# Characteristics of a Potyvirus Associated with a Mosaic-like Disease of Yellow Oat-grass

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**Abstract**

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The properties of a new filamentous virus found on yellow oat-grass (suggested name *Trisetum flavescens mosaic virus* – TFMV) were compared with those of the two potyviruses *Oat mosaic virus (*OMV) and *Oat necrotic mottle virus* (ONMV). The latter viruses were chosen because their host range, virus particles and some other characteristics are similar to TFMV. Mechanical transmission of TFMV to some OMV and ONMV host plants, drop precipitation, indirect-ELISA, DAS-ELISA, SSEM and RT-PCR were used in the study. However, there was no proof that TFMV is identical with OMV or ONMV. *Avena abyssinica* and *Bromus mollis* were found to be new experimental hosts of TFMV.

**Keywords**: yellow oat-grass virus; host range; electron microscopy; ELISA; SSEM; RT-PCR

A new, previously not described virus was isolated from yellow oat-grass [*Trisetum flavescens* (L.) P. Beauv.] from 23 localities of the Czech Republic by Vacke *et al*. (2000). The virus caused streak mosaic in leaves (Figure 1), leaf sheaths and seed covers. Filamentous particles with an average size of 650 × 12 nm were observed in crude sap from leaves by electron microscope. The cylindrical inclusions (pinwheels), characteristic for the family *Potyviridae*, were observed by electron microscope in ultra-thin sections from leaves. The virus was mechanically inoculated to species of cereals, grasses and some dicotyledonous plants. *Avena barbata* (Pott.), *A. fatua* (L.), *A. ludoviciana* (Dur.), *A. nuda* var. *chinensis* (L.), *A. sativa* (L.), *A. sterilis* (L.) and *Lagurus ovatus* (L.) were found to be hosts of the virus. While *Agrostis tenuis* (Sibth.), *Alopecurus pratensis* (L.), *Arrhenatherum elatius* (L.) P. Beauv., *Bromus mollis* (L.), *B. tectorum* (L.), *Dactylis glomerata* (L.), *Deschampsia caespitosa* (L.) P. Beauv., *Hordeum vulgare* (L.), *Lolium multiflorum* (Lam.), *L. perenne* (L.), *Phleum pratense* (L.), *Poa annua* (L.), *P. pratensis* (L.) and *Triticum aestivum* (L.) were found not to be hosts of TFMV. The same was proven for *Cucumis sativus* (L.), *Chenopodium quinoa* (Wild.), *Phaseolus vulgaris* (L.) and *Nicotiana tabacum* (L.). Transmission of the virus by the aphid

Figure 1. Leaves of yellow oat-grass with symptoms caused by TFMV (first four above), and healthy leaf (at the bottom)

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species *Rhopalosiphum padi* (L.) and *Sitobion avenae* (F.) and the beetles *Oulema melanopus* (L.) and *O. lichenis* (Voet.) failed (Vacke *et al*. 2000).

The aim of the present study was to characterise the causal agent of the mosaic disease of yellow oat-grass. The possible identity with two potyviruses infecting oats, *Oat mosaic virus*, OMV (*Potyviridae*: *Bymovirus*) and *Oat necrotic mottle virus*, ONMV (*Potyviridae*: *Tritimovirus*) was studied.

## MATERIAL AND METHODS

Plants of yellow oat-grass with mosaic symptoms from the locality Staré Břiště were used for the trials.

***Biological tests***. The virus was transmitted by sap; diluted 1:1 in phosphate buffer, pH 7 to selected cereals and grasses (Table 1) by means of the leaf-rubbing method. The plants were inoculated in the 2nd–3rd leaf stage, and maintained in a greenhouse at a temperature from 15 to 20°C, light 16 h, dark 8 h. Four weeks after inoculation the symptoms were evaluated.

Negative staining was used to confirm the presence of virus particles in crude sap from plants with mosaic symptoms and from symptomless plants by electron microscopy.

***Serological methods***. Positive controls of two potyviruses infecting oats were acquired; OMV from C. Rubies-Autonell (Italy) and ONMV from F. Rabenstein (Germany). The antibodies against OMV were obtained from M. Adams, United Kingdom (IgG), and against ONMV from F. Rabenstein, Germany (IgG and conjugate). According to Rabenstein *et al*. (2002) the antibody against ONMV is characterised by reciprocal cross-reactivity with the antibody against *Wheat streak mosaic virus*, WSMV (*Potyviridae: Tritimovirus*). Three serological methods were applied to compare TFMV with OMV.

