# MOLECULAR PROBING OF POTATO VIRUS S GENOME

## MOLEKULÁRNÍ PROBING GENOMU VIRU S BRAMBORU

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Complete genomes of three isolates of Potato virus S (PVS) were cloned and sequenced. The complete sequence of PVS genome enabled to develop an immunocapture RT-PCR probing of the PVS genome. Using this system, the sequence variability of 11 genome zones was examined for 34 PVS isolates including 15 PVS-CS variants that caused a systemic infection in *Chenopodium quinoa*. A broad variability between PVS isolates and diverse sequence variants was found. cDNA fragments covering the coat protein (CP) leader and CP-coding region (approx. 420 bp) were pooled for PVS-O and Chenopodium-systemic PVS isolates (PVS-CS) and corresponding cDNA libraries were screened for sequence variants. Genome probing suggested that the PVS-CS isolates analyzed were close to the ordinary European isolates of ordinary strain of PVS (PVS-O) but distant to the original Andean strain of PVS (PVS-A).

genome sequence; Potato virus S; RT-PCR; *Solanum tuberosum* L.; virus genome analysis

## INTRODUCTION

PVS, a member of the *Carlavirus* genus belongs to the recently created family of *Flexiviridae* (ADAMS *et al.*, 2004). The virus has 610–700 nm long flexuous filamentous particles and is transmitted by aphids in a non-persistent manner to plant species of the *Solanaceae* and *Chenopodiaceae* families. The capped single-stranded genomic RNA is polyadenylated and encapsidated in a CP of approximately 34 K (MACKENZIE *et al.*, 1989; FOSTER, 1992; MONIS *et al.*, 1987). The 3'-part of the virus genome has been characterized in detail earlier (MACKENZIE *et al.*, 1989; MATOUŠEK *et al.*, 2000b); it contains a characteristic block of three ORFs encoding 25 K, 12 K and 7 K proteins) (MOROZOV *et al.*, 1989). The virus forms minor amounts of two subgenomic RNAs (2.5 kb and 1.5 kb), the smaller of which codes for CP and 11 K protein (FOSTER and MILLS, 1992). It has been proposed that the smaller subgenomic RNA encoding the coat protein has a highly active translation enhancing leader sequence (TURNER and FOSTER, 1997). To date, only the sequence of the 3'-part of the PVS genome (MACKENZIE *et al.*, 1989; MATOUŠEK *et al.*, 2000b) and restriction analysis of complete 7.5 kb PVS genome (MONIS and DE ZOETEN, 1990) have been published. Information about the 5'-part of the PVS genome including a detailed description of ORF-1 is not available.

PVS is represented by PVS-O and highly virulent PVS-A (MACKENZIE *et al.*, 1989). Although the disease, caused by PVS-O (FOSTER and MILLS, 1992) may be symptomless on leaves and tubers of infected potato, the disease incidence may reach 100 % with yield losses of 15 %. Whereas PVS-O only causes local lesions on inoculated *Ch. quinoa* leaves, PVS-A does systemic infection. In the previous work, a broad natural sequence variability among closely related Central European PVS-O isolates has been reported (MATOUŠEK *et al.*, 2000b). This study reports sequencing of complete PVS genome and differences between the PVS-O (ordinary) and *Chenopodium*-systemic PVS-A (Andean) isolates found by molecular probing, as described previously for Potato virus Y (PVY) (PTÁČEK *et al.*, 2002).

## MATERIALS AND METHODS

### PVS isolates and plant inoculation

Nineteen PVS isolates originating from different potato cultivars were collected and *in vitro* maintained. The presence of PVS was assessed by ELISA. The isolates included those from *in vitro* germplasm collections as well as new isolates from breeding potato lines. Potato plants were *in vitro* maintained on the MURASHIGE and SKOOG (1962) medium with 0.7% agar solidified at 10oC and with an 8 hrs/day photoperiod. For the experiments new cuttings were prepared and maintained at 20 °C and a 16 hrs/day photoperiod. These were maintained on *Lycopersicum esculentum* cv. Nevski and kept in climatic chambers at a similar temperature and photoperiod. For a PVS bioassay, the isolates were first propagated

on tomato plants *L. esculentum* cv. Nevski. Indicator plants (*Ch. quinoa* and *L. esculentum* cv. Nevski) with fully developed third leaves were then mechanically inoculated with a crude sap containing each virus isolate in 50 mmol/l phosphate buffer pH 7.4. Three plants were used per isolate and maintained in a greenhouse at natural temperature and photoperiod. Inoculated plants were scored repeatedly for visual symptoms. Three PVS isolates, namely Czech isolates Kobra and Vltava, and German PVS isolate Leona were subjected to genome sequencing. All the isolates were originally collected from homonymous potato cultivars and maintained in germplasm since the 1990s.

