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Characterization of a new barley mild mosaic virus pathotype in France

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Abstract

In March 2002 in a French field, severe mosaic symptoms appeared on plants of the barley cultivar Tokyo with the rym5 locus controlling resistance to all European strains of *barley yellow mosaic virus* (BaYMV) and *barley mild mosaic virus* (BaMMV). Electron microscopic examination revealed that the disease symptoms were associated with the presence of flexuous particles which resemble bymoviruses. From these observations and after enzyme-linked immunosorbent assay analysis it was first determined that the plants could be infected by BaMMV and BaYMV. Mechanical transmission of these viruses to the barley cultivar Magie susceptible to both viruses was only possible for BaMMV. This new pathotype (BaMMV-Sil) from Sillery (Marne Department, 51, France), in contrast to another mechanically transmitted French BaMMV isolate (BaMMV-MF), could be transmitted mechanically to two barley cultivars (Tokyo, Misato Golden), *Arachis hypogaea*, *Datura stramonium* and *Lactuca sativa*. BaMMV-Sil was indistinguishable from three BaMMV isolates from Germany (G), Japan (Ka1) and France (PF) by monoclonal antibodies in ELISA while the Japanese isolate (Na1) and BaMMV-MF were distinguishable from all. The sequence of the 3′-terminal region of BaMMV-Sil RNA1 was determined. Comparison with previously published sequence data of capsid proteins indicated that BaMMV-Sil was closely related to BaMMV-Ka1, BaMMV-G and another German isolate (BaMMV-ASL1). Resistance-breaking BaMMV strains able to infect cultivars carrying the rym5 locus have also been described in Japan (BaMMV-Na1) and Korea (BaMMV-Kor). No specific amino acid differences were detected between the capsid proteins of BaMMV-Sil, BaMMV-Na1, BaMMV-Kor and those BaMMV isolates that do not overcome the rym5 resistance gene. These results indicate that BaMMV-Sil is a new pathotype of BaMMV in France and suggests that the capsid protein is not the determining factor of the pathogenicity towards the resistance gene rym5.

Introduction

Barley yellow mosaic (BaYMV) and *barley mild mosaic* (BaMMV) bymoviruses are major pathogens of winter barley in Europe and East Asia (Inouye and Saito, 1975; Huth et al., 1984). Both viruses are transmitted by the plasmodiophoraceous fungus *Polymyxa graminis* Ledingham (Toyama and Kusaba, 1970; Adams et al., 1988). They have two flexuous particles and their genome is composed of two RNAs (Usugi et al., 1989; Kashiwazaki et al., 1989). In Europe, the presence of two pathotypes of BaYMV has been reported in several countries (Adams, 1989; Huth, 1989; Hariri et al., 1990). Recently, several variants of BaYMV and BaMMV able to infect different resistant cultivars were described in France (Hariri et al., 2000). In Japan, six strains of BaYMV and two strains of BaMMV were recognized on the basis of pathogenicity towards barley cultivars (Kashiwazaki et al., 1989; Nomura et al., 1996). A Korean strain of BaMMV differing biologically and serologically from the Japanese and German isolates and several biological variants of BaYMV in China have also been recognized (Chen et al., 1992; Lee et al., 1996).

In this study, a new French pathotype of BaMMV overcoming the resistance gene rym5 was characterized and compared with known BaMMV

Table 1. BaMMV isolates used in this study

BaMMV isolates	Barley cultivars	Location	Suppliers
Sil	Tokyo	Sillery[a] (Marne) France	Mr B. Vermast
PF	Magie	Reims[b] (Marne) France	Authors
MF	Magie	Reims (Marne) France	Authors
G	Magie	Germany	Dr F. Ordon
Ka1	New Golden	Japan	Dr S. Kashiwazaki
Na1	New Golden	Japan	Dr S. Kashiwazaki

From field infected with common strains of [a]BaMMV–BaYMV and BaYMV2; [b]BaMMV–BaYMV.

strains (Table 1) from Asia and Europe by enzyme-linked immunosorbent assay (ELISA) and sequence analysis.

Materials and methods

Electron microscopy

Leaves of diseased plants were ground in phosphate buffer 0.01 M, pH 7.2. Drops of this extract were placed on a grid for 15 min, which was then rinsed, stained using 2% potassium phosphotungstate and examined by electron microscopy (Philips EM 420, 100 kV).

