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Short communication

Detection of European isolates of *Oat mosaic virus*

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Abstract

Oat mosaic virus (OMV) is a fungally-transmitted virus which causes yield losses in winter oats in five European countries. Detection of the virus has depended upon the recognition of transient symptoms or electron microscopy. Recent research has confirmed that the virus is a *Bymovirus*, yet OMV could not be reliably detected by the enzyme-linked immunosorbent assay (ELISA) using a range of antisera raised against other members of the genus. Therefore, a reverse transcription-polymerase chain reaction (RT-PCR) protocol was developed to specifically detect the virus. Using total RNA isolated from 16 field OMV isolates collected from throughout Europe, these primers were shown to reliably detect the virus in either one-step or two-step RT-PCR. The primers were specific and no PCR product was obtained with either *Oat golden stripe virus* (OGSV), which is frequently associated with OMV, or with other members of the *Bymovirus* genus. The two-step protocol was able to detect as little as 5×10^{-3} µl (10 ng) of total RNA isolated from an infected plant. Both protocols were as reliable as electron microscopy, but were more sensitive and were able to detect infection earlier than in mechanically-inoculated plants. However, this protocol did not detect three American isolates of the virus nor was amplification achieved using alternative primers raising the possibility that these isolates may represent a separate strain or virus. This protocol enables sensitive, rapid and reliable detection of OMV and will therefore assist management of the disease.

Crops of winter oats (*Avena sativa)* in Europe and the USA are frequently infected by *Oat mosaic virus* (OMV, genus *Bymovirus*). The disease was first observed in 1944 in South Carolina, USA (Atkinson, 1945) and is now found throughout the south-eastern states and in the north-west (Hebert and Panizo, 1975). In Europe, the disease was first observed in England in 1964 (MacFarlane et al., 1968) and has since been observed in Wales, Ireland, France and Italy (Monger et al., 2001). Symptoms of OMV include short chlorotic streaks or spots on the leaves which appear in early spring and develop into a general yellow mottling or chlorosis on the older leaves. Diseased plants are stunted and grain yield is reduced, often by 25–50% (Hebert and Panizo, 1975).

Based on similarities in particle morphology and disease epidemiology, OMV is classified as a *Bymovirus*. The particles are flexuous, filamentous rods which are 600–750 nm long and 12–14 nm wide (MacFarlane et al., 1968; Usugi and Saito, 1981). In common with other members of the genus, OMV is transmitted by *Polymyxa graminis* and has a narrow host range restricted to the *Poaceae*. Partial sequencing of four European OMV isolates has recently confirmed the taxonomy of the virus (Monger et al., 2001). However, OMV is either serologically unrelated (Chen and Adams, 1991; Carroll et al., 1995) or only distantly related (Usugi and Saito, 1981; Hariri et al., 1996) to other members of the group. Currently, OMV diagnosis frequently relies on the identification of transient symptoms which may be confused with other stresses such as infection with *Oat golden stripe virus* (OGSV). However, many infected crops never show obvious foliar symptoms and although

more reliable identification is possible by electron microscopy, it is time-consuming and not sufficiently sensitive to detect early infection. The aim of the research was to improve diagnostic methods for OMV by evaluating and developing serological or molecular methods. Specific antisera to OMV have been raised (Usugi and Saito, 1981; Chen and Adams, 1991) but are not commercially available, and therefore, the possibility of using antisera raised against other bymoviruses in ELISA was investigated. A second approach involved the design of specific primers and an appropriate reverse transcription-polymerase chain reaction (RT-PCR) protocol for the virus.

Oat plants showing characteristic symptoms of OMV were collected in the field during spring 2000 (Table 1). The presence of OMV particles in these plants was verified by transmission electron microscopy and, in selected isolates (Table 1), by mechanical transmission to 10 plants of *A. sativa* cv. Gerald, as described by MacFarlane et al. (1968). A Welsh (Llanishen, Monmouthshire), an Italian (Ozzano, Bologna) and the three American oat samples (Table 1) were also used to mechanically inoculate five plants of each of the following plant species: *Avena sativa* cv. Peniarth, *Avena sterilis*, *Chenopodium amaranticolor*, *Chenopodium quinoa*,