*Double-antibody sandwich ELISA (DAS-ELISA)* was used to verify the infection at 3 and 5 weeks post inoculation (p.i.) (CLARK and ADAMS, 1977). A broad-spectrum of PVS antibodies from Bioreba was employed.

**Immunocapture RT-PCR probing of PVS genome**

PCR and RT-PCR primers were designed from the full length PVS genome sequence. The latter was divided into 11 zones (A-K, Fig. 1). Each zone was subdivided into three parts (I-III) according to a strategy for the genome of PVY described earlier (PTÁČEK *et al.*, 2002). In this way three fragments ranging from 237 to 492 bp were obtained. Such fragments can be easily analyzed by thermodynamic methods (MATOUŠEK *et al.*, 2000a). In total, 33 primer pairs (Table 1) were designed. The thermodynamic properties and structural features of individual primer pairs were calculated using the Gene Runner, version 3.02 (Hastings Sofware, Inc.) to obtain minimal differences in annealing temperatures and to avoid complicated secondary structures as much as possible. Immunocapturing was performed on ELISA microplates (Nunc Maxisorp) using polyclonal antibodies (Bioreba). First strand cDNAs were synthesized with the Enhanced Avian HS RTPCR Kit from Sigma using random DNA nonamers. The RT ran at 42 °C for 60 mins according to the manufacturer‘s instructions.

In PCR (final volume of 50 µl), 5 µl of the RT reaction mixture was amplified using the abovementioned kit and primers described in Table 1 in the PTC-100 Thermocycler (MJ Research). The cycling conditions included a denaturation at 94 °C for 3 mins, 35 cycles of 94 °C/30 secs, 52–57 °C/45 secs, and 72 °C/1 min, and final extension at 72 °C for 5 mins. The PCR products were separand in 1% agarose gel stained with ethidium bromide and visualized using the EDAS 290 (Kodak) photo-documentation system.

The electrophoreograms were quantified by means of the ImageQuaNT software (Molecular Dynamics, USA). Band intensities were expressed in percents.

## RESULTS AND DISCUSSION

Full length genomes of three PVS isolates, Leona, Vltava and Kobra were cloned and genome sequence was obtained directly for only one of them, Leona. For the remaining

two isolates complete genome sequences were reconstituted from shorter cDNA fragments. The genome sequences for the isolates Leona, Vltava and Kobra were deposited in the EMBL nucleotide database under Acc. Nos. AJ863509, AJ863510 and Y15625, respectively. Complete genome sequences showed approx. 8 % differences among the three isolates.

