

Serological, Pathological and Molecular Characterisation of Hungarian Pepper Mild Mottle *Tobamovirus* (PMMoV) Isolates*

D. KÁLMÁN¹, L. PALKOVICS² and R. GÁBORJÁNYI³

¹Plant Protection Institute, Hungarian Academy of Sciences, H-1525 Budapest, P.O. Box 12, Hungary

²Agricultural Biotechnology Center, H-2101 Gödöllő, P.O. Box 411, Hungary

³Plant Protection Institute, Veszprém University, Georgikon Faculty of Agricultural Sciences,
H-8631 Keszthely, P.O. Box 71, Hungary

Last year pepper growers observed symptoms referring to virus-infection in pepper plantations in plastic tunnels. Infected plants showed mosaic symptoms or mottling of the leaves, while on the fruits necrotic spots developed. These symptoms referred to a *tobamovirus* infection. Collected samples were examined by serological and pathological methods, followed by the biological characterisation of the isolates. For serological studies the DAS-ELISA method was used, in which the pathogen was identified as pepper mild mottle *tobamovirus*. During the pathological examination different host-plants have been used including some pepper varieties containing different L genes (L⁺-L⁺). It was found, that the Hungarian isolates belonged to the P_{1,2} pathotype and were closely related to the Spanish isolate (PMMV-S). PCR-studies proved the presence of the PMMoV P_{1,2} pathotype in Hungary as well.

Keywords: Pepper mild mottle virus, *tobamovirus*, PCR.

It is well known that among pepper diseases the viruses play a major role. Pepper is a natural host of about 45 plant-pathogen viruses (Green and Kim, 1991; Gáborjányi et al., 1997, 1998). These viruses cannot be diagnosed only by the symptoms, as one virus can cause different symptoms, and similar symptoms can derive from different pathogens as well.

Tobamovirus group is an important and separate group of pepper viruses (*Table 1*), causing serious yield losses especially in the greenhouses or plastic tunnels. Its first member is the tobacco mosaic *tobamovirus* (TMV) mentioned at first more, than 100

Table 1

Pepper-pathogen *tobamoviruses*
(Viruses in bold type are officially recorded by ICTV)

Virus	First description
Tobacco mosaic tobamovirus (TMV)	Mayer, 1886
Tomato mosaic tobamovirus (ToMV)	Clinton, 1909
Tobacco mild green mosaic tobamovirus (TMGMV)	McKinney, 1929
Pepper mild mottle tobamovirus (PMMoV/ PMMV)	McKinney, 1952
Bell pepper mottle virus (BePMV)	Feldman and Oremianer, 1972
Paprika mild mottle tobamovirus (PaMMV)	Rast, 1979
Dulcamara yellow fleck virus (DYFV)	Salamon et al., 1987a,b

*Dedicated to the occasion of the 65th birthday of Professor József Horváth

years ago (Mayer, 1886; Linhart and Mezey, 1890). After the first description of TMV in Hungary (Szirmai, 1950) many papers dealt with *tobamoviruses*. Horváth (1969) tested different pepper varieties to TMV resistance but did not find resistant varieties among the cultivated sweet and red peppers. The breeders concentrated first on the incorporation of L¹ gene into different varieties (Zatykó, 1982). The first *tobamovirus* in Hungary which overcame the resistance provided by the L¹ gene was isolated in 1980 as ToMV-Ob (Csilléry and Ruskó, 1980). Later Salamon et al. (1987a,b) reported a new pathotype named dulcamara yellow fleck. Burgyán et al. (1978) drew the attention to the heterogeneity of the *tobamoviruses* isolated in Hungary, proving that isolates from infected pepper, tomato, tobacco and *Solanum dulcamara* could be grouped both by their host-plant reactions and serological features. Isolates deriving from pepper represented more independent types. Tóbiás et al. (1982; 1983) dealt especially with the symptomatological and serological behaviour of different *tobamovirus* isolates. They gave a detailed comparison of six pepper-pathogen tobamoviruses including the Hungarian ToMV-Ob isolate and the TMV-SL isolate (later separated as a new virus species, pepper mild mottle virus by Wetter, 1984a,b). Pepper mild mottle *tobamovirus* (PMMoV/PMMV) meant a new and special problem for pepper production. The virus produced slight mottling or no symptoms on the leaves, but caused chlorotic mottling, malformation and reduction in size with occasional necrotic spots on the fruits (Wetter et al., 1984a,b; Avgelis, 1986; Alonso et al., 1989).

