Genetic Variability Between Different Isolates of *Potato Mop-top Virus* (PMTV)

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Abstract

We determined partial nucleotide sequences of several different field isolates of PMTV with well-characterized biological properties from the Czech Republic and Denmark. Subsequently we were trying to elucidate the correlation between nucleotide sequences of various isolates, their biological properties and geographical origin. From the comparison with other known sequences we can conclude, that PMTV genome is relatively conserved.

Keywords: *Potato mop-top virus*; recombinant coat protein; *Escherichia coli*

INTRODUCTION

Spraing provoked by infection of *Potato mop-top virus* (PMTV) becomes a serious and increasing problem in potato production, mainly in Northern Europe. Only very limited information is available of possible genetic variation of this virus, but understanding of genetic variability of this pathogen is crucial requirement for reliable determination. PMTV was first described in Scotland and Northern Ireland, and is now known to occur in various part of Europe, South America, Canada, China and Japan (JONES 1988). In 1983 rare occurrence of PMTV was recorded also in Czech territory (NOVÁK *et al*. 1983). PMTV causes a wide range of symptoms in haulms and tubers which vary depending on the potato cultivar and environmental conditions (KURPPA 1989). This variation in symptom expression causes difficulties in the identification of the virus disease. The virus is in field conditions transmitted by the motile zoospores of the plasmodiophoromycete fungus *Spongospora subterranea* (Wallr.) Lagerh. (ARIF *et al*. 1995), which causes powdery scab on tubers. Effective and environmentally acceptable chemical control of the fungal vector is not commercially available, and there are no sources of resistance or tolerance to PMTV that have been deliberately used in breeding programs.

After reclassification of furoviruses PMTV has been placed in the proposed new genus *Pomovirus* (TORRANCE & MAYO 1997). The particles of PMTV are tubular and rigid, 18–22 nm in diameter and 100–150 nm or 250–300 nm in length. Discrepancies in reported length are probably due to the fragility of the particles, which readily disintegrate and uncoil from one end (KASSANIS *et al*. 1972). Capsid that forms the shell of the virus consists of the single type of protein subunit encoded by a distinct virus gene. The entire genome of PMTV consists of three different positive strand RNA molecules, RNA 1, 2 and 3 (KALLENDER *et al*. 1990), of approximately 6.5, 3.2 and 2.4 kb, respectively. The complete nucleotide sequence (6043 nt) of RNA 1 was determined only recently (SAVENKOV *et al*. 1999).

The data obtained by sequencing of the coat protein gene (REAVY *et al*. 1997, 1998), proved that a number of PMTV isolates seems to be highly conserved in this region. Nevertheless a considerable differences in virulence and significant variation among isolates in biological properties were already observed. As we noticed formerly, only very little is known about the

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complete nucleotide sequences of PMTV. For further characterization of this virus we designed the set of primers covering the whole genome. After nucleotide sequencing of various isolates we compared the differences between them to determine the correlation to any biological and geographical differences.

METHODS

We used two isolates of PMTV from the Czech Republic – Pacov and Korneta and 4 isolates from Denmark (kindly provided by Steen Lykke Nielsen). The isolates were propagated in *N. debneyi*.

cDNA of PMTV RNA 1, RNA2 and RNA 3 was obtained by immunocapture reverse transcription polymerase chain reaction (IC-RT-PCR) carried out on infected leaves sap using commercially available PMTV antibodies (Adgen, UK), forward and reverse primers designed for the approprite part of the genome (Table 1). The reaction was carried in 30 cycles of 30 s denaturation at 94°C, 30 s annealing at 60°C and 1 min elongation at 72°C.

The RT-PCR products were directly sequenced using PCR primers or cloned to pUC57T/A (Fermentas) using 3'-A overhangs generated by Taq polymerase and were sequenced using an ALFexpressII Sequencer with the AutoRead Sequencing Kit (AP Life Science).

RESULTS

The PCR product containing CP coding region was cloned into plazmid pUC 57. Fidelity of the clones was defined by digestion with the specific restriction enzymes and by comparing of the sizes of the resulted fragments. Then were all cloned PCR products sequenced. All sequences of different isolates were then submitted to GenBank.

Table 1. Set of primers designed for PMTV genome sequencing

1MT-0115s	AACCATGGATCAGTCTATTTTGAGtaaaatc	31
1MT-0777s	aACCATGGGCCTATGGTATGGCGATACACA	30
1MT-1004a	aAGATCTTGCTCATAGCCACAATTAGGa	28
1MT-2074s	ACCATGGATTCTCTGAAAGAGGCGTG	26
1MT-2084a	aGATCTTTCAGAGAATCAAACCAGTG	26
1MT-3169s	ACCATGGGAAAGTCGACTTATGTGGT	26
1MT-3251A	AGATCTTCAGCAGCTTCTCTACCGAT	26
1MT-4020A	AAGGATCCACACCTTCCTCATGCAAAT	27
1MT-4033s	AACCATGGCGTCAAAGTATGAGGAGATA	28
1MT-4672S	AACCATGGCTTTGTTTGGACCGATAATGAAA	31
1MT-4773A	AGGATCCAGCTCGTCAGACGTCAt	24
1MT-5659A	AAGATCTCATATAAGAAATAGTATACAAACG	31
2MT-0370S	AACCATGGAAAGCGGATTCAACGGAAGT	28
2MT-1748S	ACTCCATGGTCCGGAATAACGAAATTGGA	29
2MT-1758A	ATAGATCTTCCGGACCATACCTGTCTGTTT	30
2MT-1962S	ACCATGGATCCTCCAGTAATATTacat	27
2MT-2102A	AAGATCTCCATATGACCTGCAGCAGTCG	28
2MT-2718A	aAGATCTAAGTGAACCACGGACCGA	25
3MT-0846S	ACACCATGGAATTAACCGCTCAGGCTTTTTGGT	33
3MT-1394s	AACCATGGGGTTGCGGAAGTTCCTTACTTG	30
3MT-1580A	AGGATCCGTCTAACATTAGTCAAAc	25
3MT-2100S	ACCATGGAACGTTTGAGAAGACT	23
3MT-2362A	AAGATCTATCCTAGCAGGATCATTCAAa	28
3MT-2990A	agatcTAATCGAACCAGTCTCGAAAGGGA	29
5MT-CP	AAGAATTCCATGGCTGAAAACAGAGGTGA	29
3MT-CP	ATTACTCGAGTGCACCAGCCCAGCGTAACC	30

DISCUSSION

The complete or almost complete nucleotide sequences for all three RNAs isolated from *Potato mop-top virus* (PMTV), the type member of genus *Pomovirus*, were determined. cDNA of PMTV RNA1, 2, and 3 were obtained by immunocapture reverse transcription polymerase chain reaction (IC-RT-PCR) carried out on infected leaves sap using PMTV specific primers for demanded regions of RNAs.

The RT-PCR products were directly cloned to pUC57T/A (Fermentas) using 3'-A overhangs generated by Taq polymerase and were sequenced using an ALFexpressII Sequencer with the AutoRead Sequencing Kit (AP Life Science). From the comparison with other known sequences we can conclude, that PMTV genome is relatively conserved. This could be considered as an interesting result, comparing with other plant RNA viruses (e.g. potyviruses), commonly performing less than 98% homology by sequencing even the identical starting material.

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