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GENOME SEQUENCE ANALYSIS, RT-PCR DIAGNOSTICS AND CONSTRUCTION OF VIRAL PROTEIN-EXPRESSION CASSETES FOR KAZAKH ISOLATE OF ORDINARY AND ANDEAN STRAINS OF POTATO VIRUS S

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ABSTRACT

Potato virus S (PVS) belongs to the genus *Carlavirus* of family *Betaflexiviridae* with a positive-sense single-stranded RNA genome of 8.5 Kb. PVS is one of the most prevalent viruses of cultivated potatoes in Kazakhstan. Here we report phylogenetic analysis of complete genome sequences of Kazakh isolates of PVS from potato cultivars Fortuna and Ushkonyr, design of PVS strain-specific diagnostic PCR and construction of expression cassettes for conserved viral proteins from both isolates. The Fortuna and Ushkonyr isolates were found to share 80.1% nucleotide identity with each other and belong respectively to previously-defined ordinary and Andean strains of PVS. Based on analysis of conserved and variable regions of available PVS isolates, we designed primers for reverse transcription (RT)-duplex PCR to detect both strains in single and mixed infections and established their prevalence in Almaty region of Kazakhstan. Coding sequences of triple gene block proteins (25K, 12K, 7K), coat protein (34K) and cysteine-rich protein (11K) as well as methyltransferase, peptidase, helicase and RNA-dependent RNA polymerase domains of viral replicase were subcloned from both Fortuna and Ushkonyr genomic RNAs into the binary vector pBIN19 under the control of CaMV 35S promoter and nopaline synthetase terminator. These expression cassettes will be used to further investigate the biological properties and strain characteristics of viral proteins by their transient expression in plant cells and tissues or their stable expression in transgenic plants.

Key words: *Solanum tuberosum*, *Potato virus S*, RT-PCR, phylogenetic analysis, open reading frame.

INTRODUCTION

Viral diseases cause significant losses of crop plants worldwide. Potato viruses M, S, X and Y (PVM, PVS, PVX, PVY) and potato leafroll virus (PLRV) are the most dangerous pathogens for the global potato industry [1-6]. Integrated strategy for plant protection against viruses requires development of sensitive and efficient methods for virus detection. Currently, a classical enzyme-linked immunosorbent assay (ELISA) method allows for simultaneous detection of plant viruses in a large number of samples [2, 3, 7]. Whereas highly sensitive, more accurate and more rapid methods such as reverse transcription (RT) and polymerase chain reaction (PCR) [3, 4, 6], multiplex RT-PCR [2, 7, 8], immunocapture RT-PCR [1], quantitative RT-PCR [9, 10] are used for detection of a few copies of viral genomes not only in plant organs, but in soil samples, in embryos, in endosperm and even in seed coats [10-12].

Potato virus S (family *Betaflexiviridae*, genus *Carlavirus*) is widespread in the south and north of Kazakhstan [8, 13-15]. Most often PVS induces no visible leaf symptoms, but sometimes causes leaf folding and wrinkling, vein deformation, early leaf fall and/or death, depending on the potato variety and viral isolate. This leads to yield decreases from 10 to 20%, and to more serious yield losses in case of mixed infection with unrelated viruses [6, 16].

There are two different strains of PVS, ordinary (PVS^{O}) and Andean (PVS^{A}), which have distinct biological and molecular properties [4, 8, 15, 17]. For example, PVS^{O} causes local damage in *Chenopodium quinoa*, while PVS^{A} infects this plant systemically. PVS^{O} and PVS^{A} genomes share approximately 80% nucleotide identity [4, 17].

PVS has a positive-sense single-stranded RNA genome of 8.4 to 8.5 Kb, including 5'-untranslated region (UTR) and 3'-UTR, and a poly(A)-tail. It contains six conserved open reading frames (ORFs) [4, 17, 18]. Like in most carlaviruses, ORF1 encodes the 223K viral replicase with methyltransferase, peptidase, helicase and RNA-dependent RNA polymerase (RdRp) domains. ORF2, ORF3 and ORF4 encode triple gene block proteins (TGBp1-3; 25K, 12K and 7K) involved in cell-to-cell movement [19-21]. ORF5 encodes a coat protein (CP, 34K), while ORF6 encodes a cysteine-rich nucleic acid binding protein (NABP, 11K) presumably implicated in suppression of RNA silencing-based antiviral defence. In addition to the genomic RNA (gRNA) serving as mRNA for viral replicase, two subgenomic RNAs (sgRNAs) of 2.5 kb and 1.5 kb in length are produced. The longer sgRNA serves as mRNA for TGBp1, TGBp2 and TGBp3, while the shorter one - for CP and NABP [19].

Whole genomes of PVS strains from different countries have been sequenced and gRNA fragments have been characterized [4, 17, 18, 20, 21, 23]. Both PVS strains, ordinary (PVS^{O}) and Andean (PVS^{A}), were registered in Kazakhstan. PVS^{O} was dominant in the Almaty oblast, while PVS^{A} was widespread in the East Kazakhstan and Kostanay region [8, 14, 15]. Although PVS was identified in Kazakhstan earlier, there is no information about the molecular characteristics of Kazakh isolates. In this study, the complete genome sequences of the first two Kazakh isolates assigned to PVS^{O} and PVS^{A}

strains were characterized. A duplex RT-PCR method was optimized for the simultaneous diagnostics of PVS strains and using this method the prevalence of PVS^{O} and PVS^{A} strains in Almaty region of Kazakhstan was investigated. Expression cassettes for TGBp1, TGBp2, TGBp3, CP and NABP as well as methyltransferase, peptidase, helicase and RdRp domains of viral replicase of both Kazakh isolates were constructed.

MATERIALS AND METHODS

In our previous research [15], tubers of *Solanum tuberosum* cultivars were randomly collected in the Almaty oblast in 2015-2016. The leaves of potato plants grown from these tubers were analyzed by double-antibody sandwich (DAS)-ELISA using commercial polyclonal antibodies and kits from "BIOREBA" according to the company's recommendations. RT-PCR and sequencing were performed for ELISA-positive samples using the primers specific for ORF5 encoding CP (Table 1).

Total RNA was extracted from the leaves of PVS-infected potato cultivars Ushkonyr and Fortuna using TRI reagent (Sigma-Aldrich) according to the manufacturer's recommendations. cDNA was synthesized using 2 μg of total RNA, 1x Maxima RT-buffer (25.0 mM Tris-HCl, pH 7.5, 37.5 mM KCl, 1.5 mM $MgCl_2$, 5.0 mM DTT), 100 pmol oligo$(dT)_{18}$, 0.5 mM each dNTP and 20 U Maxima Reverse transcriptase (Thermo Fisher Scientific kit) at 50°C for 1 hr. In our previous research [15], full genomes of the PVS isolates KZ.Ushkonyr and KZ. Fortune were sequenced using a PCR approach with overlapping amplicons, and the complete nucleotide sequences were deposited in NCBI Genbank under accession numbers MK442089 and MN095414, respectively.