The presence of this pathogen in Hungary was supposed earlier. Tóbiás and Csilléry (1983) reported a new TMV pepper strain which systemically infected the peppers containing the L¹ gene. The samples were examined on indicator plants and with ELISA tests. According to these the infected samples gave positive reaction with the TMV-SL antiserum and the isolates represented at least a pathotype of P₁. However it was not further examined, if P₁, or a more aggressive pathotype was present. In a former paper Salamon (1993) mentioned the isolation of PMMV, however, no detailed description was reported about the isolate called PMMV-Nov, except the host-plant tests on the pepper varieties containing different L genes. It was indicated, that PMMV-Nov belonged to the P_{1,2,3} pathotype. Kiss (1996) reported about the infection of PMMV diagnosed by DAS-ELISA in the region of county Csongrád. Our isolates probably represent a new (P_{1,2}) pathotype of PMMoV in Hungary. The aim of our work is to characterise these isolates by different methods, to compare them with other *tobamoviruses* and work out a PCR method to identify separately the different *tobamovirus* isolates causing heavy losses in the pepper-plantations.

PMMoV became very important especially in the Mediterranean pepper-growing countries, causing more and more losses (Selassie et al., 1981; Wetter et al., 1984a,b; Avgelis, 1986; Betti et al., 1988; Alonso et al., 1989). Now it seems that it occurs often in the plastic tunnels in Hungary and causes quality degradation of the fruits. Because of the internal seed transmission (Tošić et al., 1980; Tanzi et al., 1989) its presence means a real threat for the pepper production.

As the molecular techniques became general in virology, researchers began to characterise this virus at molecular level too. The nucleotide sequence of its 5' and 3' non-

coding regions was reported in 1989 (Avila-Rincón et al., 1989), followed by the cloning and the complete nucleotide sequence of PMMV-S (Alonso et al., 1991). A procedure involving reverse transcription followed by polymerase chain reaction (RT-PCR) was developed for typing pathotypes of the *tobamoviruses* infecting the L-resistant genotypes of pepper (Tenllado et al., 1994), and to separate the two pathotypes, which cannot be differentiated by serological means. For these reasons PaMMV and PMMV specific primers were used. The discrimination between the two pathotypes was achieved by restriction enzyme analysis of the PCR products. Tenllado et al. (1997) applied the PCR method for the screening of pathotypes in field isolates.

Tobamovirus pathotypes

Differentiation of pepper-pathogen tobamoviruses is based on the resistance of wild *Capsicum* species. Resistance in the *Capsicum* species is provided by five, dominantly inherited L genes (L^+ , L^1 , L^2 , L^3 , L^4), located on the same locus and localising the infection in an incomplete way. In contrast to it the virus has four pathotypes (P_0 , P_1 , $P_{1,2}$, $P_{1,2,3}$). The different compatible or incompatible pepper-virus relationships are summarised in Table 2. The pepper containing the L^+ gene is susceptible to the infection of any pathotypes. The resistance gene L^1 provides resistance against the P_0 pathotype, the L^2 gene against the P^1 pathotype, etc. If pepper varieties of a certain resistance level are grown for a long period of time, one could observe the tendency, that the virus overcomes the resistance, and a more aggressive strain appears.

Table 2

Relationship of pepper varieties with different resistance level and *tobamoviruses* of different pathotypes (according to Rast, 1979, 1988; Boukema et al., 1980; Tóbiás et al., 1982; and Boukema, 1984)

Resistance genes	Pathotypes			
	P_0	P_1	$P_{1,2}$	$P_{1,2,3}$
L^+	S	S	S	S
L^1	R	S	S	S
L^2	R	R	S	S
L^3	R	R	R	S
L^4	R	R	R	R

S = susceptibility, R = resistance.

According to Betti et al. (1986, 1988) the reason for increasing crop losses was due to the higher level of pathogenicity of the *tobamoviruses*. If one TMV isolate (P_0) was maintained on a L^+/L^1 heterozygote plant within controlled circumstances by repeated passage, the virus overcame the L^1 resistance. Similarly, more aggressive isolate developed when the virus strain was prolonged on more resistant (L^1 or L^2) plants. The increase of pathogenicity on pepper was accompanied by the decreased pathogenicity to tobacco. The virus strain was gradually adapted to the new host and collaterally was losing its

relationship to the original host-plant. On the basis of these facts it could be theoretically supposed, that such pathological change ensued under the Hungarian growing conditions too. Our pepper production is more and more exposed to the appearance of new isolates with higher pathogenicity. The reason of increasing pathogenicity was the preference of resistant varieties, with no hypersensitive response. In these cases the pathogen could replicate at a lower level, but could adapt gradually. If more pathogenic strains became dominant in the population it could be a starting-point for heavy epidemics.

Materials and Methods

In the autumn of 1999 eighteen samples were collected from the infected peppers grown under plastic tunnels in the region of "Jászság" (Middle Hungary). Plants showed mild mosaic leaf symptoms or were symptomless, but necrotic spots developed on the fruits before ripening. First isolates were tested by serological methods followed by host-plant tests and molecular characterisation.