Table 1. Isolates of soil-borne cereal viruses used during this study

Virus	Origin	Cultivar	Source
OMV	Church Stretton, Shropshire, England*	CW07511 × LPWH9127	Dr. A. Roffey
	Church Stretton, Shropshire, England	CW04/30 × TW84130	Dr. A. Roffey
	Church Stretton, Shropshire, England	87/61CN111 × Gerald	Dr. A. Roffey
	Cranbrook, Kent, England	Peniarth	Dr. W. Monger
	Llanishen, Monmouthshire, Wales*	Gerald	Ms. M. Marshall
	Llanishen, Monmouthshire, Wales*	Grafton	Ms. M. Marshall
	Llanishen, Monmouthshire, Wales*	Viscount	Ms. M. Marshall
	Aberystwyth, Ceredigion, Wales	ND[a]	Dr. D. Jones
	Aberystwyth, Ceredigion, Wales	ND[a]	Dr. D. Jones
	Cappoquin, Waterford, Ireland*	Barra	Mr. A. Doyle
	Piltown, Kilkenny, Ireland	Barra	Mr. J. O'Mahony
	Piltown, Kilkenny, Ireland	Evita	Mr. J. O'Mahony
	Ozzano, Bologna, Italy*	Argentina	Dr. C. Rubies-Autonell
	Saunay, Indre-et-Loire, France	Fringante	Dr. D. Hariri
	Saunay, Indre-et-Loire, France	Fringante	Dr. D. Hariri
	Preaux, Indre, France*	Fringante	Ms. I. Felix
	Salisbury, North Carolina, USA*	ND[a]	ATCC PV-167
	Raleigh, North Carolina, USA*	ND[a]	Prof. S. Leath
	Raleigh, North Carolina, USA*	ND[a]	Prof. S. Leath
WSSMV	Montpellier, Hérault, France	—	Prof. P. Signoret
	Ozzano, Bologna, Italy	—	Dr. C. Rubies-Autonell
	Cornell, New York, USA	—	Prof. G. Bergstrom
WYMV	Dengzhou, Henan, China	—	Prof. J. Yu
	Morioka, Iwate, Japan	—	Dr. Y. Ohto
	Ishioka, Ibaraki, Japan	—	Dr. S. Kashiwazaki
BaYMV	Royston, Hertfordshire, England	—	Mr. T. Whitehead
	Stamford Bridge, Yorkshire, England	—	Ms. E. Metcalfe
	Newark, Nottinghamshire, England	—	Ms. E. Metcalfe
BaMMV	Royston, Hertfordshire, England	—	Mr. T. Whitehead
	Streatley, Bedfordshire, UK	—	Ms. E. Metcalfe
	Ozzano, Bologna, Italy	—	Dr. C. Rubies-Autonell
OGSV	Cranbrook, Kent, England	—	Prof. M. Adams
	Devon, England	—	Ms. E. Metcalfe
	Preaux, Indre, France	—	Ms. I. Felix

Oat mosaic virus, OMV; *Wheat spindle streak mosaic virus*, WSSMV; *Wheat yellow mosaic virus*, WYMV; *Barley yellow mosaic virus*, BaYMV; *Barley mild mosaic virus*, BaMMV; *Oat golden stripe virus*, OGSV.
*Presence of OMV verified by mechanical transmission to *A. sativa* cv. Gerald; [a]Not determined.

Datura stramonium, *Hordeum vulgare* cv. Panda, *Nicotiana benthamiana*, *Nieotiana clevelandii*, *Pisum sativum*, *Secale cereale* cv. Esprit, *Triticum aestivum* cv. Riband and *Zea mays*. No significant differences were noted between samples in the number of OMV particles seen by electron microscopy. Typical OMV symptoms developed on *A. sativa* cvs. Gerald and Peniarth, and *A. sterilis*, 2–4 weeks after mechanical inoculation with all selected isolates, as reported by MacFarlane et al. (1968). No symptoms indicative of virus infection were seen on any other species.

Further plant samples infected with *Barley yellow mosaic virus* (BaYMV), *Barley mild mosaic virus* (BaMMV), *Wheat spindle streak mosaic virus* (WSSMV), *Wheat yellow mosaic virus* (WYMV) or OGSV were obtained to ensure the specificity of the diagnostic tests (Table 1).