For cloning of viral protein-coding sequences from PVS-Fortune, forward and reverse PCR primers were designed to carry restriction sites adjacent to the start and stop codons of the respective ORFs (Table 1). For cloning of the four domains of viral replicase, translation start and stop codons were also introduced into the sequence of some forward and/or reverse primers, respectively. For cloning of coding sequences from PVS-Ushkonyr, additional primers were designed to account for differences between Ushkonyr and Fortune sequences adjacent to some of the start or stop codons (Table 1). PCR amplification of cDNA fragments was carried out in 20 μl reaction mix using 2 μl cDNA, 1x buffer for Taq-polymerase (Thermo Fisher Scientific), 2.5 mM $MgCl_2$, 0.1 μM each dNTP, 0.1 μM each primer and 0.5 U Taq-polymerase. The program was as follows: 1 cycle 94°C 5 min; 30 cycles 94°C 30 s, 58°C (for ORF2-ORF6) or 54°C (for each ORF1 domain) 30 s, 72°C 1 min (for ORF2-ORF6) or 3 min (for ORF1 domains); 1 cycle 72°C 5 min. Thermocycler GeneAmp PCR System 9700 (Applied Biosystems) was used for amplification.

5 μl of sample was analyzed in 2% agarose gel electrophoresis with 0.5 μg/ml ethidium bromide. The resulting PCR products were cloned into the binary vector pBI221 as described in the Results and Discussion section.

Restriction, elution from agarose gel and ligation (T4 ligase) of PCR products and plasmid DNA isolation were performed according to the company's recommendations (Thermo Fisher Scientific). The transformation of *Escherichia coli* DH5α strain with recombinant DNA was carried out according to the standard procedure [22].

For phylogenetic analysis, the alignment of viral genome sequences was carried out using the Clustal X program. The construction of the genetic distance and phylogenetic tree matrices was performed by the MEGA X program (neighbor-joining method, 2000 replications).

RESULTS AND DISCUSSION

Genome features and phylogenetic analysis of KZ.Fortune and KZ.Ushkonyr isolates of PVS

Two potato varieties, Fortuna and Ushkonyr, with the lowest nucleotide sequence identity of the PCR-amplified CP DNA fragments were used then for complete genome sequencing. The complete genomes were deposited in the NCBI Genbank as KZ.Fortune (MK442089) and KZ.Ushkonyr (MN095414) isolates, respectively. Their gRNAs were found to be 8467 and 8469 nts in size, respectively, including 5'- and 3'-untranslated regions and six ORFs (Table 2A).

Table 1. Nucleotide sequences of PCR primers used for diagnosis of PVS strains and for cloning of gRNA fragments of PVS isolates KZ.Fortune and KZ.Ushkonyr

The primer name contains the name of the encoded ORF, orientation, restriction site *	Nucleotide sequence of primer**	Position of primer on gRNA
Primers used for diagnosis of PVS strains		
PVS-S	5' – TGGCGAACAC CGAGCAAATG - 3'	7549-7568
PVS-AS	5' – ACTGCGCCTG TTGGGAACTC CACAG-3'	7692-7717
CP-Fw7833	5' – TTTCAATGGA ATGCACGCTT - 3'	7833-7852
CP-Rv	5' – TCATTGGTTT GCTGCATTCC - 3'	8065-8084
Primers used for cloning of the replicase domains for Fortune		
Met-Fw+SalI	5'–CGTACTCGAC ATGGCACTTACTTACAGAAGTCCAA–3'	64-88
Met-Rv+BglII	5'–CGTAAGATCTTCACCGGGGCAAAGCTGCCT CAAAG–3'	3038-3060
Pep-Fw+SalI	5' – CGTAGTCGAC ATGTTTTGGC TGGCAATCTC – 3'	2767-2782
Pep-Rv+Acc65I	5' – CGTAGGTACC TCACTCTCTA ACGTTCTCCC – 3'	3560-3576
Hel-Fw+SalI	5' – CGTAGTCGAC ATGATTGAGC ATTGTAAGAG – 3'	3313-3329
Hel-Rv+Acc65I	5' – CGTAGGTACC TCAAATCCTT TCGCCCCCAA – 3'	4490-4506
RdRp-Fw+SalI	5' – CGTAGTCGAC ATGGCAGATG AGGGCAAGAG – 3'	4510-4528

RdRp-Rv+Acc65I	5’ – CGTAGGTACC TCAAATATCC TCATACACAC – 3’	5969-5988
Additional primers used for cloning of the replicase domains for Ushkonyr		
Met-BY-Fw+XhoI	5’ – CGTACTCGAG ATGGCACTTA CTTACAGAAG – 3’	64-88
MetBY-Rv+BamHI	5’ – CGTAGGATCC TCACCGGGGC AAAGCTGCCT – 3’	3042-3064
Pep-BY-Rv+Acc65I	5’ – CGTAGGTACC TCAACCTTCT TGCAATCTCCC – 3’	3552-3570
Hel-BY-Fw+SalI	5’ – CGTAGTCGAC ATGATAGAGC ATTGCAAGAG CAA– 3’	3316-3332
Hel-BY-Rv+SacI	5’ – CGTAGAGCTC TCACCCAATT CTTTCACCTT – 3’	4493-4509
Rd-BY-Rv+Acc65I	5’ – CGTAGGTACC TCAAACCTCC TCATACACAC – 3’	5972-5991
Primers used for cloning of ORF2-ORF6 for Fortune		
25K-Fw+SalI	5’–CGTAGTCGAC ATGAGGATAT TTGATAGCTT–3	5975-5995
25K-Rv+SacI	5’–CGTAGAGCTC TTAGGCGGCG GTGTAAGTGG-3’	6686-6706
12K-Fw+SalI	5’–CGTAGTCGAC ATGCCACTTA CACCGCCGCC–3’	6684-6704
12K-Rv+Acc65I	5’–GTCAGGTACC TTAAGCACTG TGCACTCG–3’	6992-7010
7K-Fw+SalI	5’–CGTAGTCGAC ATGCTGTCCA AGGTGCAACC–3’	6974-6994
7K-Rv+Acc65I	5’–GTCAGGTACC TTACCTGTGA ACCTAAAGGT-3’	7154-7174
CP-Fw+SalI	5’-CGTAGTCGAC ATGCCGCCCA AACCGGATCC-3’	7216-7236
CP-Rv+BglII	5’-CGTAAGATCT TCATTGGTTG ATCGCATTAC GGT-3’	8077-8100
11K-Fw+SalI	5’–CGTAGTCGAC ATGAAGGCAG ACCGTTTAGC–3’	8097-8117
11K-Rv+Acc65I	5’–CGTAGGTACC TCACCTCAGT TACTCCAACC–3’	8361-8381
Additional primers used for cloning of ORF2-ORF6 for Ushkonyr		
25K-BY-Fw+SalI	5’–CGTAGTCGAC ATGAGGAGGT TTGACAGCTT–3’	5978-5997
25K-BY-Rv+SacI	5’–CGTAGAGCTC TTAGGCGGTG GTGTAAGTGG–3’	6686-6706
7K-BY-Fw+SalI	5’–CGTAGTCGAC ATGTTGCCCA AGGTGCAATC–3’	6977-6996
CP-BY-Rv+BglII	5’-CGTAAGATCT TCATTGGTTT GCTGCATTCC-3’	8084-8100
11K-BY-Fw+SalI	5’-CGTAGTCGAC ATGAGAGCGG AACGTCTAAA–3’	8100-8119

* the corresponding restriction enzymes are indicated in the primer name
** the sequences for restriction sites are underlined

Table 2A. Genome organization and nucleotide (nt) and amino acid (ac) sequence homology of the PVS isolates KZ.Fortune and KZ.Ushkonyr

