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Sequence Analysis of the Czech Potato Mop-Top Virus (PMTV) Isolate Korneta-Nemilkov

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ABSTRACT. The nucleotide sequence was determined for Czech potato mop-top virus (PMTV) isolate Korneta-Nemilkov, found in the potato field situated in South Bohemia. The nucleotide and amino acid sequences were compared with other PMTV isolates available in databases. The sequence identity was always >99 % when Czech isolate RNA 2 and RNA 3 sequences were compared with each of the 3 Danish isolates and with Sw isolate, and slightly lower when compared to Scottish isolates. Similarity of deduced proteins was 100 % for 5 out of 6 proteins used in comparison of Czech isolate with Danish isolate 54-15. The only difference between 2 isolates was found in coat protein (CP) gene. Interestingly, the CP of the Czech isolate seems to be 100 % identical to the one of Sw, while many changes were found in the region encoding TGBp2, TGBp3 and cysteine-rich protein (CRP) for these 2 isolates. The lowest similarity scores were found when comparing the Czech isolate CRP with CRP of Scottish isolates.

Abbreviations

CP	coat protein	PMTV	potato mop-top virus	TGB	triple gene block
CRP	cysteine-rich protein	ORF	open reading frame(s)		

PMTV is the type member of the genus *Pomovirus* (Torrance and Mayo 1997), occurring in potato growing regions in Europe, North and South America and Asia with cool wet climate and causing a wide range of symptoms in haulms and tubers which vary depending on the potato cultivar and environmental conditions, thus complicating the identification of the virus disease (Kurppa 1989; Sokmen *et al.* 1998). In field conditions the virus is transmitted by motile zoospores of the plasmodiophoromycete fungus *Spongospora subterranea* (WALLR.) LAGERH., which causes powdery scab on tubers (Arif *et al.* 1995; Jones and Harrison 1969). PMTV has tubular and rigid particles, measuring 18–22 × 100–150 nm or 250–300 nm (White *et al.* 1972), encapsidating three RNA components (Kallender *et al.* 1990). RNA 1, 6.0 kb long, encodes the viral RNA-dependent RNA polymerase and its read-through domain (Savenkov *et al.* 1999). The 2nd RNA, 3.0 kb long, has a single ORF encoding the 20 kDa CP and 67 kDa protein produced by read-through of an amber termination codon of CP (RT CP), which may be involved in virion assembly and virus transmission by the fungal vector (Kashiwazaki *et al.* 1995). The 3rd RNA, 2.9 kb long, contains a TGB encoding 3 proteins involved in cell-to-cell movement, and an additional ORF for a predicted CRP with unknown function (Sandgren *et al.* 2001). Molecular data on PMTV virus were based mostly on the analysis of a Swedish isolate marked as PMTV Sw whose complete nucleotide sequence was determined (Savenkov *et al.* 1999; Sandgren *et al.* 2001). Recently, the region of the RNA encoding part of read-through protein of coat protein was sequenced and compared for 9 different Danish PMTV isolates (Nielsen and Nicolaisen 2003). According to symptom development, these isolates could be grouped into 3 groups – weak, medium and strong – but no correlation between symptom grouping and nucleotide sequences was observed (Nielsen and Nicolaisen 2003). The partial data obtained by sequencing and analysis of coat proteins of different PMTV isolates proved that these viruses are highly conserved in this part of genome (Mayo *et al.* 1996; Sandgren *et al.* 2001; Čeřovská *et al.* 2003).

We sequenced the newly obtained Czech PMTV isolate Korneta-Nemilkov and compared the ensuring sequences with other PMTV isolates available in databases.