The drop precipitation test according to Jermoljev and Pozděna (1972) was used, with application of concentrated and 1/1, 1/2, 1/4, 1/8, 1/16 diluted antibody against OMV and concentrated and 1/1 diluted plant sap of oats (TFMV, OMV, healthy) and *L. ovatus* (TFMV, healthy). Rabbit serum was used as a control.

Indirect ELISA (Bar-Joseph & Garnsey 1981) was applied, using antibody against OMV diluted 1/1 000, 1/5 000, 1/10 000 and goat anti-rabbit marked AP antibody dilution 1/1 000 and 1/2 000. The samples of oats (TFMV, OMV, healthy), *L. ovatus* (TFMV, healthy) and yellow oat-grass (TFMV, healthy) were diluted 1/10 in extraction buffer.

Table 1. Results of mechanical transmission of TFMV to selected *Poaceae* species

| Species tested | Plants inoculated/with mosaic symptoms | Electron microscopy observation – no particles + particles observed | ◎ New findings ● Confirmed results |
|---|---|---|---|
| *Arrhenatherum elatius* (L.) P. Beauv. | 101/0 | – | ● |
| *Avena abyssinica* (Hochst.) | 20/12 | + | ◎ |
| *A. sativa* (L.) – cv. Abel | 152/33 | + | ● |
| *Bromus mollis* (L.) | 28/4 | + | ◎ |
| *Dactylis glomerata* (L.) | 53/0 | – | ● |
| *Festuca arundinacea* (L.) | 35/0 | – | ◎ |
| *F. pratensis* (Huds.) | 35/0 | – | ◎ |
| *Holcus mollis* (L.) | 47/0 | – | ◎ |
| *H. lanatus* (L.) | 49/0 | – | ◎ |
| *Hordeum vulgare* (L.) – cv. Okal | 20/0 | – | ● |
| *Lagurus ovatus* (L.) | 348/32 | + | ● |
| *Lolium multiflorum* (Lam.) | 29/0 | – | ● |
| *L. perenne* (L.) | 45/0 | – | ● |
| *Poa pratensis* (L.) | 35/0 | – | ● |
| *Triticum aestivum* (L.) – cv. Vlasta | 20/0 | – | ● |

The absorbance at $A_{405}$ was measured 2 h after substrate deposition.

Serologically specific electron microscopy (SSEM) according to Derrick (1973) with crude plant sap of oat (TFMV, OMV, healthy) and *L. ovatus* (TFMV, healthy) and dilution of antibody 1/500, 1/1 000, 1/2 000 and 1/5 000 was also used. The grids, coated with carbon-backed Parlodion films, were covered by diluted antibody against OMV. After washing, the grids were laid onto plant extract and incubated 60 min. After washing and drying, the grids were shadowed and observed by electron microscope Philips 208S. Negative staining of grids was used as a control.

The double antibody sandwich (DAS)-ELISA (Clark & Adams 1977) was applied to compare TFMV with ONMV. The antibody was diluted 1/100, conjugate 1/1000 (Rabenstein, pers. commun.), samples of oats (TFMV, healthy), yellow oat-grass (TFMV, healthy) and *L. ovatus* (TFMV, healthy) 1/10 each in extraction buffer. The ONMV positive control was diluted in 1ml distilled water. The absorbance at $A_{405}$ was measured 2 h after substrate deposition.

***Polymerase chain reaction***. The two-step RT-PCR according to Clover *et al.* (2002) was used to amplify the RNA isolated from TFMV-infected plants of oat and yellow oat-grass. This method enables to detect all European OMV isolates (from England, Wales, Ireland, Italy and France). Total RNA was extracted from plant tissues according to Logemann *et al.* (1987) and by means of a plant RNA extraction kit (Qiagen). The oligo-d(T)Not 1 (5′-CAATTCGCGGCCGC$(T)_{15}$-3′) and poly-T (5′-$(T)_{24}$V-3′) primers were separately used in the RT-reaction. Primers OMVCPF (5′-GACAATGGAACAGGATCAATG-3′) and oligo-d(T)Not 1 were used in PCR amplification. Following Rubies-Autonell (pers. commun.), various modifications of resuspended RNA dilution (non-diluted, 1/10, 1/20 and 1/40) were used. Thermocycling was performed as follows: 94°C for 5 min, then 30 cycles of 94°C for 1 min, 54°C for 1 min and 72°C for 1 min, followed by 72°C for 3 min. Frozen OMV-infected oat plants, obtained from C. Rubies-Autonell (Italy), were used as a positive control.