For serological examination the DAS-ELISA method was used according to Clark and Adams (1977), following the instructions of the producers of the ELISA-kit. After collecting the samples they were immediately deep-frozen, and a few days later homogenised in extraction buffer (1:2.5 g/ml), and tested with polyclonal antisera (Loewe Phytodiagnostica). Tests were made to the following viruses: tobacco mosaic *tobamovirus* (TMV), tomato mosaic *tobamovirus* (ToMV), pepper mild mottle *tobamovirus* (PMMoV), potato X *potexvirus* (PVX), potato Y *potyvirus* (PVY), alfalfa mosaic *alfamovirus* (AMV), and cucumber mosaic *cucumovirus* (CMV).

Pepper species with different resistance level and 3 different tobacco species were used for the host-plant tests (Table 3). The resistance level of pepper varieties was the following: L⁺ (Albargia, Synt. Cecei), L¹ (D. Cecei, Fehérözön), L² (Greygo), L³ (Bölény, Rapires), L⁴ (Hímes). The deep-frozen samples were homogenised in phosphate buffer (0.1 M) according to Sørensen (pH 7.0; 1:5 w/v) in a mortar with pestle, and the leaves of the host-plants were inoculated mechanically. On peppers the cotyledons, on tobaccos the lower 3–4 leaves were inoculated. To improve the efficiency of the inoculation silicon carbide (Carborundum 500 mesh) dust was added. The plants were kept in glasshouse (18–25 °C; 160 mEm⁻²s⁻¹ additional lighting for 8 hours a day; relative humidity 60%). Local symptoms were checked after 4–7 days, and systemic symptoms after 3–4 weeks.

For the molecular studies a universal primer pair was designed to recognise the most *tobamoviruses*. Sequences of the *tobamoviruses* were taken from the Genbank of EMBL Data Library, DNA Data Bank of Japan. The most important viruses under the Hungarian conditions were selected because of the great amount of the *tobamovirus* sequences. We tried to find a section, what was quite the same in every (or at least in the most important) *tobamoviruses*, where the universal primer pair was designed (Fig. 1). The 5' primer was constructed in front of the coat protein (CP) gene, it was degenerated and reverse sense. The 3' primer was also degenerated, reverse complementary and situated in the

Table 3

Symptoms of indicator plants inoculated with the samples identified as PMMoV by DAS-ELISA

Isolates*	CPSAN** L ⁺	CPSAN L ¹	CPSAN L ²	CPSAN L ³	CPSAN L ⁴	NIOTA- XNC	NIOTA- SAM	NIORU
Ca 2	-/Mot***	-/Mot	-/-	Ab/-	Ab/-	LLe/-	-/s	LLe/-
Ca 2a	-/Mot	-/Mot	-/-	Ab/N	Ab/-	LLe/-	-/M	LLe/-
Ca 4a (mixed with PVY)	-/Mot	-/Mot	-/-	Ab/-	Ab/-	LLe/ VN,N, M, Ch	-/s	LLe/-
Ca 5a	-/Mot	-/Mot	-/-	Ab/-	Ab/-	LLe/-	-/N, VN	LLe/-
Ca 5b (mixed with CMV)	-/-	-/Mot	-/-	Ab/-	Ab/-	LLe/-	-/s	LLe/-
Ca 6	-/-	-/Mot	-/-	Ab/-	Ab/-	LLe/-	-/s	LLe,N/-
Ca 7	-/Mot	-/Mot	-/-	Ab/-	Ab/-	LLe/-	-/s	LLe/-
Ca 8 (mixed with PVY)	-/Mot	-/Mot	-/-	Ab/-	Ab/-	LLe/ M, VN	Ch/ VN, N	LLe,Ch /-
Ca 10 (mixed with ToMV)	-/Mot	-/Mot	-/-	Ab/-	Ab/-	LLe/-	-/s	LLe/-

Key:

* Ca 2, Ca 2a, Ca 4a, Ca 5a, Ca 5b, Ca 6, Ca 7, Ca 8, Ca 10 = pepper isolates,

PVY = potato Y virus, CMV = cucumber mosaic virus, ToMV = tomato mosaic virus.

** Abbreviations of host-plants are used according to Horváth (1993). CPSAN = *Capsicum annum*, NIOTA-XNC = *Nicotiana tabacum* cv. Xanthi-nc, NIOTA-SAM = *Nicotiana tabacum* cv. Samsun, NIORU = *Nicotiana rustica*.*** Abbreviations of local symptoms can be seen in the numerator, while systemic symptoms are indicated in the denominator. Mot = mottling; - = no symptoms; Ab = abscission; L = local; Le = lesion; Ch = chlorosis; VN = vein necrosis; N = necrosis; M = mosaic; s = symptomless infection (detected with back inoculation to *Nicotiana tabacum* cv. Xanthi-nc).