Isolate/ ORF name/ position on gRNA (number of nucleotides (nt) or amino acids (ac))	Full genome	5’-UTR	ORF1 (methyltransferase, peptidase, helicase, RNA-depended RNA-polymerase)		ORF2 (TGBp1, protein 25K)		ORF3 (TGBp2, protein 12K)		ORF4 (TGBp3, protein 7K)		ORF5 (CP, capsid protein)		ORF6 (NABP, cistein-reach protein, protein 11K)		3’-UTR
	nt	nt	nt	ac	nt	ac	nt	ac	nt	ac	nt	ac	nt	ac	nt
KZ.Fortune	1-8467 8467	1-44 44	45-5972 5928	1975	5959-6690 732	243	6668-6994 327	108	6958-7158 201	66	7200-8084 885	294	8081-8365 285	94	8366-8467 102
KZ.Ushkonyr	1-8469 8469	1-44 44	45-5972 5928	1975	5959-6690 732	243	6668-6994 327	108	6958-7158 201	66	7200-8084 885	294	8081-8365 285	94	8366-8469 104
Identity (%)	80.1	97.7	78.2	85.7	86.9	95.5	85.9	97.2	82.6	87.9	80.0	94.6	80.7	84.0	87.5

Table 2B. Nucleotide (nt) and amino acid (ac) sequence homology of ORF1-encoded relicase domains of the PVS isolates KZ.Fortune and KZ.Ushkonyr

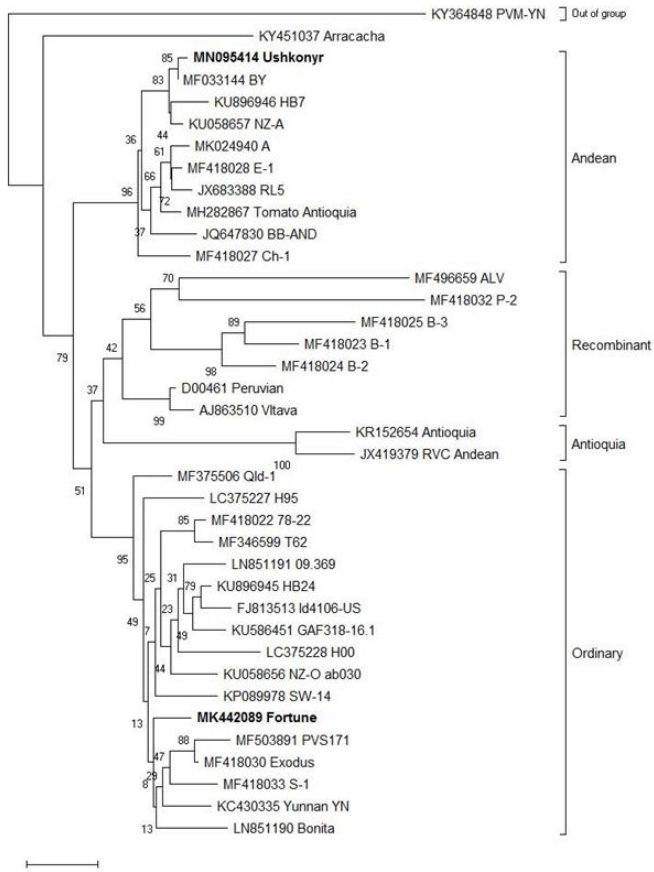
Isolate/ ORF name, domen name/ position on gRNA (number of nucleotides (nt) or amino acids (ac))	Full ORF1 sequence		ORF1-1 (methyltransferaes)		ORF1-2 (peptidase)		ORF1-3 (helicase)		ORF1-4 (RdRp)	
	nt	ac	nt	ac	nt	ac	nt	ac	nt	ac
KZ.Fortune	45-5972 5928	1976	45-3044 3000	1000	2751-3560 810	270	3297-4493 1197	399	4494-5972 1479	493
KZ.Ushkonyr	45-5972 5928	1976	45-3044 3000	1000	2751-3551 801	267	3297-4493 1197	399	4494-5972 1479	493

Genome organization was found to be identical for both KZ.Fortune (PVS^{O}) and KZ.Ushkonyr (PVS^{A}) isolates. In both isolates, ORF1 encodes the replicase with methyltransferase (pfam01660), carlavirus endopeptidase (pfam05379), RNA helicase (pfam01443) and RdRp (pfam00978) domains. ORF2 encodes a triple gene block protein 1 (TGBp1) with an RNA helicase domain (pfam01449). OFR3 encodes TGBp2 with a plant virus movement motif (pfam01307). ORF4 encodes TGBp3 with a 7kD viral motif (pfam02495). ORF5 encodes the viral coat protein with two domains (pfam08358 and pfam00286) typical for carlaviruses. ORF6 encodes a cysteine-rich protein NABP with a carlavirus putative nucleic-acid-binding motif (pfam01623).

Sequence alignments revealed that KZ.Fortune and KZ.Ushkonyr isolates share 80.1% nucleotide identity at the complete genome level (Supplementary 1). The nucleotide sequence identities of six ORFs ranged from 78.2% (replicase) to 86.9% (TGBp1), while the amino acid sequence identities of the encoded proteins ranged from 84.0% (NABP) to 97.2% (TGBp2) (Table 2A-B). These results are consistent with those reported earlier for other PVS isolates representing PVS^{O} and PVS^{A} strains [21, 22].

Phylogenetic analysis of KZ.Fortune and KZ.Ushkonyr isolates and 35 full-length PVS genomes available in the Genbank revealed three main groups (clades) containing 9 to 17 isolates and one distinct single isolate Arracacha (KY451037) (Figure 1).

KZ.Fortune and KZ.Ushkonyr isolates fell in separate clades corresponding to PVS^{O} and PVS^{A} strains, respectively. The KZ.Fortune clade contained among others the isolates Yunnan YN (KC430335) from China, Qld1 (MF375506) from Australia and H95 (LS375227) from Japan. The KZ.Ushkonyr clade contained among others the isolate BY and HB7 (MF033144 and KU896946) from China and NZ-A ab030 Lincoln (KU058657) from New Zealand. Consistent with previous reports the Peruvian isolate (D00461) and the Czech isolate Vltava (AJ863510) together fell in the clade containing PVS^{O}-PVS^{A} recombinants, two of which being from Peru (MF496659 and MF418032) and three from Bolivia (MF418023, MF418024 and MF418025) [17, 23, 24]. Interestingly, the Colombian isolates Antioquia (KR152654) and



The maximum likelihood phylogenetic tree was made using MEGA X (neighbor-joining method, 2000 replications). Potato virus M (PVM-YN) was used as an outgroup. Kazakh isolates are shown in bold.

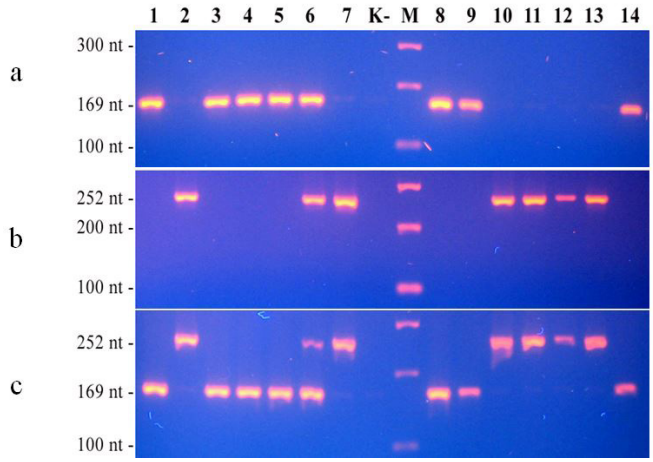
Figure 1. Phylogenetic tree of 37 full-length genomes of PVS isolates

RVC (JX419379) from *Solanum phurea* formed a sister group to the recombinants' group, although in a previous study these isolates and several other isolates from Columbia were found to be more closely related to PVS^{A} strain isolates, than to the recombinants, and grouped into a strain PVS^{P} named after the plant species epithet [17].