## RESULTS

***Biological tests***. The results of mechanical transmission of TFMV to cereals and grasses are summarized in Table 1. While the reactions of *Avena sativa* and *Lagurus ovatus* confirmed earlier results (Vacke *et al.* 2000), two species, *Avena abyssinica* and *Bromus mollis*, were found as new hosts of TFMV. Of the other inoculated species, *Festuca arundinacea*, *F. pratensis*, *Holcus mollis* and *H. lanatus* showed no symptoms. Further non-hosts were *Arrhenatherum elatius*, *Dactylis glomerata*, *Hordeum vulgare*, *Lolium multiflorum*, *L. perenne*, *Poa pratensis* and *Triticum aestivum*, confirming Vacke *et al.* (2000). By electron microscopy, filamentous virus particles were found in plants with mosaic symptoms (Figure 3), whereas they were absent from symptomless plants.

On the host plants, particularly on leaves, leaf sheaths and seed covers, streak, mottle and necrotic mosaic of varying intensity were observed (Figure 2). The virus disease was also manifested by dwarfing of infected plants; average height of flowering plants was reduced by 28% in *Avena sativa* and by 63% in *Lagurus ovatus*.

***Serology***. Tests by drop precipitation on sap of TFMV-infected plants gave negative results. The non-specific reaction was proved by indirect ELISA when $A_{405}$ of negative, positive controls and tested samples did not differ significantly. Negative results

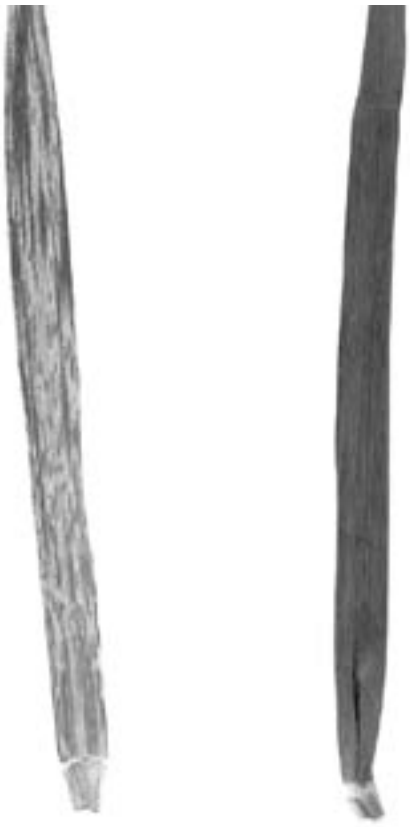

Figure 2. Oat leaf with symptoms caused by TFMV (on the left), and healthy oat leaf (on the right)

(no aggregation of virus particles) were obtained by SSEM, and aggregation of virus particles were also not observed in the OMV positive control. Filamentous particles were observed in electron microscopy by negative staining in sap from infected leaves of TFMV and OMV.

Negative results were obtained in attempts to detect TFMV through DAS-ELISA using antibodies against ONMV. The $A_{405}$ of healthy plants ranged from 0.006 to 0.028, of TFMV-infected plants from 0.001 to 0.006 and of the ONMV positive control from 1.069 to 1.159. There was cross-reactivity of antibodies against ONMV with WSMV, because the $A_{405}$ of WSMV-infected wheat plants (originated from the Czech Republic) ranged from 0.220 to 0.240. These results suggest that TFMV is not serologically related with ONMV.

***Polymerase chain reaction***. The identity of TFMV with OMV by means of two-step RT-PCR was not proved. No PCR product was obtained from TFMV-infected oat, oat-grass and healthy oat plants, whereas a product of the expected size 758 bp was obtained from OMV-infected plants. These results suggest that TFMV is not identical with previously described European isolates of OMV.