MATERIALS AND METHODS

The isolates of PMTV Korneta-Nemilkov were obtained using bait plants of *Nicotiana benthamiana* grown on soil samples from infested field (Dědič, *unpublished results*). The virus was then propagated in *N. debneyi* by mechanical inoculation using sap extracted from leaves of *N. benthamiana*.

cDNA of PMTV RNAs was obtained by immunocapture reverse transcription polymerase chain reaction (IC-RT-PCR). The tubes were coated with 100 μL anti-PMTV IgG (1 μg/mL) (*Adgen*) in coating buffer for 3 h at 37 °C. The wells were then washed (3 × 150 μL PBS+T) and 100 μL of the homogenate of PMTV infected leaves in conjugate buffer (1 : 10) was added. The samples were incubated overnight at 4 °C and washed again 3× with PBS+T buffer. After the last wash, the reverse transcription and PCR amplification with Superscript II (*Gibco*) and Taq polymerase following manufacturer's recommendations were done, using PMTV specific primers, whose sequence was based on the sequence of the Sw isolate available in *GenBank* database (accession numbers AJ238607, AJ277556, AJ243719; Savenkov *et al.* 1999; Sandgren *et al.* 2001). The PCR profile (30 cycles) was denaturation (30 s, 94 °C), annealing (30 s, 55 °C) and elongation (1 min, 72 °C).

PCR products were sequenced directly or after cloning into pUC57T/A (*Fermentas*) using 3´-A overhangs generated by Taq polymerase. In several cases it was necessary to digest cloned fragments with appropriate restriction enzymes and to religate the restricted plasmid or to reclone the chosen fragment. Sequencing was performed using an ALFexpressII Sequencer with the AutoRead Sequencing Kit (*AP Life Science*). Sequence analyses were carried out using the Genescan software (Burset *et al.* 1996), and the similarity search with sequences available in *GenBank* was performed using BLAST (Altschul *et al.* 1990). Multiple sequence alignments were done using Clustal W (Thompson *et al.* 1994).

RESULTS AND DISCUSSION

The nucleotide sequences of newly obtained PMTV Czech isolate Korneta-Nemilkov and other PMTV isolates available in databases used in the sequence comparisons are summarized in Table I.

Table I. The nucleotide sequences of newly obtained PMTV Czech isolate Korneta-Nemilkov and other PMTV isolates available in databases

Isolate	Origin	RNA[a]	Acc. no.	Reference
Korneta-Nemilkov	Czech	2 (CP)	DQ102381	–
		3 (TGB)	DQ144451	–
54-10	Danish	2 (CP)	AY277633	Pečenková *et al*. 2004
		3 (TGB)	AY426745	*ditto*
54-15	*ditto*	2 (CP)	AY196094	*ditto*
		3 (TGB)	AY187010	*ditto*
54-19	*ditto*	2 (CP)	AF487407	*ditto*
			AF508253	Nielsen and Nicolaisen 2003
			AY436586	Pečenková *et al*. 2004
		3 (TGB)	AY353719	*ditto*
S	Scottish	2 (CP)	AJ224991	Reavy *et al*. 1998
Sw	Swedish	2 (CP)	AJ243719	Sandgren *et al*. 2001
		3 (TGB)	AJ277556	–
Todd	Scottish	3 (TGB)	D30753	Scott *et al*. 1994

[a]CP – coat protein; TGB – triple gene block.

These were compared with other PMTV isolates available in databases and, based on the ORF found, it was deduced that the Czech PMTV has the same genomic organization as known PMTV isolates. The sequence of the Czech isolate was found to be highly similar to other known PMTV isolates. Relatively few nucleotide changes were found when comparing the new isolate with Danish isolates, a higher number of nucleotide substitutions was revealed by comparing the Czech PMTV with Swedish isolate Sw and the most abundant nucleotide substitutions were found in comparison with Scottish isolates S and Todd. Most of the substitutions found were transitions (Table II). No A/T, C/G nor G/T transversion was found between the Czech and Danish isolate.

In the case of RNA 2, the sequence identity was always >99 % when the Czech isolate was compared with each of the 3 Danish isolates and with Sw isolate. The analysis was performed using 1330 nucleotides from the 2nd half of this genomic RNA. In the case of Scottish PMTV isolate S this region comprised the deletion of 110 nucleotides and the sequence identity between the Czech isolate and isolate S was thus lower (97 %; Table II).

