ABSTRACT

of dissertation for the competition of Doctor of Philosophy (Ph.D.)
in the specialty "6D070100 – Biotechnology"
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"Identification of Potato Virus S proteins suppressing RNA-interference of plant cells for obtaining of potato plants with elevated resistance against Carlaviruses"

General description of work. The work is devoted to the study of the potential suppressor activity of Potato virus S (PVS) proteins, its distribution in various regions of Kazakhstan, the interaction of PVS with host plant cells, and the development of methods for protecting potatoes from virus infection.

Relevance of the research. The potato is one of the most popular crops cultivated around the world, and one of the most susceptible to infection by various pathogens, since it is propagated vegetative (by tubers). Potato plants are affected by more than 30 different viruses, of which the potato leaf curl virus (PLRV,), X-, M-, S- and Y-potato viruses (Potato virus X, M, S and Y (PVX, PVM PVS and PVY) PVS and PVM belong to the same genus Carlaviruses and are the least studied of the above, but the most common in Kazakhstan at the present time. The economic damage of potato viral diseases is estimated in crop losses up to 75% in severe cases of complex viral infections. Methods of recovery and obtaining virus-resistant potato varieties are still not sufficiently effective. From this point of view, the use of genetic engineering approaches in this area is the most promising direction for creating initial lines for further work on potato breeding. RNA interference is a natural cellular mechanism of regulation of the cell transcriptome, one of the functions of which is the fight against viral infections. To date, the study of the regulation of the cell transcriptome is one of the most relevant areas of research in molecular biology. The functions of this process are not only the regulation of transcription, expression, and post-transcriptional silencing of genes, but also protection against endogenous and foreign RNA molecules, which are represented by viruses. Most plant viruses contain an RNA molecule as a carrier of genetic information, the interaction of which with the host cell is also carried out at the level of its transcription and translation. Viruses, in turn, counteract the cellular process of RNA interference by suppressing it with suppressor proteins. The study of the interaction of viruses between themselves and host cells makes it possible to develop effective ways to combat viral infections and increase the yield of agricultural crops (potatoes) several times.

Object of the study was the Potato S virus (PVS), its genomic (g)RNA, and the proteins it encodes. In addition, different varieties of potatoes cultivated in Kazakhstan were studied.

The subject of the study was the evaluation of the suppressor properties of the proteins of the PVS virus, and the possibility of triggering the mechanism of

RNA interference in transgenic plants to protect potatoes from infection with Carlaviruses.

Research methods. The results of the work were obtained using modern molecular biological methods: isolation of RNA and DNA from plant tissue, reverse transcription (RT) and polymerase chain reaction (PCR), enzyme immunoassay (ELISA), northern blotting, Sanger sequencing and Illumina® , electrophoresis in agarose and polyacrylamide gel, cloning and gene expression, as well as modern methods of bioinformatic analysis using MEGA-X (v. 10.0.2), NCBI BLAST and GenBank, etc.

Purpose of research. To identify specific Potato S virus proteins (PVS) that cause suppression of the RNA interference process in infected host plant cells and, using genetic and cell engineering methods, develop a biotechnology for creating potato plants with genetically fixed resistance to PVS and other phytopathogenic viruses.

Research objectives:

1. Determine the presence of PVS in the Almaty and Kostanay regions of the Republic of Kazakhstan. Sequence the nucleotide sequence encoding the PVS envelope protein to identify PVS isolates common in these regions.
2. Create recombinant DNA encoding the amino acid sequences of five PVS virus proteins (25K, 7K, 12K, coat protein (CP) and 11K) into the pBIN19 vector for transient expression in *Nicotiana benthamiana* line 16c tobacco indicator plants and analyze them for their ability to suppress RNA interference.
3. Create intron-hairpin DNA constructs encoding coat protein and 25K PVS protein sequences for stable genetic transformation of potato plants based on the binary agrobacterial vector pCAMBIA2300. Test obtained transgenic plants for the presence of viruses and select resistant and healthy plant lines.

Scientific novelty of the research. For the first time, work was carried out to identify suppressor activity in PVS proteins, which turned out to be at a low level. As a result of the research, a new method of potato health improvement based on the stimulation of the natural mechanism of RNA interference was developed and convincing evidence was obtained of the effectiveness of this approach to health improvement and the production of potato plants genetically resistant to viruses. By sequencing micro(mi)RNAs and subsequent reconstruction of viral sequences in three transgenic potato lines, it was proved that it is transgene-mediated short interfering (si)RNAs that induce resistance to viral challenge.

The theoretical significance of the work lies in the assessment of the diversity of PVS virus isolates on the territory of the Republic of Kazakhstan and the study of the interaction of the poorly studied PVS virus with the host cell. As a result of the work, 3 whole genome sequences of Kazakhstan PVS isolates (MK442089, ON583978, MN095414) and 2 complete genome sequences of the PVY virus (ON583979, ON583980) were deposited in the NCBI GenBank database.

Based on the use of intron-hairpin recombinant DNA constructs, a new method was developed for virus eradication of infected potato.