Mechanical inoculation

Infected leaves of the barley cultivar Tokyo were ground in phosphate buffer 0.04 M, pH 7.2. This inoculum was used to mechanically inoculate the barley cultivar Magie at the 3-leaf stage as described by Friedt (1983).

To compare the biological properties of BaMMV-MF and BaMMV-Sil, four monocotyledonous and 16 dicotyledonous species were mechanically inoculated with these viruses. Mechanical inoculation was performed using the mechanically infected barley cultivar Magie. After inoculation, the plants were kept in growth chambers controlled at 15 °C. Daylength was adjusted to 12 h at 16 000 lux light intensity. The presence or absence of the virus in these plants was assessed by ELISA.

ELISA procedures

The leaf fragments of barley plants infected by different BaMMV isolates were ground in a citrate buffer 0.1 mol/l, pH 7.2, containing 0.5 mol/l urea and filtered through a mesh. The DAS ELISA with the polyclonal antibodies of BaMMV and BaYMV was performed as described by Clark and Adams (1977). The TAS ELISA tests were carried out with BaMMV polyclonal and monoclonal antibodies prepared at Versailles (Hariri, 1999). In this system, the microtitre plates were coated with 100 µl of 1 µg/ml anti-BaMMV in 0.05 M sodium carbonate coating buffer, pH 9.6, for 3 h at 37 °C. The samples were added to each well in 100-µl aliquots and allowed to stand at 4 °C overnight. The monoclonal antibodies diluted in PBS-T-PVP were added and incubated for 4 h at 37 °C. The plates were washed, filled with anti-mouse IgG enzyme conjugate at 2000-fold dilution in PBS-T-PVP and incubated for 3 h at 37 °C. After washing, the *p*-nitrophenyl phosphate, 1 µg/ml of substrate buffer was added and absorbance measured at 405 nm. DAS ELISA tests on mechanically inoculated plants were carried out 20 and 30 days post inoculation.

Nucleic acid extraction, PCR amplification and sequence analysis

Total RNA was extracted from naturally infected winter barley plants (cv. Tokyo) (Schenk et al., 1995). The capsid protein gene was amplified by reverse transcription–polymerase chain reaction (RT–PCR). cDNA was synthesized using Super Script II reverse transcriptase and oligo dT primers as per the manufacturer's instructions. PCR was performed using the primer pair: 5′-GATAGCCTTGTTGCACTACG 3′, identical to position 6111–6130/5′-AACCTTTCCG-GTATACA 3′, complementary to position 7245–7261 of BaMMV-MF RNA1 (Meyer and Dessens, 1996). After PCR amplification, the resulting fragment was gel purified, inserted into pGEMT-easy (Promega), and sequenced from both ends. At least two independent cDNA clones were analysed for sequence determination and the nucleotide sequence has been deposited

in the EMBL database (AJ 493272). Nucleotide and amino acid comparisons were performed using UW-GCG software version 9.

Results

Symptom expression and virus detection

The experimental field at Sillery known to be naturally infected by BaMMV, BaYMV1 and BaYMV2 has been used for more than 10 years to study the behaviour of barley cultivars. In March 2002 for the first time, severe mosaic and stunting symptoms reminiscent of an infection by bymoviruses were observed on all plants of the cultivar Tokyo. Mosaic symptoms were also visible in the two susceptible barley cultivars Hiberna and Orelie whereas the two French resistant cultivars Marne and Superchampenois showed no sign of virus infection. The cultivar Tokyo issued from the Chinese landrace Mokusekko 3 was considered to be resistant to all European isolates of these viruses (Hariri et al., 2000). Electron microscopy observations indicated the presence of flexuous particles with two modal lengths similar to those of bymoviruses (data not shown). ELISA tests were performed and confirmed that the plants of cultivar Tokyo could be infected by BaMMV alone or in mixture with BaYMV (Table 2). No virus was detected in the leaves of cultivars Marne and Superchampenois.

Mechanical inoculation of BaMMV-Sil to barley

Two weeks after mechanical inoculation, mosaic symptoms were visible in the three barley cultivars Magie, Misato Golden and Tokyo. The plants showing symptoms reacted positively to ELISA tests performed with BaMMV polyclonal antibody. No BaYMV was detected in these plants.

