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Pea Streak Virus Recorded in Europe

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Abstract

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Alfalfa (*Medicago sativa* L.) is concluded to be the principal reservoir of *Pea streak virus* (PeSV, genus *Carlavirus*) which induces necrotic streaking symptoms in pea. This virus is prevalent in pea growing areas in the USA, but in Europe it was recorded only once almost 60 years ago. Recently, filamentous virus particles 600–700 nm long have been observed in examined plant sap of alfalfa with leaf malformation, local necrotic lesions and yellow spots on leaves. Four kilo base pairs nucleotide sequence of PeSV including partial replicase gene, triple gene block, and capsid protein (CP) gene has been determined. On the nucleotide level, the sequence of the CP has about 80% identity with the North American isolates of PeSV, however, on the amino acid level the sequence has more than 94% identity. This is the first sequence-based proof of PeSV presence in Europe.

Keywords: carlavirus; alfalfa; *Pea streak virus*; partial sequence

Alfalfa (*Medicago sativa* L.) represents one of the most important and widely planted leguminous crops. In the Czech Republic there are 18 registered cultivars growing mainly as fodder crop representing about 36% of perennial green fodder produced (Czech Statistical Office, release November 2010). Beside many fungal diseases causing the highest damage of alfalfa, there are more than 30 viruses of 15 viral families listed to be able to infect alfalfa (Brunt *et al.* 1996). In the latest screening for viruses in cultivated and wild growing forage crops in the Czech Republic, *Red clover mottle virus* (RCMV, genus *Comovirus*), *Clover yellow vein virus* (genus *Potyvirus*), *Potato virus X*, *White clover mosaic virus* (both genus *Potexvirus*), new unnamed virus of the genus *Cytorhabdovirus* were identified by sequencing. Furthermore, bacilliform particles (ca. 213–533 by 44–58 nm) of the genus *Cytorhabdovirus*, *Alfalfa mosaic virus* (genus *Alfamovirus*), and particles resembling viruses of the genus *Badnavirus* (ca 30 by 220–500 nm) were observed on negatively stained preparations and ultrathin sections by electron microscopy (Fránová *et al.* 2009; Fránová & Jakešová 2012).

Pea streak virus (PeSV, genus *Carlavirus*) was first described in 1938 (Zaumeyer 1938) and was found in pea growing areas in the USA. Alfalfa latent virus has been described in 1977 (Veerisetty & Brakke 1977), but recently it is recognised as a mild strain of PeSV causing mostly symptomless infections and distinguishable from PeSV only by typical symptoms on pea plants (Hampton 1981). PeSV is presumed to be widespread in most alfalfa growing areas (Rahman & Paeden 1993), but outside America it was recorded only once by electron microscopy and by serology in white sweet clover (*Melilotus alba*) in Germany (Brandes & Quantz 1957; Wetter & Quantz 1958). Only sequences of American isolates are represented in GenBank now – two identified as ALV and originated from alfalfa host, and two identified as PeSV from pea and from unknown host (Table 1). In this work we firstly describe sequence of a PeSV isolate coming from Europe.

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Table 1. Viruses and isolates used

Acession number	Isolate	Source	Author's identification	nt	Country of origin	Reference
KP828803	VRS-541	unknown	PeSV	8041	unknown	Su *et al.* (2015)
AF354652	ATCC PV-87	*Pisum sativum*	PeSV	2658	USA, Wisconsin	Cavileer & Berger (2001, unpublished)
KP784454	ATCC PV-264	*Medicago sativa*	ALV	8041	USA, Nebraska	Nemchinov *et al.* (2015)
AY037925	ATCC PV-264	*Medicago sativa*, *Pisum sativum* lab host	ALV	2677	USA, Nebraska	Cavileer *et al.* (2001, unpublished)
HM107774	V4	*Medicago sativa*	PeSV	4046	Czech Republic	this work

MATERIAL AND METHODS

Alfalfa (*Medicago sativa*) plant originally designated as sample "V4" with leaves malformation (yellow spots becoming local necrotic lesion) has been found in Želešice (SE of the Czech Republic). Crude plant sap preparation from symptomatic leaves was negatively stained with uranyl acetate and inspected using transmission electron microscopy (JEM 1010; JEOL USA, Peabody, USA). RNA was isolated from the symptomatic plants with Nucleospin RNA II kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's recommendation and transcribed with RevertAid H Minus MMLV reverse transcriptase (Fermentas, Vilnius, Lithuania). The degenerate primer pair crlTFGf 5'-CNTTTGGNGARAGCACNGG-3' and crlKLWr 5'-YTTNARCCANGGRTCNCC-3' designed to amplify a conserved segment of carlavirus RNA polymerase gene was used as described (Petrzik 2009).