In order to characterize the PVS collection in more detail, a system of immunocapture RT-PCR probing of PVS genome was employed. This method has previously been used for PVY for quick molecular comparison of various isolates and their characterization including prediction of mutations in individual genome regions (PTÁČEK *et al.*, 2002). Complete PVS genome sequence enabled designing a similar probing system for PVS using the complete genomic sequence as a template (Fig. 1). PVS genomes were probed within 11 zones (A-K), each approached with three pairs of balanced primers to amplify three short subfragments. Thus, a specific fingerprint based on 33 primer pairs was obtained for each isolate (Table 2). A broad variability of PVS was found throughout the whole PVS genome, with characteristic patterns for individual isolates, suggesting numerous changes at the nucleotide level. This finding is consistent with previous ones of broad nucleotide variability in PVS (MATOUŠEK *et al.*, 2000b). Furthermore, numerous differences among isolates were observed, also within ORF-1 covered with primers from zone A-I to zone I-I (Fig. 1). Some nucleotide changes in ORF1 were observed also among the isolates Leona, Vltava and Kobra in the highly similar 5‘- and 3‘-parts of RPT coding sequence, as well as in LSR (data not shown). However, these changes did not include functional protein domains. 2. No specific differences in the probing patterns were observed among the isolates PVS-O and PVS-CS, suggesting that the sequence determining the “CS character” of PVS must be restricted to a short sequence(s) and protein domain(s). Although most of PVS-CS isolates showed high similarity with the PVS-O ones, four (ASS, Peru, Peruanita and Yuguima) exhibited a high divergence of probing patterns from the rest of PVS isolates. The same was true for the isolate Bograital that, according to the bioassay, was a PVS-O. The lowest probing pattern similarity was observed for the isolate ASS, with which a weak PCR band was observed for the zone G-II, only. The primers of this zone at the 5‘-end of the fragment and a stretch of conserved nucleotides at its 3‘-end. A nucleotide probing system, developed for the 3‘-portion of PVS-A (MACKENZIE *et al.*, 1989), also showed that this strain was very divergent from other PVS isolates tested (data not shown). In previous studies on a limited number of isolates (FOSTER, 1991; MATOUŠEK *et al.*, 2000b), it was proposed that major differences between PVS-A and PVS-O genomes concerned the region of genes for 7 K and 11 K protein. For detailed investigations by thermodynamic analysis and sequencing the fragment J-III (now designated 7KCPLCP) of approximately 420 bp that covered the region from the C-terminal part of the 7 K protein to the N-terminal part of CP, including the CP translational enhancer (CP leader, CPL; TURNER *et al.*, 1999) was chosen. 7KCPCPL fragments from positive PVS-O and PVS-CS variants were pooled separately to form PVS-O- and PVS-CS-specific fragment pools. In total, 17 and 18 distinct

sequence variants were identified in the PVS-O and PVS-CS pools, respectively. It was found that both cDNA pools differed in distinct nucleotide positions especially within the 5‘-end of the CP gene, suggesting some divergence of these two groups of sequences. For instance, G or C at the position 232 of the 7KCPLCP fragment in the PVS-O group was replaced by A in all the PVS-CS variants. Also, distinct mutations accumulated at high frequency in other positions and in total, 23 such positions were detected (data not shown).

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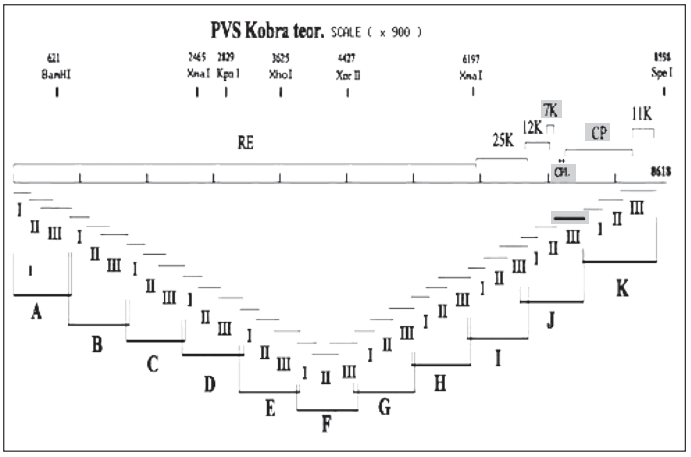


