ABSTRACT

of thesis for the degree of doctor of philosophy (Ph.D.) specialty 6D060700 - Biology

GRITSENKO DILYARA ALEKSANDROVNA

Development of viral vector for expression of heterologous proteins in plants and investigation of their expression efficiency

The thesis is devoted to the development of a viral vector based on the genome of grapevine virus A (GVA) for the production of recombinant proteins in plants. Two vectors were developed, one is developed on the basis of the full genome of the virus, the second is developed by deconstructing the genome – by replacing the open reading frame (ORF) 4 with a heterologous gene. The efficiency of vectors was tested by introducing heterologous genes: green fluorescent protein (GFP) gene; capsid protein gene of the apple chlorotic leafspot virus (ACLSV).

The aim of the study was to develop a viral vector based on the genome of the grapevine virus A for the production of recombinant proteins in plants and to study the efficiency of their expression**.**

To achieve the goal, the following **tasks** were assigned:

- to conduct a phylogenetic analysis of the PA-3 isolate of grapevine virus A
- to modify the viral genome of GVA by introducing a heterologous gene between ORF4 encoding the capsid protein of the virus and ORF5 encoding the suppressor of RNA interference
- to modify the viral genome of GVA by replacing the ORF4 encoding the capsid protein of the virus with a heterologous gene
- to study the expression of a heterologous protein in a viral vector based on the full viral genome (insertion of a heterologous gene between ORF4 and ORF5)
- to develop transgenic plants carrying the capsid protein gene of GVA
- to study the expression of a heterologous protein in a viral vector based on the deconstructed viral genome (replacement of ORF4 with a heterologous gene)

Two vectors were developed, one is developed on the basis of the full genome of the virus, and the second is developed by deconstructing the genome – by replacing the open reading frame 4 with a heterologous gene. The target gene was in the frame with ORF4 and ORF5 of GVA for the first vector, and for the second vector, in the frame with the overlapping region ORF3 and ORF4 and with ORF5. For the first time for GVA, heterologous genes have been introduced under the control of the subgenomic ORF4 promoter. The recombinant protein was co-translationally cleaved from the viral proteins by using 2A peptides. To analyze the efficiency of viral vectors, the gene encoding the capsid protein of the ACLSV and the eGFP gene were inserted. The efficiency of expression of heterologous genes was analyzed at the level of transcription and translation. The results of

hybridization of viral RNA with a specific probe for two viral vectors showed the presence of subgenomic RNA encoding a heterologous gene. Densitometric analysis for the viral vector with the replacement of ORF4 showed that the amount of subgenomic RNA of heterologous genes in agroinfiltrated non-transgenic plants is 7 times lower compared to the amount of subgenomic RNA in agroinfiltrated transgenic plants carrying the capsid protein gene of GVA. A similar pattern was observed when comparing the amount of subgenomic RNA of a heterologous gene in a viral vector for agroinfiltrated non-transgenic plant and the amount of subgenomic RNA of the capsid protein gene of GVA for agroinfiltrated non-transgenic plant with unmodified GVA. The lack of expression of the capsid protein of GVA in the viral vector leads to the prevention of local and, accordingly, systemic transport, rapid degradation of both genomic and subgenomic RNA, despite the presence of a suppressor of RNA interference. In view of this, transgenic plants bearing capsid protein the gene of GVA were created to increase the amount of the target protein. When the genome is deconstructed, as a rule, the expression of viral proteins, whose genes have been removed from the viral genome, is restored in trans-systems. Due to the removal of viral genes, it is possible to introduce extended heterologous genes and the lack of competition for energy resources. Analysis of the expression of recombinant proteins for the two developed viral vectors showed that the target proteins are expressed separately from the viral proteins. The amount of heterologous protein for a vector based on the full genome corresponds to the amount of the GVA capsid protein of the unmodified virus. Analysis of the expression efficiency of the target protein in the viral vector with the replacement of ORF4 showed that the amount of the target protein in agroinfiltrated non-transgenic plants is 7 times lower compared to the amount of the target protein in the agroinfiltrated transgenic plants. The lack of expression of the capsid protein significantly affects the accumulation of both the subgenomic RNA encoding the heterologous gene and the target protein itself. Improving the expression efficiency of the recombinant protein is possible through the restoration of the expression of the GVA capsid protein in trans-systems, in this case by creating transgenic plants. The developed viral vectors based on the GVA genome are stable and safe for the environment, since the GVA is able to infect only grapes and tobacco; the chances of transgene transfer are very low. Expression of heterologous proteins is maintained at the native level of viral expression of the capsid protein.