3' non-coding region. The universal primer pair was successfully tried out with own and authentic isolates. The authentic isolates originated from the stock-collection of the Plant Protection Institute. The PCR was carried out according to the following description:

Nicotiana tabacum cv. Samsun plants were mechanically infected as mentioned above with the own and authentic isolates. Samples of 0.1 g were taken about 3–4 weeks after the inoculation from the systemically infected leaves. Total RNA was extracted from plant tissues, using the following method: samples were homogenised in 600 µl extraction buffer (pH 7.6 containing 5% TRIS (1.0 M), 2% NaCl (5.0 M), 0.2% EDTA (0.5 M), 5% SDS (10%), 0.3% mercaptho-ethanol in 100 ml distilled water) adding 600 µl phenol, then centrifuged for 5 minutes (15000 rpm). 300 µl phenol and 300 µl chloroform was added to the supernatant and centrifuged for 5 minutes. After adding 600 µl chloroform to the supernatant the centrifugation was repeated, and adding 1 µl absolute alcohol to the supernatant was followed by centrifugation (10 min) again. The total RNA was precipitated and washed with ethanol (70%), then after drying suspended in sterile distilled water (60 µl).

a. The 5' end of the primer pair

TMV-Ob TATGAGTCAG GACGGTAGCT CAAGCGAGAT ATCATCCGAT TCGTTTATTT
 TMV-U1 .ATCGATGAT GATTCGGAGG CTACTIONTGC CAAATCCGGAT TCGT...TTT
 PMMoV .ATTGGGCAG AACTCGGAGT CATCGGACGC CGAGTCTTCT TCGT...TTT
 Univers. 5' GACGC GGAGTCGGAT TCGT ATT
 T C T T T

↓ CP start

TMV-Ob AATCATGCCT TATACTGTTA CGTCTCCAAG CCAACTAGTT TATTTTGGTT
 TMV-U1 AATATGTCCT TACAGTATCA CTACTCCATC TCAGTTCGTG TTCTTGTCAT
 PMMoV AACTATGGCT TACACAGTTT CCAGTGCCAA TCAATTAGTG TATTTAGGTT
 Univers. AATATG 3'

b. The 3' end of the primer pair

TMV-Ob TACGACTATA AAAATAAGAA GGGGTTTCGAA TCCCCCCTG ACCCCCGGTA
 TMV-U1 CAGGCACGT. AATAAGCGA GGGGTTTCGAA TCCCCCCTG ACCCCCGGTA
 PMMoV AACGACGTAA TTATTTTTCA GGGGTTTCGAA TCCCCCCTG
 ACCCGCGGTA

TMV-Ob GGGGCCCCA
 TMV-U1 GGGGCCCCA
 PMMoV GCGGCCCCA

Universal: 5' TGGGCC (GC) CTACC (GC) G (GC) GG 3'

Fig. 1. The universal primer pair

a. The TMV-Ob specific primer.

TMV-Ob ATAGTATAGG AGTTTCCAAA CCTGTCCGTT TGGAAAGAAA TAAAGTAAGG
 TMV-U1 AAAATAGTAG TAATGATCGG TCAGTGCCGA ACAAGAATA TAGAAATGTT
 PMMoV AGAGAAATGA TAATAAGGGT T.TGA ATAAGGAAGG GAAGCTGTTT

b. The TMV-U1 specific primer.

TMV-Ob GGAATCAGT TTCAAACACA GAATGCGAGA ACTACTGTAC AACAGCAGTT
 TMV-U1 GGAATCAGT TTCAAACACA ACAAGCTCGA ACTGTCTTTC AAAGACAATT
 PMMoV GGCAATCAGT TTCAAACACA ACAGGCTAGA ACTACGGTTC AACAGCAGTT

TMV-Ob CAGTGATCTG TTTAAGACTG TTCCCACTAG GACCAATAGG TTCAATGATG
 TMV-U1 CAGTGAGGTG TGGAACCTT CACCACAAGT AACTGTTAGG TTCCCTGACA
 PMMoV CTCTGATGTG TGGAAGACTA TTCCGACCGC TACAGTTAGA TTTCTGTCTA

c. The PMMoV specific primer.

TMV-Ob CTGTTTGGGC GGATCCGATT ACATTTATCG ATCTTTGTAC CGTGGCCTTG
 TMV-U1 CAGCGTGGGC CGACCCAATA GAGTTAATTA ATTTATGTAC TAATGCCTTA
 PMMoV CTGTATGGGC TGATCCATTA GAGTTACAAA ATCTGTGTAC TTCCGGCGTTA
 T

Fig. 2. The virus-specific primers

For cDNA synthesis, 1 μ l 3' primer (100 pmol/ μ l cc.) and 3.75 μ l distilled water was added, and denaturised at 65 °C for 5 minutes. Afterwards 2 μ l reverse-transcriptase buffer (5x), 0.25 μ l reverse-transcriptase enzyme and 1 μ l dNTP mix (5mM) was added to the mixture, and kept at 42 °C for one hour. In the final reaction tube 2 μ l of the former synthesised DNA, 5 μ l PCR buffer (10x), 3 μ l MgCl₂ (25 mM), 2 μ l dNTP mix (5mM), 1–1 μ l of both primers, and 36 μ l distilled water was mixed and 0.5 μ l Taq polymerase enzyme was added. The enzymes and reagents were produced by Fermentas. The amplifications were carried out using the following programme: 94 °C denaturation for 3 minutes, 35 cycles (one cycle was consisted of 94 °C for 15 seconds, 60 °C annealing for 30 seconds, 72 °C extension for 2 minutes) and 10 minutes final extension at 72 °C. The PCR-products were analysed in agarose gel (1.2%) in the presence of 0.001% ethidium bromide, using 45 mM Tris-borate pH 8.0 1 mM EDTA (TBE) as electrophoresis buffer (Sambrook et al., 1989) at 120 mA.