**Fig. 1:** Analysis of cDNA fragments from PVS Kobra isolate using selected primers

**Table 1:** Characteristics of primers

| | Primer designation and sequence Position | Gene region in PVS genome | Ann. t. (°C) |
|---|---|---|---|
| Zone A | A-I-1: 5´TCACTATAGGGCGAATTGGG3´ | 3-22 | RPT 57.0 |
| | A-I-2: 5´CTCTTGAAAGCTGCTGGTGG3´ | 255-236 | RPT 57.0 |
| | A-II-1: 5´ATCCCTAATTTCCAACGTCG3´ | 216-235 | RPT 56.0 |
| | A-II-2: 5´TCGGCACTGCTCACATACC3´ | 539-521 | RPT 55.6 |
| | A-III-1: 5´CGGTGTGCAAGACATTGGA3´ | 371-389 | RPT 56.3 |
| | A-III-2: 5´CCAACGAATATCCCCGTTG3´ | 788-770 | RPT 57.0 |
| Zone B | B-I-1: 5´TTGTGCACCATTGTGTACCC3´ | 751-770 | RPT 55.6 |
| | B-I-2: 5´GGTGTGATCAAATCGCCCT3´ | 1022-1004 | RPT 56.4 |
| | B-II-1: 5´GCAAATTCGCCCATCATCT3´ | 971-989 | RPT 57.1 |
| | B-II-2: 5´ATCATGACTCTCAAAGTGCCAG3´ | 1286-1265 | RPT 55.8 |
| | B-III-1: 5´CGACAAGCAGTCTGCAATGG3´ | 1164-1183 | RPT 58.5 |
| | B-III-2: 5´CACCCACCTCCTATCCATAGC3´ | 1533-1513 | RPT 57.8 |
| Zone C | C-I-1: 5´GACATGATTGAAGCTATGGATAG3´ | 1501-1523 | RPT 53.4 |
| | C-I-2: 5´CGAGAAGCGAGCAACTTG3´ | 1759-1742 | RPT 53.3 |
| | C-II-1: 5´CCTACGTGGCTCAGATCGC3´ | 1721-1739 | RPT 57.5 |
| | C-II-2: 5´CCCACTCCGTGAAAGCTGTA3´ | 2033-2014 | RPT 57.4 |
| | C-III-1: 5´CCGATTGGGCAAGGTACAA3´ | 1922-1940 | RPT 58.0 |
| | C-III-2: 5´AGTACCATGCAGCACGCTTG3´ | 2284-2265 | RPT 58.4 |
| Zone D | D-I-1: 5´AAGGGGGTTCCAGGCAA3´ | 2251-2267 | RPT 56.6 |
| | D-I-2: 5´GACTGACCTCCAACCTCAGC3´ | 2510-2491 | RPT 55.2 |
| | D-II-1: 5´TGATGGAGGCCAGTTCATCC3´ | 2451-2470 | RPT 59.0 |
| | D-II-2: 5´CTCATTGGGCTTGGAACTCC3´ | 2751-2732 | RPT 58.4 |
| | D-III-1: 5´CTGCCACAACGCCAGGTG3´ | 2711-2728 | RPT 59.4 |
| | D-III-2: 5´GCACTCAGCACCACGATCCT3´ | 3050-3031 | RPT 59.2 |

