus'	D±/D–[5]	D–	D+	D–	D–	D–	D+
'Shallot virus X' (virus preparation)	D+	D+	D+	D–	D+	D+	D+
'Shallot virus X' (recombinant coat protein)	D+	D+	D–	D–	D+	D+	D–

[1] For cultivars and accessions see Van Dijk et al. [1991].

[2] The first and second symbol denote the reaction of inoculated and non-inoculated leaves, respectively: L = local lesions; l = latent local infection; S = systemic symptoms; s = latent systemic infection; – = no infection; –* = no symptoms, but not tested for latent infection; ( ) = reaction very poor or variable; • = not tested.

[3] Rakkyo mentioned in an earlier publication [Van Dijk et al., 1991] concerned Utrechtse Sint-Jan's onion (unidentified *Allium* species) [Van Dijk, 1993a].

[4] Antisera suppliers and reidentification of viruses indicated by quotation marks as follows:
- 'garlic latent virus' (Dr K. Graichen, Institut für Phytopathologie, Aschersleben, Germany), reidentified as a mixture of the carlavirus garlic common latent virus and the garlic strain of OMbLV [Van Dijk et al., 1991; Van Dijk, 1993b];
- 'shallot virus X' (Dr S.K. Zavriev, Institute of Agricultural Biotechnology, Moscow, Russia), reidentified as OMbLV, SMbLV, or a mixture of both viruses [Van Dijk, 1993a; Van Dijk and Van der Vlugt, this paper]; antiserum to partially purified virus preparation and antiserum to recombinant coat protein;

[5] Reactions in electron microscope decoration tests as follows: D+ = strong to medium rate of decoration; D± = weak decoration; D– = no decoration.

Aa147–178). Some morphologically similar accessions had been collected by CPRO-DLO in former Yugoslavia (Aa179, Aa181) and Pakistan (Aa180) in the 1980s. Accessions were identified as *A. commutatum* and to a lesser extent as *A. ampeloprasum*, *A. bourgeaui*, and *A. porrum*, all belonging to the *A. ampeloprasum* complex [Von Bothmer, 1974]. Of 89 plants investigated – identified as *A. ampeloprasum* and *A. commutatum*, or not yet identified – 29 showed clear elongate stripes (Fig. 1a). None of the plants reacted in ELISA with antiserum to LYSV [Van Dijk, 1993a]. *C. quinoa* reacted with chlorotic local lesions (Fig. 1b) about one week after inoculation with sap from plants which all but one (Aa164) showed symptoms, and not with sap from symptomless plants. Striped plants contained highly flexuous virus particles that were absent in symptomless plants.

Sprouting bulbs, stored at CPRO-DLO for about four months at 20 °C, only incidentally contained single or very few mites. Therefore, mites reared on tulip cv. Yokohama at the Bulb Research Centre, Lisse, the Netherlands, were used for experimental virus transmission. No virus particles were detected in those tulip bulbs when investigated by electron microscopy. Slices of heavily mite-infested scales of tulip bulbs were laid upside down on tops of wild leek bulbs placed in glass beakers. The beakers were thereafter put in cardboard boxes and stored in the dark at ca. 30 °C. After one week, moderate to low numbers of mites were present on