Specific primers were designed to the most important *tobamoviruses* (Fig. 2). These primers were created to a section, where the other *tobamoviruses* were different. (The 3' primer of the universal primer pair was used as the other half of these specific primer pairs.) The TMV-Ob specific primer was situated in front of the CP gene, while the TMV-U1 specific primer and the PMMoV specific primer were located in the CP gene. During the designing of the specific primers we had the aim to construct primers recognising different-sized PCR products (Fig. 3). Later we would like to use them in a multiplex-PCR, in which the *tobamoviruses* could be identified according to the size of the PCR products. The specific primers were also tested with more isolates following the description detailed above.

Universal primer pair:

in case of PMMoV: 699 bp

in case of TMV-U1: 709 bp

in case of TMV-Ob: 782 bp

Specific primers:

in case of PMMoV: 613 bp

in case of TMV-U1: 555 bp

in case of TMV-Ob: 899 bp

Fig. 3. The estimated length of the sections recognised by the primers

Results

In ELISA tests 9 samples were infected by PMMoV, from which 4 were infected with other viruses too. Altogether 3 samples were infected by CMV, 4 by PVY, and 1 by ToMV. In some cases complex-infection was detected, but no pathogen was found in 5 samples.

Tests were continued with samples containing the PMMoV. Symptoms were summarised in *Table 3*. Most isolates caused systemic mottling on the pepper varieties containing the L⁺ or L¹ gene, while no mottling could be detected on the pepper varieties of L² resistance level. Pepper varieties containing the L³ or L⁴ gene were resistant, responded to the infection with leaf-abscission (hypersensitive response). On *Nicotiana tabacum* cv. Xanthi-*nc* plants local lesions were observed in every case, no systemic infection occurred except in the case of mixed-infection, where the other viruses of the complex (CMV, PVY) could be responsible for the systemic symptoms. On the *Nicotiana rustica* plants local lesions developed, but no symptoms were observed at all on *Nicotiana tabacum* cv. Samsun.

A lot of examinations have been carried out with the universal primer pair to find the best parameters and to prove that this primer pair was able to recognise the *tobamovirus* isolates. *Fig. 4* demonstrates the results of a PCR examination where different authentic and own isolates were recognised by the universal primer pair. On the basis of PCR products every isolate proved to be a *tobamovirus*. On the other hand, the universal primer pair was able to recognise every *tobamovirus* isolates.

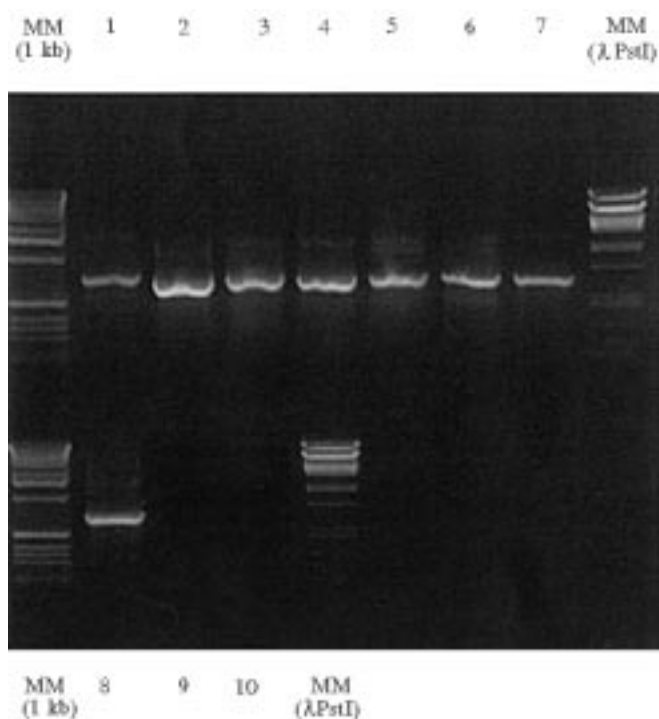


Fig. 4. PCR examination of the Ca 2 isolate using the universal and the PMMoV virus-specific primers. PCR products were analysed in agarose gel (1.2%). Line 1: isolate Ca 2 with the universal primer pair (699 bp); lines 2–3: isolate Ca 2 with the PMMoV-specific primer (in repetition, 613 bp); line 4: TMV-U1 isolate with the TMV-U1 specific primer (as comparison, 555 bp); MMa: size-marker (1 kb); MMb: size-marker (λ PstI)

Fig. 5 shows a PCR test. One of our isolates, called Ca 2 (identified as PMMoV with ELISA and test plants) was tested with the universal and PMMoV specific primers. PCR products were detected by using the universal primer pair, as well as with the PMMoV specific primer. It confirmed the former results, that the isolate Ca 2 was PMMoV. Naturally, the isolate Ca 2 was tested with the TMV-U1 and TMV-Ob primer as well, but in these cases – as it was expected – no PCR products were detected.