RESULTS AND DISCUSSION

Filamentous particles with modal length of 620 nm typical for carlaviruses have been observed in crude sap preparation from symptomatic leaves.

RT-PCR using degenerate carlavirus-specific primers and a walking primer approach has been applied and resulted with 4046 nt contig of the 3'-terminal part of the virus genome (Acc. No. HM107774). This part contained C-terminal part of RNA-polymerase gene, triple gene block (TGB), CP gene and, the 3' terminal noncoding sequence excluding the poly(A) tail. The Cys-rich protein of 11–16 kDa encoded by ORF6 of carlaviruses was not present there.

Nucleotide sequence comparison of the CP gene revealed about 80% identity with sequences deposited as PeSV and ALV in GenBank (Table 2), however, amino acid sequence comparison of that gene revealed about 95% identity with the PeSV and ALV sequences, respectively. The next closest species from the genus *Carlavirus* was *Red clover vein mosaic virus* (FJ685618) which was 59.2% identical in nucleotide sequence and 56.8% identical in amino acid sequence of the CP, however this virus differed in the presence of ORF6. As the ICTV stated *Pea streak virus* as a species of the genus *Carlavirus*, the virus from alfalfa sample "V4" collected in the Czech Republic was identified as a strain of PeSV, therefore. Phylogenetic analysis based on the amino acid sequences performed using MEGA software (Tamura *et al.* 2011) placed this sequence in PeSV/ALV cluster close to *Red clover vein mosaic virus* (FJ685618) (Figure 1). We propose that the sequences KP828803/AF354652 and KP784454/AY037925 are variants of the same isolates: the first one the PeSV isolate from Wisconsin (Su *et al.* 2015), the second one the PV-264 isolate from Nebraska (Nemchinov *et al.* 2015). Despite different names

Table 2. Nucleotide (above diagonal) and amino acid (below diagonal) identities among capsid protein gene of PeSV isolates and RCVMV (%)

	HM107774	AF354652	AY037925	KP784454	KP828803	FJ685618 (RCVMV)
HM107774		80.0	79.9	79.9	79.8	59.2
AF354652	94.6		82.4	81.7	98.5	59.9
AY037925	95.6	94.9		98.9	82.4	60.3
KP784454	96.3	94.9	99.3		81.7	60.3
KP828803	94.6	100.0	94.9	94.9		59.3
FJ685618 (RCVMV)	56.8	57.5	56.5	56.8	57.5	

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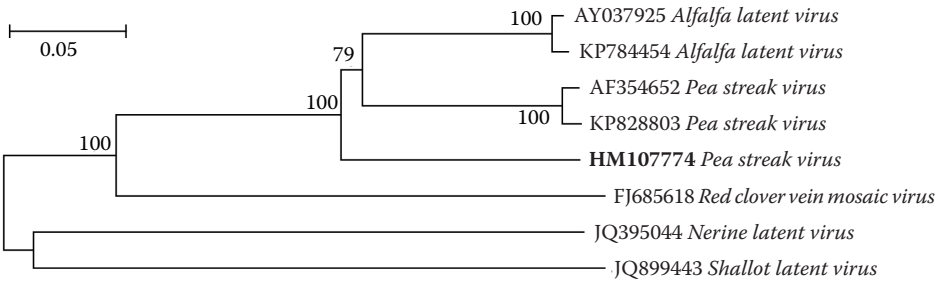

Figure 1. Phylogenetic tree of the capsid protein amino acid sequences of PeSV/ALV isolates and related sequences of carlaviruses. The tree was constructed in MEGA5 software using the minimum evolution method and 1000 bootstrap replications

of the viruses used by authors, the Czech sequence joined them in a monophyletic cluster of PeSV isolates. The Czech sequence differs substantially from the Nebraska and Wisconsin isolates and possesses unique $Q \rightarrow A_{(20)}$,$S \rightarrow Q_{(26)}$, $S \rightarrow A_{(27)}$, $S \rightarrow T_{(67)}$ amino acid substitutions concentrated on the N-terminal part of CP.

The $Pi_{(a)}/Pi_{(s)}$ mean diversity parameter, where $Pi_{(a)}$ is the number of non-synonymous substitutions per site and $Pi_{(s)}$ is the number of synonymous substitutions per site computed for five available CP sequences (882 sited for 294 codons) was 0.0453 (0.0270/0.5969). This low value (smaller than 1) suggests that strong purifying selection which conserved the protein sequences occurred there despite the occurrence of nucleotide polymorphism.

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