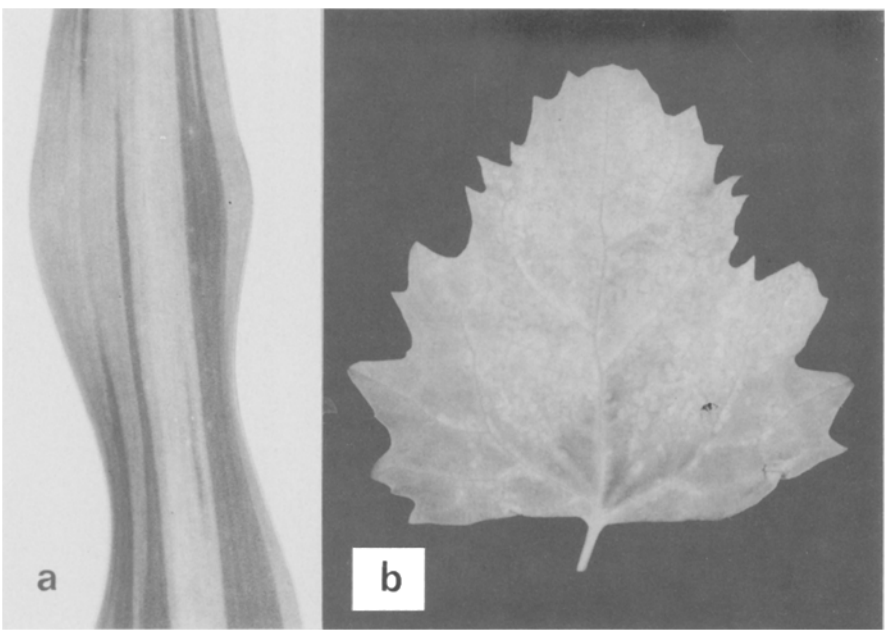

Fig. 1. Chlorotic stripes in leaf of a wild leek species from Pakistan (sample Aa180) due to mite-borne viruses (a), and chlorotic local lesions in leaf of *Chenopodium quinoa* three weeks after inoculation with one of these viruses (isolate Aa179) (b).

the wild leek sprouts and on adjacent parts of fleshy scales. The mites moved onto small plants of crow garlic (*A. vineale*) and leek after placing the infested bulb parts in paper cones around the leaves of these test plants as in earlier experiments [Van Dijk et al., 1991]. Electron microscopy at least two weeks later showed high concentrations of very flexuous particles in part of the crow-garlic plants, but no virus particles in leek. Flexuous viruses are not known to occur in crow garlic in the Netherlands [Van Dijk et al., 1991; Van Dijk, 1993a,b]. The antisera to 'shallot virus X' reacted only to part of the flexuous particles in the crow garlic plants, or to the particles in one plant and not to those in a second one. Virus isolates from selected crow garlic plants and wild leek plants containing only one type of particles were studied further by sap inoculation onto a limited number of test plants and by serology, and could be divided into two groups (Table 1). The first group including Aa147I and Aa181 induced local lesions in *Atriplex hortensis* and *Chenopodium* spp., and they did not react with any of the three antisera. The second group including Aa147II and Aa164, did not react in any of the test plants and reacted only with the antisera to 'shallot virus X'. Clear symptoms of plants singly infected by Aa181 and Aa164 indicate that both groups induce streaking in wild leek. Only one of the groups has serological similarities with OMbLV and SMbLV (Table 1).

The virus complex from rakkyo

Sprouting rakkyo bulb samples Ach14 and Ach15, originating from Thailand and Indonesia, respectively, were not infested by mites. Mites from tulip were transferred for virus transmission as described above. Poor survival of mites on the rakkyo sprouts prevented virus isolation from Ach14, but isolates Ach15II and Ach15III, differing in their reaction with the antiserum to recombinant coat protein of 'shallot virus X' and with the German antiserum (Table 1), were transmitted from the other sample in mixed or single infections to five crow garlic plants. Sample Ach14 contained isolate Ach14III, resembling Ach15II in serology, mixed with carlaviruses. The scarce data obtained suggest serological similarities of Ach15II and Ach14III with OMbLV, SMbLV, and one of the isolate groups from wild leeks, and of Ach15III with the new shallot virus Ac183III (Table 1).

Virus isolation by transfer of mites at Wageningen [Van Dijk et al., 1991; Van Dijk and Van der Vlugt, this paper], and investigations in Germany [Barg et al., 1994] and probably also those in Japan [Sumi et al., 1993], show that filamentous mite-borne viruses are common in vegetatively propagated *Allium* spp., and that their identification is complicated. Their occurrence in complexes implies that great care must be taken to ensure purity of virus cultures for identification and antiserum production. Purity of the new virus isolates reported here, and of isolates of SMbLV, OMbLV, and OMbLV-G described earlier [Van Dijk et al., 1991], is uncer-

tain, and further precision is therefore needed. Biological and molecular-biological approaches are both essential. Some viruses can be separated from complexes by repeated transfer via single lesions of *Chenopodium* spp. Other viruses may be filtered out by repeated transfer of single mites or by repeated transfer in sap of low infectivity. Crow garlic has proved highly susceptible to all *Allium* viruses isolated at IPO-DLO [Van Dijk et al., 1991; Van Dijk, 1993a,b], including all of the present isolates tested on this species (Table 1). The species therefore is often indispensable for virus isolation and culture. Culturing of dry bulb mites on tulip 'Yokohama' appeared useful for virus transmission from hosts with insufficient natural mite infestation.