*pokračování*

| | | | |
|---|---|---|---|
| Zone E | E-I-1: 5´CGCAACAAGTAAGCAATACCG3´ | 3006-3026 | RPT 58.4 |
| | E-I-2: 5´CTTCACCCTTCAGCACATCCT3´ | 3268-3248 | RPT 58.1 |
| | E-II-1: 5´CGCGCAGTTGGGTAATGAG3´ | 3222-3240 | RPT 58.9 |
| | E-II-2: 5´GTTGTACGGTAGCAACGTGC3´ | 3528-3508 | RPT 58.9 |
| | E-III-1: 5´GTGCATGATGCTAGCTGTGA3´ | 3451-3471 | RPT 55.4 |
| | E-III-2: 5´CAAGCTCATTGAAAGAATCT3´ | 3799-3778 | RPT 56.5 |
| Zone F | F-I-1: 5´ATCTACGTATCCCCAAGGAAG3´ | 3751-3771 | RPT 54.8 |
| | F-I-2: 5´TCGCTTTGGCACGGATC3´ | 4013-3997 | RPT 56.3 |
| | F-II-1: 5´CTCTATGTTGGCACCAGCG3´ | 3954-3972 | RPT 55.9 |
| | F-II-2: 5´AAGAGCTCGGGAAGTAAGTCT3´ | 4300-4280 | RPT 54.0 |
| | F-III-1: 5´CACAATCATTGAGGGCATTG3´ | 4188- 4207 | RPT 55.6 |
| | F-III-2: 5´GGCAACAACATCTTGAGGTC3´ | 4550-4531 | RPT 53.7 |
| Zone G | G-I-1: 5´GCTCAAAACAGCAAAGATTGAT3´ | 4509-4530 | RPT 56.2 |
| | G-I-2: 5´CTTTTCAACCCACCTTGCC3´ | 4782-4764 | RPT 56.7 |
| | G-II-1: 5´ATGGTTTCGCACACATTTGC3´ | 4719-4738 | RPT 58.1 |
| | G-II-2: 5´GAAATGGCCCATACATCTC3´ | 5029-5011 | RPT 58.6 |
| | G-III-1: 5´AAGATTGAGGTTTTCGGACC3´ | 4959-4978 | RPT 55.0 |
| | G-III-2: 5´GCGGCATAGGACGCTATG3´ | 5298-5281 | RPT 55.7 |
| Zone H | H-I-1: 5´GCAGCACAAACCATTGTCTG3´ | 5254-5273 | RPT 55.7 |
| | H-I-2: 5´TCAATGCTAACTCAAACGCC3´ | 5500-5481 | RPT 54.1 |
| | H-II-1: 5´GACTATGAAGCCTTTGACGC3´ | 5440-5459 | RPT 54.6 |
| | H-II-2: 5´TGAATAGAGCCCTGTTGGC3´ | 5731-5713 | RPT 56.1 |
| | H-III-1: 5´CTTGTTCAACACAATGGCCA3´ | 5619-5638 | RPT 56.1 |
| | H-III-2: 5´TCTCGGACTTCAGCAAATGC3´ | 6049-6030 | RPT 57.5 |
| Zone I | I-I-1: 5´TTGTGTGAGGGTGATTATTAAG3´ | 6003-6024 | RPT-25K 51.8 |
| | I-I-2: 5´ACTTAAGCAACTCCCGAATT3´ | 6240-6221 | RPT-25K 53.0 |
| | I-II-1: 5´AGCTGGTAAAAGTTCTGCAA3´ | 6203-6222 | 25K 52.1 |
| | I-II-2: 5´AATCTTGACCTTCTGCCTGT3´ | 6531-6512 | 25K 52.5 |
| | I-III-1: 5´AGTACCGCTGTGAGCCCAT3´ | 6411-6429 | 25K-12K 56.4 |
| | I-III-2: 5´GTGTAGTTAGGCGGCGGT3´ | 6800-6783 | 25K-12K 54.7 |
| Zone J | J-I-1: 5´CTGGTTGATAAGGCTGCTGC3´ | 6702-6720 | 25K-12K 57.4 |
| | J-I-2: 5´GCTTTAGTGCCGTCGCGA3´ | 6938-6921 | 25K-12K 59.3 |
| | J-II-1: 5´ACTGCCAATTGTTGGGGAT3´ | 6878-6888 | 12K-7K 56.1 |
| | J-II-2: 5´TCCCCAGTAATGAGTAGGACG3´ | 7180-7160 | 12K-7K 56.0 |
| | J-III-1: 5´TAAGTTGGTATGCGCTCAGG3´ | 7123-7142 | 7K-CPL-CP 55.6 |
| | J-III-2: 5´GGGTCGGCTCAAGCGA3´ | 7544-7529 | 7K-CPL-CP 55.8 |
| Zone K | K-I-1: 5´GGTAGGCCCTCGCTTGAG3´ | 7520-7537 | CP 56.6 |
| | K-I-2: 5´CACAGTCCCTGCTGGATCTAG3´ | 7783-7763 | CP 56.0 |
| | K-II-1: 5´GAAAGTGGTGATCATGTGTGC3´ | 7720-7740 | CP 54.3 |
| | K-II-2: 5´TGAGCTATTGCTTCCTCAGGT3´ | 8055-8035 | CP 56.0 |
| | K-III-1: 5´CGTAGAGGGGCTCATACGC3´ | 8008-8026 | CP-11K 57.1 |
| | K-III-2: 5´CTCTGACTTTGCACCATGGG3´ | 8499-8480 | CP-11K 57.3 |