Table 2. ELISA detection of BaMMV and BaYMV in different barley cultivars from an infected field at Sillery

Cultivars	04.03.02		27.03.02	
	BaMMV	BaYMV	BaMMV	BaYMV
Hiberna	nt[a]	nt	7/10[b]	5/10
Marne	nt	nt	0/10	0/10
Orelie	nt	nt	10/10	10/10
Superchampenois	nt	nt	0/10	0/10
Tokyo	9/10	4/10	10/10	0/10

[a]Not tested.
[b]Number of infected plants/number of plants tested.

Host range of BaMMV-Sil and BaMMV-MF

The reactions of different monocotyledonous and dicotyledonous species to BaMMV-Sil and BaMMV-MF after mechanical inoculation are summarized in Table 3. Symptoms were not observed in any of the dicotyledonous plants tested nor in wheat or oat. In ELISA, BaMMV was detected in new emerging leaves of *Arachis hypogae*, *Lactuca sativa* and *Datura stramonium* inoculated with BaMMV-Sil. The plants of maize infected with each isolate showed yellow mosaic symptoms.

ELISA

The serological relationships of BaMMV-Sil with four other BaMMV isolates were examined by using BaMMV polyclonal and monoclonal antibodies. In DAS ELISA, the polyclonal antiserum of BaMMV reacted with all isolates tested. In TAS ELISA, four monoclonal antibodies of BaMMV (Hariri, 1999) reacted with BaMMV-Sil, BaMMV-PF, BaMMV-G and BaMMV-Ka1. As noted previously, in this system BaMMV-MF and BaMMV-Na1 can be distinguished from one another and also from the other BaMMV isolates (Table 4).

Sequence analysis

In order to better characterize BaMMV-Sil, its capsid protein gene was sequenced. A fragment of about 1.1 kb corresponding to the 3′ end region sequence of RNA1 was amplified by RT–PCR and fully sequenced. The sequence analysis indicated that the coat protein (CP) gene of BaMMV-Sil is identical in length (753 nt, 251 aa) to that of isolates from France (Dessens and Meyer, 1995), Germany (Schlichter et al., 1993), the UK (Peerenboom et al., 1997), Japan (Kashiwazaki et al., 1992) and Korea (Lee et al., 1996). The sequence of the CP gene of a Chinese BaMMV isolate has also been reported (Zheng et al., 1999). However, because the numbers of nucleotides and amino acids of the CP gene reported in the text are different from those shown in the figure and in the EMBL database, this isolate has not been included in our studies.

Previous BaMMV CP sequence comparisons indicate that these isolates belong to three distinct strain

Table 3. The plant species mechanically inoculated with BaMMV-Sil and BaMMV-MF

Plant species	Leaf detection of the virus in ELISA	
	BaMMV-Sil	BaMMV-MF
Arachis hypogaea	2/5[a]	0/5
Avena sativa	0/5	0/5
B. chinesis	0/5	0/5
Beta vulgaris	0/5	0/5
Brasicca napus	0/5	0/5
Chenopodium quinoa	0/5	0/5
Cucumis sativus	0/5	0/5
Datura stramonium	3/5	0/5
Hordeum vulgare (cv. Magie)	5/5	5/5
H. vulgare (cv. Marne)	0/15	0/15
H. vulgare (cv. Misato Golden)	5/15	0/15
H. vulgare (cv. Superchampenois)	0/15	0/15
H. vulgare (cv. Tokyo)	7/15	0/15
Lactuca sativa	1/5	0/5
Lycopersicon esculentum	0/5	0/5
Nicotiana banthamiana	0/5	0/5
Nicotiana tabacum	0/5	0/5
Petunia hybrida	0/5	0/5
Pisum sativum	0/5	0/5
Spinacia oleracea	0/3	0/3
Tetragonia tetragonioides	0/5	0/5
Triticum aestivum	0/5	0/5
Vicia faba	0/5	0/5
Zea mays	5/5	5/5

Samples are considered to be positive when the OD value is equal or more than three times that of the healthy control.
[a]Number of infected plants/number of plants tested.