The new isolates from wild leeks, inducing clear striping in their natural host, are of special interest. Their isolation has unambiguously shown that not all mite-borne *Allium* viruses are latent and that they may be of economic importance. A mosaic disease of a vegetatively propagated leek type in the region Krasnodar of the former USSR was also associated with a mite-borne virus [Galochkina and Ivashchenko, 1981]. Mites were numerous there on bulbs during storage and on leaves in the field, and the wide dissemination of the disease was ascribed to spread of the mites by air. The wild leek viruses now isolated in Wageningen likely are the cause of the disease of the leek type in Krasnodar. They do not endanger leek culture in Western Europe because the leek cultivated there is resistant to them and is not vegetatively propagated. The rigid host specialization of the mite-borne filamentous viruses of *Allium* resembles the specialization of the potyviruses of these species, where the resistance of leek to the strain of LYSV present in great-headed garlic (*A. ampeloprasum* var. *ampeloprasum*) was striking [Van Dijk, 1993a].

Acknowledgements

The authors would like to thank Ir Q.P.M. van der Meer and Ir W.D. Smilde for providing samples of rakkyo and wild leeks, respectively, Mr C.G.M. Conijn for providing *Aceria tulipae* on tulip bulbs, Drs K. Graichen and S.K. Zavriev for supplying antisera, Mr C. Cuperus for technical assistance and Dr L. Bos for critically reading the manuscript.

References

Barg E, Lesemann D-E, Vetten HJ and Green SK (1994) Identification, partial characterization and distribution of viruses infecting *Allium* crops in south and south-east Asia. Acta Horticulturae 358: 251–258

Conijn CGM and Van Aartrijk J (1994) Flower bulbs. In: Lindquist E, Helle W and Sabelis MW (eds.) Eriophyid mites: their biology, natural enemies and control. World Crop Pests, Volume 6 (in press), Elsevier, Amsterdam

Galochkina LA and Ivashchenko II (1981) Onion mosaic in leek. Zashchita Rastenii 10: 29 (in Russian)

Kanyuka KV, Vishnichenko VK, Levay KE, Kondrikov DYu, Ryabov EV and Zavriev SK (1992) Nucleotide sequence of shallot virus X RNA reveals a 5′-proximal cistron closely related to those of the potexviruses and a unique arrangement of the 3′-proximal cistrons. Journal of General Virology 73: 2553–2560

Sumi S-I, Tsuneyoshi T and Furutani H (1993) Novel rod-shaped viruses isolated from garlic, *Allium sativum*, possessing a unique genome organization. Journal of General Virology 74: 1879–1885

Van Dijk P (1993a) Survey and characterization of potyviruses and their strains of *Allium* species. Netherlands Journal of Plant Pathology 99, Supplement 2: 1–48

Van Dijk P (1993b) Carlavirus isolates from cultivated *Allium* species represent three viruses. Netherlands Journal of Plant Pathology 99: 233–257

Van Dijk P (1994) Virus diseases of *Allium* species and prospects for their control. Acta Horticulturae 358: 299–306

Van Dijk P, Verbeek M and Bos L (1991) Mite-borne virus isolates from cultivated *Allium* species, and their classification into two new rymoviruses in the family *Potyviridae*. Netherlands Journal of Plant Pathology 97: 381–399

Vishnichenko VK, Konareva TN and Zavriev SK (1993) A new filamentous virus in shallot. Plant Pathology 42: 12–126

Von Bothmer R (1974) Studies in the Aegean flora XXI. Biosystematic studies in the *Allium ampeloprasum* complex. Opera Botanica 34: 1–108