Double antibody sandwich (DAS)-ELISA was carried out on the OMV samples using three antisera raised against WSSMV (Carroll et al., 1995; Marie-Jeanne et al., 1999; DSMZ, Braunschweig, Germany) or BaYMV (Adams, 1991; Sanofi, Paris, France; DSMZ, Braunschweig, Germany), and an antiserum raised against BaMMV (Adams, 1991), according to the published protocols. None of these antisera were able to detect OMV in DAS-ELISA. Further ELISA tests were done using triple antibody sandwich ELISA and a BaYMV antiserum as described by Hariri et al. (1996). A slight reaction was observed occasionally with the French and Italian OMV-infected samples using the BG3 and FD3 monoclonal antibodies as described by Hariri et al. (1996). However, this reaction was not consistent nor was any reaction observed with other isolates of the virus. Replacing the BaYMV antiserum raised by Hariri et al. (1996) with the WSSMV, BaYMV or BaMMV antisera used in DAS-ELISA did not improve the reliability with which either monoclonal antibody detected OMV.

Since it was not possible to detect OMV reliably using commercially available antisera, a molecular protocol to diagnose the virus was devised. Total RNA was extracted from 200 mg of leaf tissue as described by Clover and Henry (1999) and the resulting pellet was resuspended in 100 µl of sterile distilled water. Multiple sequence alignment of the 3′-terminal part of RNA 1 of 4 OMV isolates (Accessions AF314536, AF314537, AF318180 and AF318181) with the available sequence of other bymovirus species was done using the Clustal V method from the MegAlign package (DNA Star, Madison, USA). Areas of divergent sequence between the species were identified and the primer OMVCPF (5′-GACAATGGAACAGGATCAATG-3′) was designed to complement part of the putative coat protein gene of OMV (equivalent to 971–991 nt of Accession AF314536). For two-step RT-PCR, cDNA was produced using 1 µl template RNA, 1 µl dNTPs (10 mM), 2 µl of 5 × MMLV reaction buffer, 2 µl of 5 µM oligo-d(T)Not1 primer (5′-CAATTCGCGGCCGC$(T)_{15}$-3′) and 100 units MMLV reverse transcriptase (Promega, Southampton, UK) in a 10 µl volume at 37 °C for 1 h. PCR amplification was performed in 50 µl reaction volumes containing 10 µl cDNA, 1 µl dNTPs (10 mM), 5 µl of 10 × *Taq* reaction buffer, 3 µl $MgCl_2$ (25 mM), 2.5 units of *Taq* DNA polymerase (Promega, Southampton, UK) and 2 µl OMVCPF (5 µM) primer. Thermocycling was performed as follows: 94 °C for 5 min, then 30 cycles of 94 °C for 1 min, 58 °C for 1 min and 72 °C for 1 min, followed by 72 °C for 3 min. One-step RT-PCR was carried out using 1 µl template RNA, 2 µl oligo-d(T)Not1 and OMVCPF primers (5 µM), 1 µl dNTPs (10 mM), 5 µl of 10 × *Taq* reaction buffer, 3 µl $MgCl_2$ (25 mM), 2.5 units of *Taq* DNA polymerase and 10 units of MMLV reverse transcriptase (Promega, Southampton, UK) in a 50 µl volume. Thermocycling was performed as before except that the tubes were first incubated at 37 °C for 30 min. The sensitivities of the two RT-PCR methods were evaluated using an extraction from infected oat leaves collected from Llanishen-Wales (Table 1) diluted serially in extractions from healthy oat. The reliability of the one- and two-step RT-PCR protocols was investigated by comparing the diagnostic results obtained using these methods and those obtained using electron microscopy on 45 test plants. Five plants of the three cultivars collected from Llanishen-Wales (Table 1) were tested with 10 plants of *A. sativa* cv. Gerald which had been mechanically inoculated with one infected plant of each of the three field-collected cultivars. Mechanically inoculated plants were tested 2 and 4 weeks after inoculation.