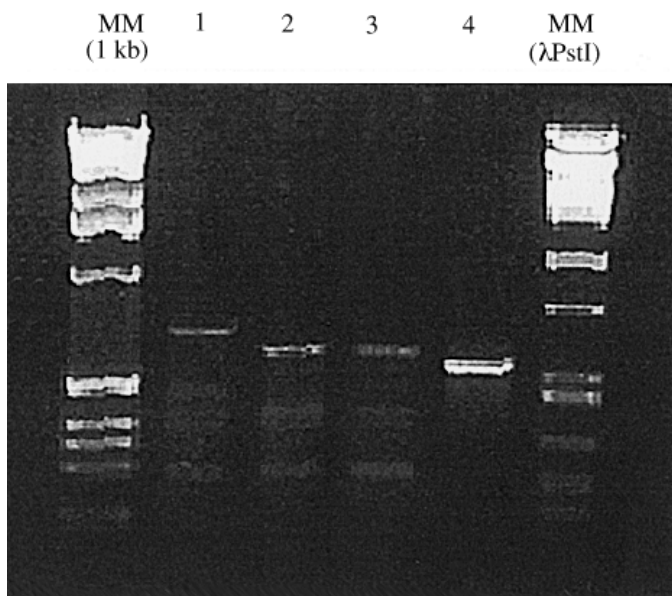


Fig. 5. PCR examination of *tobamovirus* isolates by using the universal primer pair. Lines 1–5: authentic *tobamovirus* isolates, line 1: SL 5x; line 2: SL; line 3: TMV-U1; line 4: ToMV-Ob; line 5: ToMV D/H; lines 6–8: own isolates; line 6: And; line 7: Ca 6; line 8: Ca 5a; line 9: healthy plant; line 10: control; MMA: size-marker (1 kb); MMb: size-marker (λ PstI)

Discussion

PMMoV (*versus* PMMV) was first described by McKinney in 1952. Former synonym names of the virus were: Samsun latent strain of TMV, pepper mosaic virus, *Capsicum* mosaic virus, pepper unusual strain, isolate P 8, isolate P 14, SL-TMV.

Serological tests carried out with the specific antiserum of different *tobamovirus* confirmed our hypothesis that a *tobamovirus* was present. Within the *tobamovirus* group the pathogen was identified as pepper mild mottle *tobamovirus* (PMMoV).

Two important PMMoV/PMMV isolates were separated so far, which differ in their pathotype (Boukema et al., 1980; García-Luque et al., 1990, 1993). The first one

was the Spanish isolate (PMMV-S) a pathotype of $P_{1,2}$, and the other was the Italian isolate (PMMV-I) with higher level of pathogenicity ($P_{1,2,3}$). Spanish isolate caused resistance only on the peppers containing the L^3 or L^4 gene, while the peppers of L^1 and L^2 resistance level were susceptible to its infection. The infection was symptomless on the *Nicotiana tabacum* cv. Samsun, while local lesions could be detected on the *Nicotiana tabacum* cv. Xanthi-nc and *Nicotiana rustica* plants. Similar symptoms could be observed in the case of the Italian isolate with the exception that only the L^4 pepper was resistant to its infection.

Comparing our results to the literature data it was concluded, that our samples were infected by PMMoV. The symptoms developed on the tobacco plants were more or less coincide with the data in the literature (Tóbiás et al., 1983), while the symptoms on pepper plants decided, that from the two PMMoV isolates in Hungary the less aggressive $P_{1,2}$ pathotype was found. According to the literature this pathotype caused systemic mottling on the L^2 peppers, but we did not observe symptoms at all. It was probably due to the fact, that this virus – as it is shown in its name – often causes very weak or no symptoms. No leaf-abscission was observed as in case of the resistant varieties, therefore the peppers of resistance level L^2 are susceptible to its infection.

Examination of the PMMoV isolates in nucleic acid level confirmed the results of our former serological and pathological studies. Tests with the universal primer pair demonstrated that the isolates belonged to the *tobamovirus* group, while tests carried out with virus-specific primers proved that the isolates were identical with PMMoV. The PCR-based method was efficient to identify the Hungarian *tobamovirus* isolates rapidly and easily using universal and virus-specific primers.

Acknowledgement

This work was partly supported by the AKP (98-53 3,1), and FEFA 0056/1999.