Primer design and RT-PCR detection of PVS strains in potato samples from Kazakhstan

The most variable regions in 11 and 9 isolates representing respectively PVS^{O} (KC430335, HF571059, KF011280,

HQ875132, JX183954, AB364946, DQ315387, HQ875142, GU233454, KC818635, AY512653) and PVSA (AJ863510, D0061, JQ647830, JX183955, JX13956, KF011272, KF225470, MF033144, JX683388) strains were aligned and searched for conserved and variable regions within all protein-coding sequences. As a result, two primer pairs were designed within the CP-encoding ORF5 for strain-specific PCR diagnostics: the primers PVS-S and PVS-AS are specific for PVS^{O}, while the primers CP-Fw7833 and CP-Rv are specific for PVS^{A} (Table 1). Using these two pairs of primers for PCR and the KZ.Fortune (PVS^{O}) and KZ.Ushkonyr (PVS^{A}) samples as positive controls, discrete bands of the expected sizes of 169 and 252 bp, respectively, were amplified (Figure 2; lanes 1, 2).



PCR products amplified using the PVS^{O}-specific primers PVS-S and PVS-AS (a), the PVS^{A}-specific primers CP-Fw7833 and CP-Rv (b) or both pairs of the primers (c) were separated by gel electrophoresis in 2% agarose gel and stained with ethydiume bromide. Positive control samples of Fortuna (PVS^{O}) and Ushkonyr (PVS^{A}) isolates were loaded in lanes 1 and 2, respectively. "K-" – negative control (ELISA-negative for PVS). "M" – molecular marker ("Thermo Fisher Scientific", O'RangeRuler 100 bp): positions of 100 bp, 200 bp or 300 bp marker DNA bands as well as expected PCR products of 169 bp (PVS^{O}) and 252 bp (PVS^{A}) are indicated on the left. Single PVS^{O} was detected in 7 samples (lanes 1, 3-5, 8, 9, 14), single PVS^{A} – in 7 samples (lanes 2, 7, 10-13), and mix infection – in one samples (lanes 6).

Figure 2. RT-PCR analysis of Solanum tuberosum leaf samples ELISA-positive for PVS

A total of 70 samples of potato plants collected in the Almaty region of Kazakhstan in 2021 and scored ELISA-positive for PVS were then analyzed by RT-PCR. The PVS^{A} strain was detected in 52 samples, while the PVS^{O} strain in 5 samples. The mixed infection with PVS^{O} and PVS^{A} was identified in 13 samples. The representative gels of this large-scale PCR analysis are shown in Figure 2. Interestingly, our previous surveys in the Almaty region in 2015 and 2016 revealed PVS^{A} in 20 samples and PVS^{O} in 44 samples, while the mixed infection with PVS^{O} and PVS^{A} was identified only in 3 samples [15]. Taken together, both PVS strains are still circulating in the Almaty region, but the cases of mix infection have increased and PVS^{A} strain has become dominant.

Multiplex RT-PCR techniques have previously been reported for potato virus identification [16, 21, 25, 26]. A large variety of isolates and an increasing number of strains of individual viruses, as well as the presence of mixed infections with two or more viruses and/or several strains of the same virus in one sample makes it difficult to perform a qualitative diagnosis using multiplex RT-PCR. Therefore, guided by the results in ELISA and the published recommendations on the design of specific primers, we successfully achieved detection of two PVS strains in single and mixed infections using the optimized duplex RT-PCR method.

Construction of viral protein-expression cassettes for PVS^{O} and PVS^{A} isolates

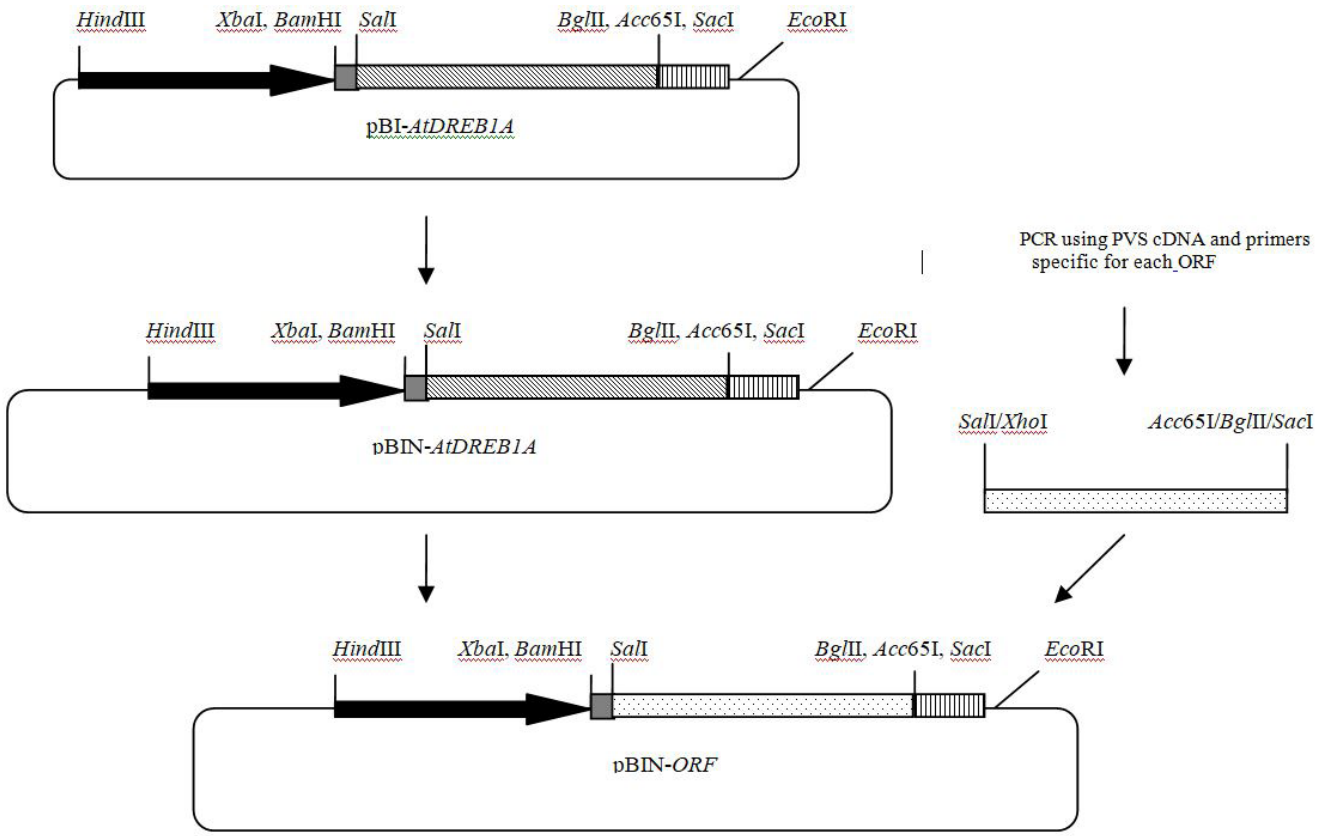
A derivative of the plasmid vector pBI221 (NCBI GenBank Acc. No. AF502128) containing the coding sequence of *AtDREB1A* under the control of cauliflower mosaic virus (CaMV) 35S promoter, the 5'-untranslated sequence (UTR) of tobacco mosaic virus (TMV) and the terminator of nopaline synthetase gene (*nos*) (Figure 3; pBI-*AtDREB1A*) constructed in our previous study was used for introduction of the coding sequences of PVS ORFs in place of the *AtDREB1A* sequence. To this end the plasmid DNA of pBI-*AtDREB1A* was digested with *Hind*III and *Eco*RI and the short fragment containing the expression cassette "*HindIII - 35S CaMV-XbaI, BamHI –TMV 5'UTR – SalI - AtDREB1A - SacI - nos – EcoRI*" was subcloned into the plant binary plasmid vector pBIN19 (NCBI Acc. No. U09365). Coding sequences of viral proteins encoded by ORF2 to ORF6 were amplified by RT-PCR from KZ.Fortune and KZ.Ushkonyr isolates using the strain-specific primers (Table 2) and cloned between sites *Sal*I (all ORFs) and *Acc*65I (ORF3, ORF4, ORF6), *Sac*I (ORF2) or *Bgl*II (ORF5) (Figure 3).