Table 4. Reaction of polyclonal and monoclonal antibodies of BaMMV with different isolates of this virus in DAS and TAS ELISA

Antibodies	Isolates of BaMMV							Healthy control
	ELISA	Sil	MF	PF	G	Ka1	Na1	
Monoclonal								
1B12		1.86	1.98	2.16	1.56	1.34	0.09	0.11
5C8		1.43	1.51	1.56	0.98	0.77	0.71	0.09
1D5	TAS	1.57	2.18	1.81	1.12	1.13	0.42	0.08
1A12		0.08	2.19	0.06	0.08	0.09	0.05	0.07
3A9		0.60	0.90	0.74	0.65	0.49	0.08	0.07
Polyclonal	DAS	1.68	2.67	1.76	0.72	0.35	0.67	0.09

Samples are considered to be positive when the OD value is equal or more than three times that of the healthy control.

subgroups: one including the German and Japanese (Ka1) isolates, one including the French and UK isolates, and one including the Korean and Japanese (Na1) isolates (Zheng et al., 1999). Pairwise comparisons of the BaMMV-Sil CP ORF sequence were made with those of seven other BaMMV isolates (Table 5). Nucleotide identities ranged from 97.6% to 87.1% and clearly grouped the Sillery isolate with two German (G and ASL1) and one Japanese (Ka1) isolates. This relatedness was confirmed by amino acid sequence comparisons (100% and 99.6% identity for isolate G and isolates ASL1-Ka1, respectively). The number of

Table 5. Pairwise comparisons (using the GAP Program) of the capsid protein region of different isolates of BaMMV

BaMMV isolates	% homology capsid protein								
	Sil	G	ASL1	Ka1	UKF	MF	UKM	Na1	Kor
Sil	—	100	99.6	99.6	96.8	96.4	96.0	94.4	93.2
G	96.9	—	99.6	99.6	96.8	96.4	96.0	94.4	93.2
ASL1	97.5	96.3	—	100	96.4	96.0	95.6	94.0	92.8
Ka1	97.6	96.4	99.9	—	96.4	96.0	95.6	94.0	92.8
UKF	90.0	90.3	89.9	89.8	—	99.6	99.2	94.4	93.2
MF	90.0	90.3	89.9	89.8	99.2	—	99.6	94.0	92.8
UKM	90.2	90.4	90.0	89.9	99.1	99.6	—	93.6	92.8
Na1	88.3	88.8	88.7	88.8	89.4	89.4	89.5	—	97.2
Kor	87.1	87.6	87.5	87.6	87.7	87.7	88.0	95.7	—

EMBL accession numbers of the sequences used are BaMMV-G: X 69204; BaMMV-ASL1: AJ 242725; BaMMV-Ka1 and Na1: D 10947 BaMMV-UKF: Y 10973: BaMMV-UKM: Y 10974; BaMMV-MF: L 49381; BaMMV-Kor: D 83410.

amino acid differences with the seven other BaMMV isolates vary from 1 to 17 and the majority of the amino acid changes occur in the N-terminal regions of the capsid proteins (Table 6).

Discussion

In this paper, we report for the first time the presence of a BaMMV in France that is able to overcome the resistance of the barley cultivar Tokyo carrying the resistance rym5 locus. At present, this new French BaMMV variant has been found only at one location (Sillery). This work completes a previous study in which the existence of other French BaMMV and BaYMV variants able to infect cultivars with different loci (rym3, rym4, rym6, rym8, rym9, rym10, rym11) encoding resistance to BaMMV and/or BaYMV has been described (Hariri et al., 2000).

Some biological, serological and molecular properties of the BaMMV-Sil isolate have been characterized. Like another French isolate (BaMMV-MF), BaMMV-Sil is easily transmitted by mechanical inoculation to the susceptible barley cultivar 'Magie'. But, these two isolates can be differentiated by their host range and their serological properties. In particular, BaMMV-Sil can infect two resistant barley cultivars and three dicotyledonous species. This is the first time that a successful inoculation of dicotyledonous species by a bymovirus has been reported.

The resistance gene rym5 was introduced from a Chinese landrace of barley Mokusseko 3 in a number of two-rowed barley cultivars like Misato Golden, Mikamo Golden in Japan (Konishi et al., 1997) and Tokyo in Europe (J. Stragliati and L. Hanneton, Company Nickerson France, personal communication). These Japanese resistant cultivars were found to be susceptible to Japanese (BaYMV III, BaMMV-Na1) and Korean (BaMMV-Kor) isolates (Nomura et al., 1996; Lee et al., 1996; Ogawa et al., 1987).