Literature

- Alonso, E., García Luque, I., Avila-Rincón, M. J., Wicke, B., Serra, M. T. and Diaz-Ruiz, J. R. (1989): A *tobamovirus* causing heavy losses in protected pepper crops in Spain. *J. Phytopathology*, 125, 67–76.
- Alonso, E., García-Luque, I., De La Cruz, A., Wicke, B., Avila-Rincón, M. J., Serra, M. T., Castresana, C. and Diaz-Ruiz, J. R. (1991): Nucleotide sequence of the genomic RNA of pepper mild mottle virus, a resistance-breaking *tobamovirus* in pepper. *Journal of General Virology*, 72, 2875–2884.
- Avgelis, A. D. (1986): A pepper strain of TMV who is new in Crete (Greece). *Phytopath. Medit.*, 25, 33–38.
- Avila-Rincón, M. J., Ferrero, M. L., Alonso, E., García-Luque, I. and Diaz-Ruiz, J. R. (1989): Nucleotide sequences of 5' and 3' non-coding regions of pepper mild mottle virus strain S RNA. *Journal of General Virology*, 70, 3025–3031.
- Betti, L., Tanzi, M. and Canova, A. (1986): Evolutionary changes in TMV pepper strains as a result of repeated host passages. *Phytopath. Medit.*, 25, 39–43.
- Betti, L., Tanzi, M. and Canova, A. (1988): Pepper mosaic virus strains and their adaptation to the host. I. Biological and serological behaviour. *Phytopath. Medit.*, 27, 7–17.

- Boukema, I. W., Jansen, K. and Hofman, K. (1980): Strains of TMV and genes for resistance. *Capsicum*, Eucarpia Working Group. Synopses of the 4th Meeting, Wageningen, 44 pp.
- Boukema, I. W. (1984): Resistance to TMV in *Capsicum chacoense* Hunz. is governed by an allele of the L-locus. *Capsicum* Newsletter No. 3, 47–48.
- Burgyán, J., Beczner, L. and Gáborjányi, R. (1978): Relationship among some *tobamoviruses*. I. Symptomatological and serological comparison. *Acta Phytopathol. Acad. Sci. Hung.*, 13, 75–85.
- Clark, M.F. and Adams A.N. (1977): Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol.*, 34, 475–483.
- Clinton, (1909): *Rep. Conn. Agric. Exp. Stn.* 1907–1908, 854.
- Csilléry, G. and Ruskó, J. (1980): The control of a new tobamovirus strain by linked to anthocyanin deficiency in pepper (*Capsicum annuum*). Eucarpia Vth Meeting, 1983, Plovdiv, Bulgaria, 40–43.
- Feldman, J. M. and Oremianer, S. (1972): An unusual strain of tobacco mosaic virus from pepper. *Phytopath. Z.*, 75, 250–267.
- Gáborjányi, R., Pogány, M. and Horváth J. (1997): A vírusok szerepe a paprikapusztulásban (Role of viruses in pepper decline). *Növényvédelem*, 33, 181–185.
- Gáborjányi, R., Horváth J., Kovács J. and Kazinczi, G. (1998): Role of viruses in pepper decline in Hungary. Xth EUCARPIA Meeting on Genetics and Breeding of *Capsicum* and Eggplant. Avignon, France. Abstr., 129–132.
- García-Luque, I., Serra, M. T., Alonso E., Wicke, B., Ferrero, M. L. and Díaz-Ruiz J. R. (1990): Characterization of a Spanish strain of pepper mild mottle virus (PMMV-S) and its relationship to other *tobamoviruses*. *J. Phytopathology*, 129, 1–8.
- García-Luque, I., Ferrero, M. L., Rodríguez, J. M., Alonso, E., De La Cruz, A., Sanz, A. I., Vaquero, M. T., Serra, M. T. and Díaz-Ruiz, J. R. (1993): The nucleotide sequence of the coat protein genes and 3' non-coding regions of two resistance-breaking *tobamoviruses* in pepper shows that they are different viruses. *Arch. Virol.*, 131, 75–88.
- Green, S. K. and Kim, J. S. (1991): Characteristics and control of viruses infecting peppers: A literature review. Technical Bulletin No 18. Asian Vegetable Research and Development Center, Taipei, pp.60.
- Horváth, J. (1969): Adatok a paprikafajták vírusokkal szembeni fogékonyságához és a paprikapatogén vírusok differenciálásához (Contributions to the susceptibility of paprika varieties to viruses and the differentiation of paprika pathogene viruses). *Növénytermelés*, 18, 79–88.
- Horváth, J. (1993): A list of proposed letter codes for hosts and non-hosts of plant viruses. *Acta Phytopathol. Acad. Sci. Hung.*, 28, 21–58.
- Kiss, F. E. (1996): Virus diseases of greenhouse pepper in South Hungary. International Workshop on Biological and Integrated Pest Management in Greenhouse Pepper, Hódmezővásárhely, Hungary, Abstr. 119.
- Linhardt, Gy. and Mezey, Gy. (1890): A dohány mozaikbetegsége (The mosaic diseases of tobacco). *Mezőgazdasági Szemle*, 1–10.
- Mayer, A. (1886): Über die Mosaikkrankheit des Tabaks. *Landwirtsch. Vers. Sta.*, 32, 451–467.
- McKinney, H. H. (1929): Mosaic diseases in Canary Islands, West Africa and Gibraltar. *J. Agric. Res.*, 39, 557–558.
- McKinney, H. H. (1952): Two strains of tobacco mosaic virus, one of which is seed-borne in an etch-immune pungent pepper. *Plant Dis. Rep.*, 36, 184–187.
- Rast, A. Th. B. (1979): Pepper strains of TMV in the Netherlands. *Med. Fac. Landb. Wet. Rijksuvin. Gent.*, 44, 617–622.
- Rast, A. Th. B. (1988): Pepper *tobamovirus* and pathotypes used in resistance breeding. *Capsicum* Newsletter, 7, 22.
- Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989): *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Salamon, P. (1993): *Tobamovirus* rezisztenciagének a *Capsicum* nemzetségben és a paprika rezisztencianemesítés hazai eredményei (*Tobamovirus* resistance genes in the genus *Capsicum* and Hungarian results of the breeding of paprika). In: *Integrált termesztés a kertészetben (Integrated production in horticulture)* 14, Budapest, 104–113.