Using total RNA extracted from OMV-infected leaf tissue, in either one- or two-step RT-PCR, a PCR product of the expected size (758 nt) was obtained from all 16 European OMV isolates tested (Table 1) (Figure 1, lanes 3–10). Following gel electrophoresis, there was no discernible difference in the size of the products obtained from different isolates, and no non-specific bands were observed. No PCR products were produced using total RNA extracted from

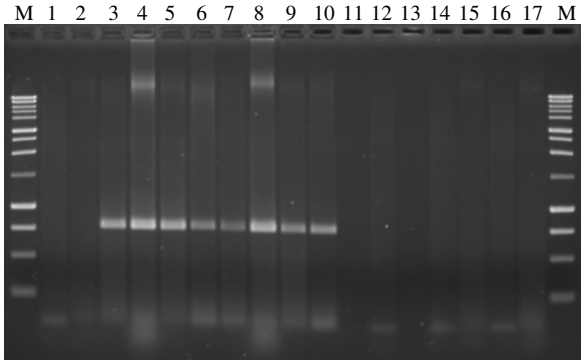

Figure 1. Detection of OMV from field isolates by one-step RT-PCR with primers oligo-d(T)Not1 and OMVCPF. Ethidium bromide stained 1.2% agarose gel in TBE. M, Promega 1 kb DNA ladder (13 fragments; 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9 and 10 kb); 1–10, OMV, Salisbury-USA, Raleigh-USA, Cranbrook-England, Church Stretton-England, Llanishen-Wales, Aberystwyth-Wales, Cappoquin-Ireland, Morellazzo-Italy, Saunay-France, Preaux-France respectively; 11, WSSMV, Montpellier-France; 12, MYMV, Dengzhou-China; 13, BYMV, Royston-England; 14, BMMV, Morellazzo-Italy; 15, OGSV, Cranbrook-England; 16, healthy oats; 17, no template (see Table 1 for further details).

healthy oat leaves (Figure 1, lane 16) or from plants infected with WSSMV, WYMV, BaYMV, BaMMV or OGSV (Figure 1, lanes 11–15). The one- and two-step protocols were able to detect dilutions of total RNA extractions from infected oat leaves to a limit of 1×10^{-2} µl (20 ng) and 5×10^{-3} µl (10 ng), respectively. Comparison of the two RT-PCR protocols with electron microscopy on 15 field-grown plants showed that both molecular methods were as reliable as microscopy, infection being detected in the same 13 of the 15 plants by all methods. Similarly all methods detected infection in the same 7 of the 30 mechanically infected oat plants 4 weeks after inoculation. However, both one- and two-step RT-PCR were able to detect infection in three of the plants 2 weeks after inoculation which were diagnosed as being uninfected by electron microscopy until 4 weeks after infection.

Neither the one-step nor two-step protocols described above for OMV diagnosis were able to detect the American isolates (Figure 1, lanes 1–2), therefore two new OMV-specific forward primers were designed to complement part of the putative coat protein gene, OMVCPF1 (5′-TCCTCTCAACAAATTGGC-3′) and OMVCPF2 (5′-ATAGCCAATGAGTCTGAGAGG-3′) (equivalent to 754–771 and 809–829 nt of Accession AF314536, respectively). These primers were used in two-step RT-PCR as described above at a range of annealing temperatures but although able to detect European OMV isolates, they detected none of the American isolates. Similarly the American isolates were not detected using the primers used previously to amplify European isolates of OMV (Monger et al., 2001) or with universal *Potyviridae* (Gibbs and Mackenzie, 1997) or *Bymovirus* primers (Badge et al., 1997). Therefore, it seems likely that the virus present in the American oat samples either represents a separate strain of OMV or a completely different virus. Two strains of the virus have previously been reported, one producing an ‘apical mosaic’, the other ‘eyespot’ symptoms. These strains are more common in North America and Europe respectively. However, both strains occur in both continents and these variable symptoms may relate to environmental conditions or oat cultivar (Hebert and Panizo, 1975). Furthermore, all European and American isolates which were mechanically transmitted to *A. sativa* and *A. sterilis* during the current study produced similar chlorotic streaks on the leaves similar to those described by MacFarlane et al. (1968), and neither obvious differences were observed between isolates from the two continents, nor was any virus transmissible to any other mechnical indicator.

This protocol enables more sensitive, rapid and reliable detection of European isolates of OMV than has been possible previously and should therefore, assist management of the disease, for example, in breeding programmes and maintenance of quarantine.

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