Comparison of the capsid protein sequences shows that the Japanese (Na1) and the Korean (Kor) isolates belong to the same subgroup of BaMMV strains. This is not the case for the Sillery isolate, which is much more closely related to strains forming another subgroup, including the Japanese (Ka1) and the two German (G and ASL1) isolates. Thus, there is no apparent correlation between the pathogenicity and the degree of relationship between the BaMMV strains. On the other hand, by using pseudorecombinants between the Japanese (Ka1 and Na1) isolates, it has been demonstrated that the pathogenicity and symptomatology are determined by BaMMV RNA1 (Kashiwazaki and Hibino, 1996).

The reported capsid protein sequence of BaMMV-Sil differs from those of BaMMV-Na1 and BaMMV-Kor by 14 and 17 amino acids, respectively. But no specific amino acid difference exists between the capsid protein of these three resistance breaking BaMMV strains and other BaMMV isolates like Ka1 and MF which do not infect cultivars carrying the resistance gene rym5. This suggests that the capsid protein gene is not involved in the pathogenicity of this virus towards cultivars with the rym5 resistance locus.

Table 6. Amino acid differences among the capsid sequences of nine BaMMV isolates

	Amino acid position																								
BaMMV isolates	1	3	4	5	13	14	21	27	29	30	37	45	46	50	53	61	67	70	83	91	92	101	134	177	180
Sil	A	H	E	E	V	P	M	D	R	R	I	S	L	K	T	N	N	D	S	A	A	D	G	R	H
G	—*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
ASL1	—	—	—	—	—	—	—	—	—	K	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Ka1	—	—	—	—	—	—	—	—	—	K	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
UKF	—	—	—	—	A	S	—	—	—	—	—	—	M	T	—	K	D	E	—	—	T	—	—	—	—
MF	—	—	—	—	A	S	—	—	—	—	—	—	M	T	—	K	D	E	—	—	T	—	S	—	—
UKM	—	—	—	—	A	S	—	—	K	—	—	—	M	T	—	K	D	E	—	—	T	—	S	—	—
Na1	S	K	D	D	—	S	—	—	—	—	V	N	—	—	M	—	D	E	T	—	T	N	—	—	Q
Kor	S	K	D	—	—	S	L	A	T	—	V	N	—	—	M	—	D	E	T	T	T	—	—	P	Q

*Amino acids identical to BaMMV–Sil.

The presence of BaYMV in barley plants (cv. Tokyo) detected by ELISA suggests that a variant of BaYMV may also exist at Sillery. In Japan, a BaYMV pathotype (BaYMV-III) infecting resistant cultivars like Misato Golden and Mikamo Golden has been described (Saeki et al., 1999). Further work is needed to confirm the existence of this possible new French BaYMV pathotype and to study its relationship with this Japanese variant.

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References

Adams MJ, Swaby AG and Jones P (1988) Confirmation of the transmission of barley yellow mosaic virus (BaYMV) by the fungus *Polymyxa graminis*. Annals of Applied Biology 112: 133–141

Adams MJ (1989) Deployment of resistance to the barley yellow mosaic virus in the UK. Proceedings of the 4th International Plant Virus Epidemiology Workshop, Resistance to viruses and vectors – Temperate and Tropical Plants (pp 77–78) Montpellier

Chen J, Adams MJ, Zhu F, Shi C and Chen H (1992) Responses of some Asian and European barley cultivars to UK and Chinese isolates of soil-borne barley mosaic viruses. Annals of Applied Biology 121: 631–639

Clark MF and Adams AN (1977) Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. Journal of General Virology 34: 475–483

Dessens JT and Meyer M (1995) Characterization of fungally and mechanically transmitted isolates of barley mild mosaic virus: Two strains in competition. Virology 212: 383–391

Friedt W (1983) Mechanical transmission of soil-borne barley yellow mosaic virus. Phytopathologische Zeitschrift 106: 16–22

Hariri D (1999) Production of monoclonal antibodies to barley mild mosaic virus and their use for strain differentiation. Journal of Phytopathology 147: 353–357

Hariri D, Fouchard M and Lapierre H (1990) Resistance to barley yellow mosaic virus and to barley mild mosaic virus in barley. In: Koenig R (ed) Proceedings of the First Symposium of the International Working group on plant Viruses with Fungal Vectors (pp 109–112) Stuttgart, Ulmer