- Salamon, P. and Beczner L. (1987a): Dulcamara yellow fleck virus (DYFV): a *tobamovirus* csoport új, *Solanum dulcamara* populációkban elterjedt, Magyarországon endemikus tagja (Dilcamara yellow fleck virus (DYFV): a new member of the *tobamovirus* group distributed in populations of *Solanum dulcamara* and endemic in Hungary). Növényvédelmi Tud. Napok 1987, Budapest, 83.
- Salamon, P., Beczner L. and Hamilton R. I. (1987b): Dulcamara yellow fleck virus (DYFV): a new member of the *tobamovirus* group isolated in Hungary. VIIIth International Congress of Virology. Edmonton, National Res. Council, Ottawa, Canada, pp. 329.
- Selassie, K. G., Dumas De Vaulx R., Marchoux G. and Pochard E. (1981): Le virus de la mosaïque du tabac chez le piment. I. Apparition en France du Pathotype P1,2. *Agronomie*, 1(10), 853–858.
- Szirmai, J. (1950): Vírusok és vírusbetegségek (Viruses and virus diseases). In: Ubrizsy, G. (ed.): Növénykórtan (Plant Pathology). Akadémiai Kiadó, Budapest, 1952. pp. 193–232.
- Tanzi, M., Betti, L. and Canova, A. (1989): PepMV infection in pepper seeds. Biological characterisation of some strains and their localisation in the seed. *Phytopath. Medit.*, 28, 204–209.
- Tenllado, F., García-Luque, I., Serra, M. T. and Díaz-Ruiz, J. R. (1994): Rapid detection and differentiation of *tobamoviruses* infecting L-resistant genotypes of pepper by RT-PCR and restriction analysis. *Journal of Virol. Methods*, 47, 165–174.
- Tenllado, F., García-Luque, I., Serra M. T. and Díaz-Ruiz, J. R. (1997): Pepper resistance-breaking *tobamoviruses*: can they co-exist in single pepper plants? *European Journal of Plant Pathology*, 103, 235–243.
- Tóbiás, I. and Csilléry, G. (1983): Virus diseases of pepper in greenhouse and plastic tunnel in Hungary. *Eucarpia Vth Meeting*, 1983, Plovdiv, Bulgaria.
- Tóbiás, I., Rast, A. Th. B. and Maat, D. Z. (1982): *Tobamoviruses* of pepper, eggplant and tobacco: comparative host reactions and serological relationships. *Neth. J. Pl. Path.*, 88, 257–268.
- Tóbiás, I., Rast A. Th. B. and Maat, D. Z. (1983): Comparison of some *tobamoviruses* from pepper with U1 strain of TMV by test plants and serology. *Proc. Int. Conf. Integr. Plant Prot.*, Budapest, 1983, 3, 46–52.
- Tošić, M., Šutić, D. and Pešić, Z. (1980): Transmission of tobacco mosaic virus through pepper (*Capsicum annuum* L.) seed. *Phytopath. Z.*, 97, 10–13.
- Wetter, C., Conti, M., Altschuh, D., Tabillion, R. and Van Regenmortel, M. H. V. (1984a): Pepper mild mottle virus, a *tobamovirus* infecting pepper cultivars in Sicily. *Phytopathology*, 74, 405–410.
- Wetter, C. (1984b): Serological identification of four *tobamoviruses* infecting pepper. *Plant Disease*, 68 (7), 597–599.
- Zatykó, L. (1982): Paprikatermesztés (Production of paprika). *Mezőgazdasági Kiadó*, Budapest, 388 pp.