The ORF1 of all carlaviruses encodes the 223K polyprotein containing methyltransferase, peptidase, helicase and RdRp domains. Five sites for autoproteolysis of the ORF1-encoded polyprotein have been identified in most carlaviruses. According to Lawrence et al. [27], the first two of them do not affect proteolytic processing *in vitro*, although mutations at these sites lead to delay in symptom development in infected plants. The last three are thought to be required for viral replication. All the functional domains were found in the ORF1-encoded polyproteins of the KZ.Fortune and KZ.Ushkonyr isolates, and the conserved autoproteolitic sites were identified at positions 902C, 993H, 1003-1004CV, 1084H and 1483G in the polyprotein of KZ.Fortune. Based on positions of three putative functional autoproteolitic sites, the ORF1 sequence was divided into four overlapping coding regions without changing the reading frame and primer pairs for their RT-PCR amplification were designed for both KZ.Fortune and KZ.Ushkonyr isolates (Table 2B and Materials and Methods).

PCR amplicons with these coding regions were named as follows: ORF1-1 (methyltransferase), ORF1-2 (peptidase), ORF1-3 (helicase) or ORF1-4 (RdRp) (Table 2B).

The sense primer for each coding sequence contained the AUG codon (natural in the case of ORF1-1, or introduced in the case of ORF1-2, ORF1-3 and ORF1-4) and the site for restrictase *Sal*I on 5'-end, while the antisense primer contained the stop codon (introduced in the case of OFR1-1, ORF1-2 and ORF1-3, or natural in the case of ORF1-4), and the site for the restrictase *Acc*65I (for peptidase and RdRp domains) or *Bgl*II (for helicase domain), respectively (Table 1).

The cloning scheme of PCR-amplified DNA fragments carrying the peptidase, helicase, and RdRp domains was the



The *35S CaMV* promoter is shown as a black arrow, *5'-TMV* as a gray rectangle, the coding sequence *AtDREB1A* as a rectangle with oblique lines, the *nos*-terminator as a rectangle with vertical lines, the ORF coding sequence as a rectangle with dots, and the plasmid pBI221 or pBIN19 bodies as ovals.

Figure 3. The scheme used for cloning of PVS ORFs 2 to 6

same as the one used from the cloning of each ORF2-ORF6 (Figure 3), while it had to be modified for the methyltransferase domain, because the coding sequences carrying the KZ.Ushkonyr and KZ.Fortune methyltransferase domains were the longest (3003 bp) with multiple internal restriction sites that differ in their locations. The methyltrasferase PCR product ORF1-1-1 amplified using the Fortune cDNA and a pair of primers Met-Fw+SalI and Met-Rv+BglII (Table 1) was trimmed with *Sal*I and *Bgl*II restrictases and used for ligation with a short DNA fragment obtained after digestion of pBI-*AtDREB1A* with *Hind*III and *Sal*I (Figure 3). This short fragment contained the sequences of *CaMV 35S* promoter and *5'-TMV*. After ligation the elongated fragment *"35S CaMV-5'TMV-ORF1-1-1"* containing all three sequences between the *Hind*III and *Bgl*II was obtained. Then this elongated fragment was cloned into the plant vector pBIN19 carrying the *nos* terminator. As a result, the Fortune methyltrasferase expression cassette *"35S-5'TMV-ORF1-1-1-nos"* was constructed.

The metyltrasferase PCR product ORF1-1-2 amplified using the Ushkonyr cDNA and a primer pair Met-BY-Fw+XhoI and MetBYRv+BamHI (Table 1) was digested with *Xho*I and *Bam*HI and ligated with the longer fragment obtained after digestion of pBI-*AtDREB1A* with *Sal*I and *Bgl*II (Figure 3). As a result, a DNA construct contained the ORF1-1-2 sequence under the control of the *CaMV 35S* promoter, *5'-TMV* and the *nos* terminator in the pBI221 backbone. In this construct, the cloning sites at the 5'- (*Sal*I/*Xho*I) or 3'-ends (*Bam*HI/*Bgl*II) were not restored. In next step, this construct was digested with *Xba*I and *Sma*I and a short fragment containing the *5'-TMV* and the methyltransferase coding sequence ORF1-1-2 was ligated with the long fragment obtained after digestion of pBIN-*AtDREB1A* (Figure 3) with *Xba*I and *Ecl*136II (*Sac*I and *Ecl*136II are isoschizomers). As a result, the Ushkonyr methyltrasferase expression cassette *"35S-5'TMV-ORF1-1-2-nos"* was introduced in the binary vector pBIN19.

CONCLUSION

In this study, we performed molecular characterization of the complete genomes of the first two Kazakh isolates KZ. Fortune and KZ.Ushkonyr belonged to the ordinary and Andean strains of PVS, respectively. Phylogenetic analysis revealed their relationship to other isolates of these PVS strains and their recombinants from several countries and continents around the world. Furthermore, we designed the duplex RT-PCR method to distinguish between the two PVS strains and, using this method, we found that, over the last five years since our previous surveys, both PVS strains are still circulating in the Almaty region of Kazakhstan, but the cases of their mixed infections have increased and PVS^{A} has become dominant. The validated primer pairs for PVS^{o} and/or PVS^{A} detection and standardized RT-PCR conditions described in this study can be used by both research and commercial diagnostic laboratories that test for economically important potato viruses, as well as by potato seed certification agencies that monitor of potato seed quality. Finally, we constructed the expression cassettes for four functional domains of the viral replicase and all other viral proteins of the KZ.Fortune and KZ.Ushkonyr isolates in order to perform functional analysis of PVS proteins *in vitro* and *in vivo*, to establish differences (if any) between the functions of the same protein from different PVS

strains, and to study interactions between the virus and the host plant.

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LITERATURE

1 Chikh-Ali, Gray, S., Karasev, A. // An improved multiplex IC-RT-PCR assay distinguishes nine strains of Potato virus Y // Plant Disease. - 2013. - V. 97. - P. 1370-1374. http://dx.doi.org/10.1094/PDIS-02-13-0161-SR.