Hariri D, Meyer M, Le Gouis J, Bahrman N, Fouchard M, Forget C and Andre A (2000) Characterisation of BaYMV and BaMMV pathotypes in France. European Journal of Plant Pathology 106: 365–372

Huth W, Lesemann DE and Paul HL (1984) Barley yellow mosaic virus: Purification, electron microscopy, serology, and other properties of two types of the virus. Phytopathologische Zeitschrift 111: 37–54

Huth W (1989) Ein weiterer Stamm des Barley yellow mosaic virus (BaYMV) gefunden. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes 41: 6–7

Inouye T and Saito Y (1975) Barley yellow mosaic virus. CMI/AAB Description of Plant Viruses, No. 143

Kashiwazaki S, Ogawa K, Usugi T, Omura T and Tsuchizaki T (1989) Characterization of several strains of barley yellow mosaic virus. Annals of Phytopathological Society of Japan 55: 16–25

Kashiwazaki S, Nomura K, Kuroda H, Ito K and Hibino H (1992) Sequence analysis of the 3′-terminal halves of RNA 1 of two strains of barley mild mosaic virus. Journal of General Virology 73: 2173–2181

Kashiwazaki S and Hibino H (1996) Genomic reassortment of barley mild mosaic virus: Evidence for the involvement of RNA1 in pathogenicity. Journal of General Virology 77: 581–585

Konishi T, Ban T, Iida Y and Yoshimi R (1997) Genetic analysis of disease resistance to all strains of BaYMV in a Chinese barley landrace, Mokusekko 3. Theoretical and Applied Genetics 94: 871–877

Lee KJ, Kashiwazaki S, Hibi T and So IY (1996) Properties and capsid protein gene sequence of a korean isolate of barley mild mosaic virus. Annals of Phytopathological Society of Japan 62: 397–401

Meyer M and Dessens JT (1996) The complete nucleotide sequence of barley mild mosaic virus RNA1 and its relationship with other members of the potyviridae. Virology 219: 268–273

Nomura K, Kashiwazaki S, Hibino H, Inoue T, Nakata E, Tsuzaki Y and Okuyama S (1996) Biological and serological properties of strains of barley mild mosaic virus. Journal of Phytopathology 144: 103–107

Ogawa K, Watanabe K, Iida Y, Toshima I, Kashiwazaki S and Tsuchizaki T (1987) On the infection of a BaYMV resistant cv. Misato Golden. Annals of Phytopathological Society of Japan 53: 123

Peerenboom E, Cartwright EJ, Foulds I, Adams MJ, Stratford R, Rosner A, Steinbiss H-H and Antoniw JF (1997) Complete RNA1 sequence of two UK isolates of barley mild mosaic virus: A wild-type fungus-transmissible isolate and a non-fungus-transmissible derivative. Virus Research 50: 175–183

Saeki K, Miyazaki C, Hirota N, Saito A, Iito K and Konishi T (1999) RFLP mapping of BaYMV resistance gene rym3 in barley (*Hordeum vulgare*). Theoretical and Applied Genetics 99: 727–732

Schenk PM, Antoniw JF, deBatista M, Jacobi V, Adams J and Steinbiss H-H (1995) Movement of barley mild mosaic and barley yellow mosaic viruses in leaves and roots of barley. Annals of Applied Biology 120: 291–305

Schlichter U, Sohn A, Peerenboom E, Schell J and Steinbiss H-H (1993) Molecular analysis of the capsid protein gene of a German isolate of barley mild mosaic virus. Plant Cell Reports 12: 237–240

Toyama A and Kusuba T (1970) Transmission of soil-borne barley yellow mosaic virus. 2. *Polymyxa graminis* Led as vector. Annals of Phytopathological Society of Japan 36: 223–229

Usugi T, Kashiwazaki S, Omura T and Tsuchizaki T (1989) Some properties of nucleic acids and coat proteins of soil-borne filamentous viruses. Annals of Phytopathological Society of Japan 55: 26–31

Zheng T, Cheng Y, Chen JP, Antoniw JF and Adams MJ (1999) The occurrence of barley mild mosaic virus (BaMMV) in China and the nucleotide sequence of its coat protein gene. Journal of Phytopathology 147: 229–234