## DISCUSSION

Some new findings on the host range of TFMV, on the intensity of symptoms on these hosts, and further characteristics of the filamentous virus causing yellow oat-grass mosaic symptoms were obtained in this study. TFMV was found in *Trisetum flavescens* and successfully transmitted by mechanical inoculation to some members of the genus *Avena* and to *Lagurus ovatus* (Vacke *et al*. 2000). In the present study, *Avena abyssinica* and *Bromus mollis* were found as new hosts of TFMV. The failure to transmit TFMV to wheat and barley, the manifestation of mosaic symptoms on oats, the manner of transmission as well as the morphology of particles and inclusion bodies (Vacke *et al*. 2000) suggested that TFMV could be identical or closely related with previously described potyviruses – OMV or ONMV. *Oat mosaic virus* first described in South Carolina, USA, in 1944 (Atkinson 1945), observed in Great Britain in 1964 (MacFarlane *et al*. 1968), infects only some species of the genus *Avena* (Clover *et al*. 2002). The reactions of *Lagurus ovatus* and *Trisetum flavescens* to OMV have not been studied yet. *Bromus mollis* was shown to be non-host of the American type of OMV (Bruehl & Damsteegt 1961). According to Clover *et al*. (2002) it seems likely that the virus present in American oat samples either represents a separate strain of OMV or a completely different virus. However, *Poa pratensis*, one of the hosts of ONMV (Rabenstein *et al*. 2002), appears to be a non-host of TFMV. ONMV was first described in Canada (Gill 1967), and recently found in Germany (Rabenstein *et al*. 2002).

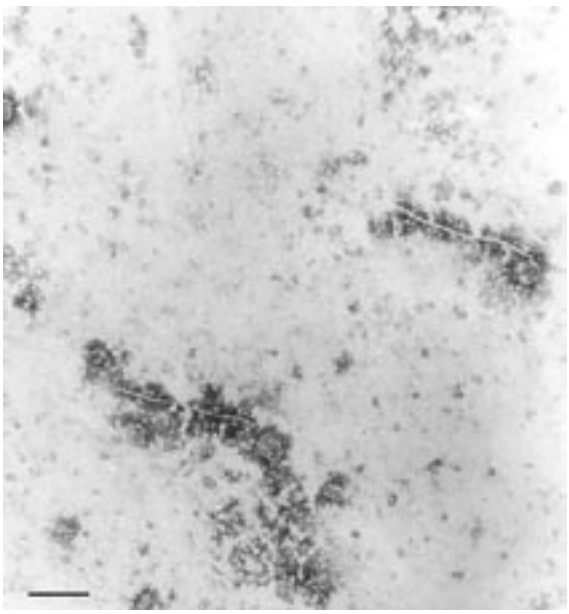

Figure 3. Filamentous particles of TFMV observed by electron microscope; bar represents 300 nm

Tests to find TFMV identical with OMV by drop precipitation, indirect ELISA and SSEM were not reliable; therefore RT-PCR was used. To establish the identity of TFMV with OMV by means of RT-PCR failed, even though this protocol enables sensitive, rapid and reliable detection of European isolates of OMV (Clover *et al*. 2002). Therefore, it is possible to conclude that the examined virus is not identical with European isolates of OMV.

The identity of TFMV with ONMV by DAS-ELISA was not proved. The reactivity of the antibody against ONMV with WSMV was confirmed. In any case, TFMV is serologically not identical with ONMV.

Further molecular analyses are necessary to complete the identification of TFMV.

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## Souhrn

Širlová L., Vacke J., Jokeš M. (2004): **Identifikace potyviru způsobujícího mozaikové onemocnění trojštětu žlutavého**. Plant Protect. Sci., **40**: 37–41.

Byla zkoumána možná totožnost nového vláknitého viru, který způsobuje mozaikové onemocnění trojštětu žlutavého (navrhovaný název virus mozaiky trojštětu žlutavého – *Trisetum flavescens mosaic virus* – TFMV) se dvěma potyviry: virem mozaiky ovsa (*Oat mosaic virus* – OMV) a virem nekrotické skvrnitosti ovsa (*Oat necrotic mottle virus* – ONMV). Tyto viry přicházely v úvahu jako možní původci onemocnění, protože jejich hostitelský okruh, velikost virionů a některé další vlastnosti jsou podobné jako u TFMV. V identifikačních testech byly použity mechanické přenosy, elektronová mikroskopie, kapková precipitace, SSEM, nepřímá ELISA, DAS-ELISA a RT-PCR. Totožnost TFMV s oběma potyviry však nebyla prokázána. Byly zjištěny dva nové druhy experimentálních hostitelů, *Avena abbysinica* a *Bromus mollis*.

**Klíčová slova**: virová mozaika trojštětu žlutavého; experimentální hostitelský okruh; elektronová mikroskopie; ELISA; SSEM; RT-PCR

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