2 Bai, Y., Han, S., Gao, Y., Zhang, W., Fan, G., Qiu, C., Nie, X., Wen, J. // Genetic diversity of *Potato virus Y* in potato production areas in Northeast China // Plant Disease. - 2019.- V. 103. - P. 289-297, https://doi.org/10.1094/PDIS-04-18-0687-RE.

3 Massumi, H., Poormohammadi, S., Pishyar, S., Maddahian, M., Heydarnejad, J., Hosseini-Pour, A., Bysterveldt, K., Varsani, A. // Molecular characterization and field survey of Iranian potato virus X isolates // Virus Disease. - 2014. - V. 25. - P. 338-344. https://doi.org/10.1007/s13337-014-0222-z.

4 Lin, Y-H, Abad, JA, Maroon-Lango, CJ, Perry, KL, Pappu, HR // Molecular characterization of domestic and exotic potato virus S isolates and a global analysis of genomic sequences // Arch Virol. - 2014. - V. 159. - P. 2115-22, https://doi.org/10.1007/s00705-014-2022-6.

5 Xu, H., D'Aubin, J., Nie, J. // Genomic variability in *Potato virus M* and the development of RT-PCR and RFLP procedures for the detection of this virus in seed potatoes // Virology Journal. – 2010. - V. 7. - P. 25, http://doi.org/virologyj.com/content/7/1/25.

6 Rashid, M., Li, J.-H., Liu, Q., Wang, Y., Han, C.-G. // Molecular detection and identification of eight potato viruses in Gansu province of China // Current Plant Biology. - 2021. - V. 25. - P. 100184, https://doi.org/10.1016/j.cpb.2020.100184.

7 Karasev, A., Hu, X., Brown, C., Kerlan, C., Nikolaeva, O., Crosslin, J., Grey, S. // Genetic diversity of the Ordinary strain of *Potato virus Y* (PVY) and Origin recombinant PVY strains // Phytopathology. - 2011. - V. 101. - P. 778-785, https://doi.org/10.1094/PHYTO-10-10-0284.

8 Khassanov, V.T., Vologin, S.G. // Occurrence of the Ordinary and the Andean Strains of Potato Virus S Infecting Potatoes in the Eastern Region of Kazakhstan // Plant Disease. - 2018. - V. 102. - P. 2052, https://doi.org/10.1094/PDIS-12-17-2000-PDN.

9 MacKenzi, T., Nie, X., Singh, M. // RT-PCR and real-time RT-PCR methods for detection of Potato virus Y in potato leaves and tubers // Methods of Mol. Biol. - 2015. - V. 1236. - P. 13-26. https://doi.org/10.1007/978-1-4936-1743-3_2.

10. Schumpp, O., Srechon, A., Brodard, J., Dupuis, B., Farinelli, L., Frei, P., Otten, P., Pellet, D. // Large-scale RT-qPCR diagnostics for seed potato sertification // Potato Research. – 2021. – V. 64. – P. 553-569, https://doi.org/10.1007/s11540-021-09491-3.

11 Rubio, L., Galipienso, L., Ferriol, I. // Detection of plant viruses and disease management: relevance of genetic diversity and evolution // Frontiers in plant Science. - 2020. - V. 11. - P. 1092. https://doi.org/10.3389/fpls.2020.01092.

12 McLeish, M., Fraile, A., Garcia-Arenal, F. // Population genomics of plant viruses: the ecology and evolution of virus emergence // Phytopathology. - 2021. - V. 111. - P. 32-39. https://doi.org/10.1094/PHYTO-08-20-0355-FI.

13 Loebenstein, G. and Manadilova, A. // Virus and virus-like diseases of major crops in developing countries. - Springer, Dordrecht, Netherlands. - 2003, https://doi.org/10.1007/978-94-007-0791-7_8.

14 Alexandrova, A.M., Karpova, O.V., Nargilova, R.M., Kryldakov, R.V., Nizkorodova, A.S., Zhigaylov, A.V., Yekaterinskaya, E.M., Kushnarenko, S.V., Akbergenov, R.Z., Iskakov, B.K. // Distribution of potato (*Solanum tuberosum*) viruses in Kazakhstan // Int J Biol Chem. - 2018. - V. 11. - P. 33-40, https://doi.org/10.26577/ijbch-2018-1-311.

15 Karpova, O., Alexandrova, A., Yeriskina, E., Kryldakov, R., Gritsenko, D., Galiakparov, N., Iskakov, B. // Andean and Ordinary strains of Potato virus S infecting potatoes in southern Kazakhstan // Plant Disease. - 2020. - V. 104. - P. 599. https://doi.org/10.1094/PDIS-09-19-1822-PDN.

16 Hameed, A., Iqbal, Z., Asad, S., Mansoor, S. // Detection of multiple potato viruses in field suggests synergistic interactions among potato viruses in Pakistan // Plant Pathol. J. - 2014. - V. 30. - P. 407-415, https://doi.org/10.5423/PPJ.OA.05.2014.0039.

17 Vallejo, D.C., Gutierrez, P., Marin, M. // Genome characterization of Potato virus S (PVS) variant from tuber sprouts of *Solanum phureja* // Juz et Buk Agronomia Colombiana. - 2016. - V. 34(1). – pp. 51-60, https://doi.org/10.15446/agron.colomb.v34n1.53161.

18 Matoušek, J., Schubert, J., Ptaček, J., Kozlova, P., Dedič, P. // Complete nucleotide sequence and molecular probing of potato virus S genome // Acta Virologica. - 2005. - V. 49. - P. 195-205. PMID:16178517.

19 Foster, G., Mills, P. // The 3'-nucleotide sequence of an ordinary strain of potato virus S // Virus Genes. -1992. -V. 6. -P. 212-220, PMID:1413539 https://doi.org/10.1007/BF01702560.

20 Duarte, P., Galvino-Costa, S., Ribeiro, S., Figueira, A. // Complete genome sequence of the first Andean strain of potato virus S from Brazil and evidence of recombination between PVS strains // Arch Virol. - 2012. - V. 157. - P. 1357–1364, https://doi.org/10.1007/s00705-012-1289-8.

21 Wang, J., Meng, F., Chen, R., Liu, J., Nie, X., Nie, B. // RT-PCR differentiation, molecular and pathological characterization of Andean and ordinary strains of *Potato virus S* in Potatoes in China // Plant Disease. - 2016. - V. 100. - P. 1580-1585. http://dx.doi.org/10.1094/PDIS-11-15-1257-RE.

22 Sambrook, J., Russel, D.W. // Molecular cloning: A laboratory manual: 3 volumes. - Third edition. - New York: Cold Spring Harbor Laboratory Press. - 2001. (CSHLP).

23 Geraldino, P., Galvino-Costa, S., Ribeiro, S., Figueira,

A. // Complete genome sequence of the first Andean strain of Potato virus S from Brazil and evidence of recombination between PVS strains // Arch. Virol. - 2012. - V. 157. - P. 1357-1364. https://doi.org/10.1007/s00705-012-1289-8.

24 Santillan, F., Fribourg, C., Adams, I., Gibbs, A., Boonham, N., Kehou, M., Maina, S., Jones, R. // The biology and Phylogenetics of Potato virus S isolates from the Andean Region of South Africa // Plant Disease. - 2018. - V 102. - P. 869-885. https://doi.org/10.1094/PDIS-09-17-1414-RE.