The practical significance of the research. The results obtained in the course of the work can be used to develop biotechnology for obtaining new varieties and hybrids of potatoes with genetically fixed resistance to certain viral infections, as well as one of the tools for improving the health of potatoes. The use of plants obtained in this way as planting material will reduce the use of expensive insecticidal preparations to protect potatoes from aphids, the main carrier of viral infections, which will increase the yield and productivity of potatoes.

At present, the introduction of new approaches to increasing the productivity of agricultural crops through the development of resistance to various infections is a promising direction. There are several reasons for this – climate change, intensification of agriculture, increased food security through the cultivation of own released varieties of agricultural crops, the constant development of resistance in insects to the chemical plant protection products used, etc. The method proposed in the work for obtaining virus-resistant plants can be widely used in biotechnology and agriculture, since, as a result of genetic transformation, there is no transgene expression product in the form of a protein, which suggests that there are no negative consequences of using such genetically modified organisms as a human food and feed for farm animals.

Basic provisions of doctoral thesis:

1. The most common viral infections in Kazakhstan are PVS (more than 35%) and PVM (more than 80%) – representatives of the genus *Carlavirus*. PVS most often occurs in complex infection with PVM. On the territory of Kazakhstan, there is a wide variety of PVS isolates belonging to two strains – Ordinary ("Fortune", NCBI GenBank acc. nos. MK442089; "PVS_ALYU-75" ON583978) and Andean ("Ushkonyr", MN095414).
2. The coat protein (CP), as well as the 25K and 11K proteins of the PVS^{A} (Andean) virus, exhibit weak suppressor activity on the verge of detection under conditions of transient expression in tobacco indicator plants *Nicotiana benthamiana* line 16c. In the proteins of the PVS^{O} (Ordinary) strain, synthesized from the second to the sixth open reading frames (ORF) were not possible to register pronounced suppressor properties in this system.
3. The use of intron-hairpin DNA constructs as an RNA interference inducer contributed to the development of multiple resistance to related viruses in transgenic potatoes, and provides a healing effect on infected plants at the level of small RNAs.

Main results of the work and conclusions. The coding sequences of five ORFs of the PVS^{A} virus (CP, 25K and 11K) showed weak suppressor activity on the verge of detection in experiments on the induction of RNA interference in the *Nicotiana benthamiana* line 16c system. In the PVS^{O} strain, the proteins exhibited such a weak suppressor activity that it was not possible to detect it in the *N. benthamiana* 16c system. Four variants of recombinant hairpin DNA constructs

based on the pCAMBIA2300 vector were obtained, encoding the nucleotide sequences of the coat protein (CP) and the “movement” of the 25K PVS protein in forward and reverse orientations, which were used for the stable transformation of 4 virus-free and 12 infected varieties of Kazakh and foreign selection. Based on potato varieties infected with single and complex viral infections, 50 lines of transformants were obtained. Part of the lines showed release from complex viral infection after 6 months of cultivation in the greenhouse. Subsequent 3-year field trials demonstrated the resistance of 20 transgenic lines to infection not only with PVS, but also with PVM. The molecular analysis of RNA of transgenic potato lines showed the presence of transgene-mediated siRNAs in three potato lines (#119, #61, and #103). Subsequent miRNA sequencing proved that the resistance of these lines to infection is due to the expression of the 25K PVS transgene insert.

Based on the results obtained, the following **conclusions** can be drawn:

1. The most common viral infections affecting potatoes in Kazakhstan are two representatives of the genus of Carlaviruses – PVS and PVM.
2. Coat protein (CP), 25K and 11K proteins PVS^{A} (Andean) show weak suppressor activity on the verge of detection under conditions of transient expression in tobacco indicator plants *Nicotiana benthamiana* line 16c. In the proteins of the PVS^{O} (Ordinary) strain, synthesized from the second to the sixth ORF, it was not possible to register pronounced suppressor properties in this system. The proteins of the PVS^{O} strain synthesized from the second to the sixth ORF did not show pronounced suppressor properties in this system.
3. The use of intron-hairpin recombinant DNA for plant transformation leads not only to the production of lines that are genetically resistant to viral infections, but also can heal infected plants by triggering the mechanism of RNA interference.

Connection of dissertation work with scientific research. Scientific research of the dissertation work was carried out within the framework of research projects AP05131133 "Identification of S proteins of the potato virus, which suppress the process of RNA interference of host cells, in order to study the molecular mechanisms of the interaction between the virus and the plant and the recovery of viral material" and OR11465447 "Evaluation of the epidemiological situation of potato viral lesions in various regions of Kazakhstan and the identification of molecular genetic features of local isolates”.

Approbation, approval of the results of the work and personal contribution of the author.

The results of the work were published in 27 publications, including three articles published in international peer-reviewed journals with a high rating and citation index. Based on the results of the work done, three patents of the Republic of Kazakhstan were obtained. The personal contribution of the author was the main one in the published works.

Structure and scope of the dissertation work. The dissertation is presented on 136 pages, consists of definitions, designations and abbreviations, introduction,

literature review, research materials and methods, results and discussion, conclusion, list of sources used.