Table II. The number of nucleotide changes found when the newly obtained sequences of Czech PMTV isolate Korneta-Nemilkov were aligned and compared to other PMTV isolates available in databases[a]

Isolate RNA[b]		A/C	A/G	A/T	C/G	C/T	G/T	Σ	Δ
RNA 2[c] (CP)	54-10	1	0	0	0	0	0	1	0
	54-15	0	1	0	0	2	0	3	0
	54-19	0	1	0	0	1	0	2	0
	S[d]	2	9	0	2	7	1	22	110
	Sw	0	0	0	1	5	0	6	0
RNA 3 (TGB)	54-10	0	2	0	0	1	0	3	0
	54-15	0	1	0	0	0	0	1	0
	54-19	0	3	0	0	1	0	4	0
	Sw	3	23	8	2	19	3	58	0
	Todd	4	27	8	1	24	7	71	0

[a]Σ – total number of nucleotide changes found; Δ – deletion. [b]CP – coat protein; TGB – triple gene block.
[c]The analysis was performed using 1330 nucleotides from the 2nd half of RNA 2.
[d]This region comprises the deletion of 110 nucleotides.

The nucleotide sequences for the RNA encoding the TGB (RNA 3) of the Czech isolate was determined and for the purpose of sequence alignment with other isolates the length was adjusted to the shortest one (54-10, 2286 nucleotides). Again, >99 % sequence identity was found between the Czech and Danish isolates. The nucleotide identity was ≈97 and 96 % when Czech isolates were compared to Sw and Todd, respectively.

The identity and similarity of the newly sequenced Czech PMTV isolate Korneta-Nemilkov with other PMTV isolates available in the databases in terms of deduced amino acid sequences was also determined (Table III). A sequence similarity of 100 % was found between Czech and Danish isolate 54-15 for all proteins encoded by RNA 3 and >99 % for CP. A similarity >99 % was found when the Czech isolate was compared to other Danish isolates, while comparison with Swedish and Scottish isolate gave slightly lower similarity scores. The CP of the Czech isolate seems to be 100 % identical to that of Sw.

Table III. The identity (%, *first columns*) and similarity (%, *second columns*) of deduced amino acid sequence between the newly sequenced Czech PMTV isolate Korneta-Nemilkov and other PMTV isolates available in databases[a]

Isolate	54-10		54-15		54-19		S		Sw		Todd	
CP	–	–	99.3	98.7	99.3	98.7	–	–	100	100	–	–
RT CP	100	100	100	100	100	100	98.4	97.7	99.3	99.3	–	–
TGB 1	99.8	99.8	100	100	100	100	–	–	98.1	98.1	97.7	97.2
TGB 2	99.2	99.2	100	99.2	100	98.3	–	–	97.5	97.5	97.5	97.5
TGB 3	99.5	100	100	100	99.5	99.5	–	–	97.7	96.9	94.8	94.8
CRP	100	100	100	100	100	98.5	–	–	92.8	88.4	92.8	88.4

[a](–) – unavailable data.

The lowest similarity scores were found when Czech isolate CRP was compared with the CRP of Sw and Todd. Similarly to the situation found for Danish isolates (Pečenková *et al.* 2004), the CRP ORF of Korneta-Nemilkov isolate has the first codon for Met mutated to the codon for Val.

The newly obtained nucleotide sequence of the Czech PMTV isolate described here thus confirms the quite high stability of the PMTV genome. The coat protein of the Czech isolate is 100 % identical to the Swedish isolate, while many nucleotide changes were found for regions encoding TGBp2 and TGBp3.

The discrepancy found in the mutation rate for two RNAs makes us speculate that the evolution of these isolates could include a pseudorecombination event. The interchange of RNAs between these isolate ancestors could explain the higher similarity with Swedish isolate for RNA 2 and with Danish isolates for RNA 3. This explanation could be valid if different isolate ancestors had met in the nature, possibly as the consequence of the mobility of sources and technology in the potato cultivation.

According to our analysis, Czech PMTV seems to be the most similar to the Danish 54-15 isolate. Since this isolate belongs to the group of isolates which provokes very strong symptoms during the host plant infection, it remains to be elucidated whether the Czech isolate exhibits the same behavior when propagated on indicator plants. Should this be confirmed, the significance of control of this pathogen in the area where it was found should be a priority goal, since the decreases in the quality of potato production caused by this pathogen could induce severe economical losses.

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