**Tab. 2:** Molecular probing of PVS isolates

| ISOLATES | strain | PRIMER ZONES (corresponding to Tab. 1) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A-I | A-II | A-III | B-I | B-II | B-III | C-I | C-II | C-III | D-I | D-II | D-III | E-I | E-II |
| Alka | o | – | + | – | + | + | – | – | + | (+ | – | – | – | + | + |
| Arran Banner | o | + | – | – | – | – | – | – | + | – | – | – | – | + | (+ |
| Karin | o | – | + | + | + | + | – | – | + | + | + | + | + | + | + |
| Kobra | o | – | – | – | + | + | – | – | + | + | + | – | – | + | + |
| Kordoba | o | + | + | (+) | + | + | – | – | + | + | + | (+ | (+ | + | + |
| Nordchip 54 | o | + | + | – | + | + | – | + | + | + | + | – | – | + | + |
| Oddesky | o | + | + | – | + | + | – | – | – | – | – | – | – | + | + |
| Amarilla del Centro | o | + | + | + | + | + | + | – | + | + | + | + | + | + | + |
| Aquila | o | + | + | (+ | + | + | + | – | + | + | (+ | (+ | – | – | + |
| Bograital | o | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Centinial Russet | o | – | + | – | + | + | + | – | + | + | + | + | – | + | + |
| Fransen | o | (+ | + | + | + | + | + | – | + | + | + | + | – | + | + |
| Heideniese | o | (+ | + | + | + | + | + | – | + | + | + | + | – | + | + |
| Linzer Rose | o | (+ | + | + | + | + | + | – | + | + | + | + | + | + | + |
| Long Blue | o | – | + | – | + | + | + | – | + | + | + | + | – | + | + |
| Purple and White | o | + | + | + | + | + | + | – | + | + | + | + | – | + | + |
| Reichskanzler | o | + | + | + | + | + | + | – | + | + | + | + | (+ | + | + |
| VAC 67 | o | + | + | – | + | – | + | – | + | + | + | (+ | + | + | + |
| Wohltmann | o | + | + | – | + | + | + | – | + | + | + | (+ | – | + | + |
| ASS | cs | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Peru | cs | (+ | – | – | – | – | – | – | – | – | – | – | – | (+) | – |
| Dutch | cs | – | + | – | + | + | – | – | + | + | (+ | – | – | + | + |
| Germ | cs | – | + | – | + | + | – | – | + | + | + | – | – | + | + |
| I/47 | cs | + | – | – | + | + | – | + | – | + | (+ | – | – | + | + |
| I/84 | cs | + | + | – | + | + | – | – | + | + | + | + | (+) | + | + |
| II/24 | cs | (+ | + | – | + | + | – | – | + | + | + | – | – | + | + |
| II/31 | cs | – | + | + | + | + | – | – | + | + | + | + | + | + | + |
| 10 II/35 | cs | + | + | – | + | + | – | – | + | + | + | – | – | + | + |
| Dunbar Rover | cs | + | + | (+) | + | + | – | – | + | + | (+) | + | (+) | + | + |
| Tarpan | cs | + | + | – | + | + | – | – | + | + | + | – | – | + | + |
| Blaue Mandel | cs | + | + | + | + | – | + | – | + | + | + | + | (+ | + | + |
| Clivia | cs | + | + | + | + | + | + | – | + | + | + | + | + | + | + |
| Peruanita | cs | + | – | – | – | – | + | – | – | – | – | – | – | + | – |
| Yuguima | cs | + | – | – | + | – | + | – | – | – | – | – | – | + | – |
| Desiree VF | | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| E-III | F-I | F-II | F-III | G-I | G-II | G-III | H-I | H-II | H-III | I-I | I-II | I-III | J-I | J-II | J-III | K-I | K-II | K-III |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| + | + | (+ | + | + | + | + | + | (+) | – | – | (+) | + | + | + | + | + | – | + |
| – | + | – | + | – | (+ | – | + | – | – | – | + | – | – | – | + | (+ | – | (+ |
| + | + | + | + | + | + | + | (+ | – | + | – | + | + | + | + | + | + | + | + |
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**Vědecké práce – Výzkumný ústav bramborářský Havlíčkův Brod, 2019, *25*: 29-38**

Byly klonovány a sekvenovány kompletní genomy tří izolátů viru S bramboru (PVS). Kompletní sekvence PVS genomu umožnila vyvinout imunovazebnou RT-PCR metodu testování PVS genomu. Pomocí tohoto systému byla prokázána variabilita sekvence 11 genomových zón u 34 PVS izolátů včetně 15 variant PVS-CS, které způsobily systémovou infekci na *Chenopodium quinoa*. Byla zjištěna široká variabilita mezi PVS izoláty a různými sekvenčními variantami.

Izoláty PVS (PVS-CS) a odpovídající knihovny cDNA byly testovány pro sekvenční varianty. Genomové testování naznačilo, že analyzované izoláty PVS-CS se nacházejí v blízkosti běžných evropských izolátů běžného kmene PVS (PVS-O), ale vzdálené od původního andského kmene PVS (PVS-A).

sekvence genomu; virus S bramboru; RT-PCR; *Solanum tuberosum* L.; analýza virového genomu

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