25 Crosslin, J., Hamlin, L. // Standardized RT-PCR Conditions for Detection and Identification of eleven viruses of potato and Potato spindle tuber viroid // Am. J. Pot Res. - 2011. - V. 88. - P. 333–338. . https://doi.org/10.1007/s12230-011-9198-z.

26 Rashid, M., Wang, Y., Han, C. // Molecular Detection of Potato Viruses in Bangladesh and Their Phylogenetic Analysis // Plants. - 2020. - V. 9. - P. 1413. http://dx.doi.org/10.3390/plants9111413.

27 Lawrence, D., Rozanov, M., Hillman, B. // Autocatalytic processing of the 223-kDa protein of Blueberry Scorch Carlavirus by papain-like proteinase // Virology. - 1995. - V. 207. - P. 127-135. PMID:7871721 https://doi.org/10.1006/viro.1995.1058.

REFERENCES

1 Chikh-Ali, Gray, S., Karasev, A. // An improved multiplex IC-RT-PCR assay distinguishes nine strains of Potato virus Y // Plant Disease. - 2013. - V. 97. - P. 1370-1374. http://dx.doi.org/10.1094/PDIS-02-13-0161-SR.

2 Bai, Y., Han, S., Gao, Y., Zhang, W., Fan, G., Qiu, C., Nie, X., Wen, J. // Genetic diversity of *Potato virus Y* in potato production areas in Northeast China // Plant Disease. - 2019.- V. 103. - P. 289-297, https://doi.org/10.1094/PDIS-04-18-0687-RE.

3 Massumi, H., Poormohammadi, S., Pishyar, S., Maddahian, M., Heydarnejad, J., Hosseini-Pour, A., Bysterveldt, K., Varsani, A. // Molecular characterization and field survey of Iranian potato virus X isolates // Virus Disease. - 2014. - V. 25. - P. 338-344. https://doi.org/10.1007/s13337-014-0222-z.

4 Lin, Y-H, Abad, JA, Maroon-Lango, CJ, Perry, KL, Pappu, HR. // Molecular characterization of domestic and exotic potato virus S isolates and a global analysis of genomic sequences // Arch Virol. - 2014. - V. 159. - P. 2115-22, https://doi.org/10.1007/s00705-014-2022-6.

5 Xu, H., D'Aubin, J., Nie, J. // Genomic variability in *Potato virus M* and the development of RT-PCR and RFLP procedures for the detection of this virus in seed potatoes // Virology Journal. – 2010. - V. 7. - P. 25 , http://doi.org/virologyj.com/content/7/1/25

6 Rashid, M., Li, J.-H., Liu, Q., Wang, Y., Han, C.-G. // Molecular detection and identification of eight potato viruses in Gansu province of China // Current Plant Biology. - 2021. - V. 25. - P. 100184, https://doi.org/10.1016/j.cpb.2020.100184.

7 Karasev, A., Hu, X., Brown, C., Kerlan, C., Nikolaeva, O., Crosslin, J., Grey, S. // Genetic diversity of the Ordinary strain of *Potato virus Y* (PVY) and Origin recombinant PVY strains // Phytopathology. - 2011. - V. 101. - P. 778-785, https://doi.org/10.1094/PHYTO-10-10-0284.

8 Khassanov, V.T., Vologin, S.G. // Occurrence of the Ordinary and the Andean Strains of Potato Virus S Infecting Potatoes in the Eastern Region of Kazakhstan // Plant Disease. - 2018. - V. 102. - P. 2052, https://doi.org/10.1094/PDIS-12-17-2000-PDN.

9 MacKenzi, T., Nie, X., Singh, M. // RT-PCR and real-time RT-PCR methods for detection of Potato virus Y in potato leaves and tubers // Methods of Mol. Biol. - 2015. - V. 1236. - P. 13-26, https://doi.org/10.1007/978-1-4936-1743-3_2.

10. Schumpp, O., Srechon, A., Brodard, J., Dupuis, B., Farinelli, L., Frei, P., Otten, P., Pellet, D. // Large-scale RT-qPCR diagnostics for seed potato sertification // Potato Research. – 2021. – V. 64. – P. 553-569, https://doi.org/10.1007/s11540-021-09491-3.

11 Rubio, L., Galipienso, L., Ferriol, I. // Detection of plant viruses and disease management: relevance of genetic diversity and evolution // Frontiers in plant Science. - 2020. - V. 11. - P. 1092. https://doi.org/10.3389/fpls.2020.01092.

12 McLeish, M., Fraile, A., Garcia-Arenal, F. // Population genomics of plant viruses: the ecology and evolution of virus emergence // Phytopathology. - 2021. - V. 111. - P. 32-39, https://doi.org/10.1094/PHYTO-08-20-0355-FI.

13 Loebenstein, G. and Manadilova, A. // Virus and virus-like diseases of major crops in developing countries. - Springer, Dordrecht, Netherlands. - 2003, https://doi.org/10.1007/978-94-007-0791-7_8.

14 Alexandrova, A.M., Karpova, O.V., Nargilova, R.M., Kryldakov, R.V., Nizkorodova, A.S., Zhigaylov, A.V., Yekaterinskaya, E.M., Kushnarenko, S.V., Akbergenov, R.Z., Iskakov, B.K. // Distribution of potato (*Solanum tuberosum*) viruses in Kazakhstan // Int J Biol Chem. - 2018. - V. 11. - P. 33-40, https://doi.org/10.26577/ijbch-2018-1-311.

15 Karpova, O., Alexandrova, A., Yeriskina, E., Kryldakov, R., Gritsenko, D., Galiakparov, N., Iskakov, B. // Andean and Ordinary strains of Potato virus S infecting potatoes in southern Kazakhstan // Plant Disease. - 2020. - V. 104. - P. 599, https://doi.org/10.1094/PDIS-09-19-1822-PDN.

16 Hameed, A., Iqbal, Z., Asad, S., Mansoor, S. // Detection of multiple potato viruses in field suggests synergistic interactions among potato viruses in Pakistan // Plant Pathol. J. - 2014. - V. 30. - P. 407-415, https://doi.org/10.5423/PPJ.OA.05.2014.0039.

17 Vallejo, D.C., Gutierrez, P., Marin, M. // Genome characterization of Potato virus S (PVS) variant from tuber sprouts of *Solanum phureja* // Juz et Buk Agronomia Colombiana. - 2016. - V. 34(1). - P. 51-60, https://doi.org/10.15446/agron.colomb.v34n1.53161.

18 Matoušek, J., Schubert, J., Ptaček, J., Kozlova, P., Dedič, P. // Complete nucleotide sequence and molecular probing of potato virus S genome // Acta Virologica. - 2005. - V. 49. - P. 195-205. PMID:16178517.

19 Foster, G., Mills, P. // The 3'-nucleotide sequence of an ordinary strain of potato virus S // Virus Genes. -1992. -V. 6. -P. 212-220. PMID:1413539 https://doi.org/10.1007/BF01702560

20 Duarte, P., Galvino-Costa, S., Ribeiro, S., Figueira, A. // Complete genome sequence of the first Andean strain of potato virus S from Brazil and evidence of recombination be-

tween PVS strains // Arch Virol. - 2012. - V. 157. - P. 1357–1364, https://doi.org/10.1007/s00705-012-1289-8.

21 Wang, J., Meng, F., Chen, R., Liu, J., Nie, X., Nie, B. // RT-PCR differentiation, molecular and pathological characterization of Andean and ordinary strains of *Potato virus S* in Potatoes in China // Plant Disease. - 2016. - V. 100. - P. 1580-1585, http://dx.doi.org/10.1094/PDIS-11-15-1257-RE.

22 Sambrook, J., Russel, D.W. //Molecular cloning: A laboratory manual: 3 volumes. - Third edition. - New York: Cold Spring Harbor Laboratory Press. - 2001. (CSHLP)

23 Geraldino, P., Galvino-Costa, S., Ribeiro, S., Figueira, A. // Complete genome sequence of the first Andean strain of Potato virus S from Brazil and evidence of recombination between PVS strains // Arch. Virol. - 2012. - V. 157. - P. 1357-1364, https://doi.org/10.1007/s00705-012-1289-8.

24 Santillan, F., Fribourg, C., Adams, I., Gibbs, A., Boonham, N., Kehou, M., Maina, S., Jones, R. // The biology and Phylogenetics of Potato virus S isolates from the Andean Region of South Africa // Plant Disease. - 2018. - V 102. - P. 869-885, https://doi.org/10.1094/PDIS-09-17-1414-RE.

25 Crosslin, J., Hamlin, L. // Standardized RT-PCR Conditions for Detection and Identification of eleven viruses of potato and Potato spindle tuber viroid // Am. J. Pot Res. – 2011. - V. 88. - P. 333–338, https://doi.org/10.1007/s12230-011-9198-z.

26 Rashid, M., Wang, Y., Han, C. // Molecular Detection of Potato Viruses in Bangladesh and Their Phylogenetic Analysis // Plants. - 2020. - V. 9. - P. 1413, http://dx.doi.org/10.3390/plants9111413.

27 Lawrence, D., Rozanov, M., Hillman, B. // Autocatalytic processing of the 223-kDa protein of Blueberry Scorch Carlavirus by papain-like proteinase // Virology. - 1995. - V. 207. - P. 127-135. PMID:7871721, https://doi.org/10.1006/viro.1995.1058.

АНАЛИЗ ГЕНОМНЫХ ПОСЛЕДОВАТЕЛЬНОСТЕЙ, ДИАГНОСТИКА С ПОМОЩЬЮ ОТ-ПЦР И КОНСТРУИРОВАНИЕ ЭКСПРЕССИОННЫХ КАССЕТ ВИРУСНЫХ БЕЛКОВ КАЗАХСТАНСКИХ ИЗОЛЯТОВ S ВИРУСА КАРТОФЕЛЯ, ПРИНАДЛЕЖАЩИХ К ШТАММАМ ORDINARY И ANDEAN

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АБСТРАКТ

S вирус картофеля (PVS) принадлежит к роду *Carlavirus* семейства *Betaflexiviridae*, геном которого представлен одноцепочечной положительно-смысловой РНК размером около 8.5 тысяч оснований. PVS является одним из самых распространенных вирусов картофеля, культивируемого в Казахстане. Здесь мы сообщаем о результатах филогенетического анализа полных геномных последовательностей казахстанских изолятов PVS, выделенных из сортов картофеля Фортуна и Ушконыр, оптимизации штамм-специфичной диагностики PVS методом ПЦР и конструировании экспрессионных кассет для консервативных вирусных белков обоих вариантов. Обнаружено, что изоляты Fortuna и Ushkonyr имеют 80.1% идентичности нуклеотидов друг с другом и принадлежат, соответственно, к известным штаммам PVS, ordinary и Andean. На основании анализа консервативных и вариабельных областей этих изолятов PVS, были подобраны праймеры для дуплексной ПЦР после реакции обратной транскрипции (ОТ), позволяющие выявлять оба штамма при одиночной и смешанной инфекциях и установить их распространение в Алматинской области Казахстана. Для обоих геномных РНК Fortuna и Ushkonyr, кодирующие последовательности белков тройного генного блока (25K, 12K, 7K), белка оболочки (34K) и белка, богатого цистеином (11K), а также фрагменты, кодирующие домены метилтрансферазы, пептидазы, хеликазы и РНК-зависимой РНК-полимеразы были субклонированы в бинарный вектор pBIN19 под контролем 35S промотора CaMV и терминатора гена нопалинсинтетазы. Эти экспрессионные кассеты будут использованы для дальнейшего исследования биологических свойств и характеристик вирусных белков разных штаммов путем их временной экспрессии в растительных клетках и тканях или их стабильной экспрессии в трансгенных растениях.

Ключевые слова: *Solanum tuberosum*, S вирус картофеля, ОТ-ПЦР, филогенетический анализ, открытая рамка считывания.

ГЕНОМДЫ РЕТТІЛІГІН ТАЛДАУ, КТ-ПТР ДИАГНОСТИКАСЫ ЖӘНЕ КАРТОП S ВИРУСЫНЫҢ ORDINARY ЖӘНЕ ANDEAN ШТАММДАРЫНЫҢ ҚАЗАҚСТАНДЫҚ ИЗОЛЯТТАРЫ ҮШІН ВИРУСТЫҚ АҚУЫЗ-ЭКСПРЕССИЯЛЫҚ КАССЕТАЛАРДЫҢ ҚҰРАСТЫРЫП ШЫҒАРУ

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ТҮЙІН

Картоп S вирусы (PVS) *Carlavirus* тұқымдасына, *Betaflexiviridae* отбасына жатады, оның геномы бір тізбекті, 8,5 кб жуық оң мағыналы РНҚ болып табылады. PVS – Қазақстанда өсірілетін картоптың ең көп таралған вирустарының бірі. Мұнда біз Фортуна және Үшқоңыр картоп сорттарынан алынған қазақстандық PVS изоляттарының толық геномдық тізбектерінің филогенетикалық талдауын, PVS штаммына спецификалық диагностикалық ПТР дизайнын және екі изоляттың консервіленген вирустық ақуыздары үшін экспрессиялық кассеталардың құрылысын баяндаймыз. Fortuna және Ushkonyr изоляттары бір-бірімен 80.1% нуклеотидтік сәйкестікті бөлісетіні және сәйкесінше бұрын анықталған PVS ordinary және Andean штамдарына жататыны анықталды. Қол жетімді PVS изоляттарының консервіленген және ауыспалы аймақтарын талдау негізінде біз кері транскрипция (КТ)-дуплексті ПТР үшін праймерлерді әзірледік, бұл

бойдақ және аралас инфекциялардағы екі штаммды анықтауға мүмкіндік берді және олардың Қазақстанның Алматы облысында таралуын анықтады. Fortuna және Үшқоңыр геномдық РНҚ үшін үш гендік блок ақуыздарының (25K, 12K, 7K), қабықтік ақуыздың (34K) және цистеинге бай ақуыздың (11K) кодтау реттілігі, сондай-ақ метилтрансфераза, пептидаза, геликаза және РНҚ-тәуелді РНҚ полимераза домендерін кодтайтын фрагменттер 35S CaMV промоторы мен нопалин синтетаза генінің терминаторының бақылауымен pBIN19 екілік векторына субклондалған. Бұл экспрессиялық кассеталар өсімдік жасушалары мен тіндеріндегі өтпелі экспрессиясы немесе трансгендік өсімдіктердегі тұрақты экспрессиясы арқылы әртүрлі штаммдарға арналған вирустық ақуыздардың биологиялық қасиеттері мен сипаттамаларын әрі қарай зерттеу үшін пайдаланылады.

Негізгі сөздер: *Solanum tuberosum*, картоп S вирусы, КТ-ПТР, филогенетикалық талдау, ашық тізбекті жақтау