The main conditions for the defense:

1. The PA-3 isolate of GVA is distributed into group I by phylogenetic analysis, the isolates of this group have medium pathogenicity and do not cause extensive necrosis.

2. The expression level of heterologous proteins in the viral vector based on the full GVA genome corresponds to the expression level of the capsid protein of unmodified GVA.

3. The virus based on the full genome of GVA is able to move through the plant both locally and systemically. An additional 19 amino acids at the C-terminus of GVA capsid protein do not prevent the packaging of the virus in the capsid.

4. The accumulation level of subgenomic RNA encoding a heterologous gene and heterologous proteins in transgenic plants is 7 times higher than the accumulation level of RNA and proteins in non-transgenic plants for the viral vector with the replacement of the open reading frame 4 with a heterologous gene. Developed transgenic plants carry the GVA capsid protein gene.

The theoretical and practical significance of the work.

The obtained results of the research significantly expand the knowledge of the molecular biology of the grapevine virus A. For the first time it has been shown that a heterologous gene can be located under the control of the subgenomic promoter of the GVA, despite the overlapping reading frames of the viral genome. It has been determined that the insertion of heterologous genes into the genome of GVA between ORF4 and ORF5 does not affect the transcription and amplification of the virus. The possibility of packaging the virus in the capsid and the movement of the virus through the plant in the presence of additional 19 - amino acids at the C-terminus of GVA capsid protein is shown. It was determined that the removal of the capsid protein from the GVA genome significantly affects the accumulation of viral RNA and proteins. Production of recombinant proteins in plants using a vector with the replacement of ORF4 can only be carried out when restoring expression of GVA capsid protein in trans - systems.

The developed viral vectors based on the genome of GVA can be used to produce recombinant proteins in plants for the needs of medicine and agriculture. Viral vectors have a leading position in the creation of vaccines and the expression of antibodies, peptide hormones. The obtaining of recombinant protein can be carried out in 4-10 days using modern magnification technology. 2A peptides in the developed viral vectors will allow introducing more than one heterologous gene; it is relevant for the expression of antibodies or virus-like particles (VLP) in the development of vaccines.

The developed viral vectors can be used for scientific purposes, to study the processes of RNA interference, molecular mechanisms of virus movement, host – pathogen interaction, functional genomics of grapes.

Based on the obtained results, the following conclusions were made:

1. It was established that the PA-3 isolate of GVA was distributed into the 1st group according to the results of phylogenetic analysis using the amino acid sequences of RNA - dependent RNA - polymerase and capsid protein.

2. A viral vector was developed based on the full genome of GVA by introducing a heterologous gene between ORF4 and ORF5.

3. A viral vector was developed by deconstructing the genome of GVA (replacement of ORF4 with a heterologous gene).

4. It is established that the expression level of the heterologous protein in the viral vector based on the full GVA genome corresponds to the expression level of the capsid protein in the unmodified virus. Expression of heterologous proteins occurs in cleaved form from viral proteins. GVA with an additional heterologous gene between ORF4 and ORF5 is able to move through the plant despite the presence of an additional 19 amino acids at the C-terminus of the capsid protein.

5. Transgenic *Nicotiana benthamiana* carrying the capsid protein gene of GVA have been developed. Expression of the capsid protein is confirmed by immunoblotting.

6. It was established that the expression level of heterologous proteins in the viral vector with the replacement of ORF4 by agroinfiltration of transgenic plants is 7 times higher than the expression level of heterologous proteins in non-transgenic plants. Efficient production of recombinant proteins in the vector with the replacement of ORF4 is possible only by restoring the expression of GVA capsid protein in trans - systems.

The main results of the research were reported:

- at the International Conference “Plant biology and biotechnology. International conference "(2014, Almaty, Kazakhstan);
- at the Global Genome Bodiversity Network (GGBN) International Conference (2014, London, England);
- at the International Conference "Global Climate Change and Biodiversity" (2015, Almaty, Kazakhstan);
- at the IV International Farabi Readings (2017, Almaty, Kazakhstan);
- at the 4th International Conference “Genetics, Genomics, Bioinformatics, and Plant Biotechnology” (2017, Almaty, Kazakhstan);
- at the Global Summit on Plant Science International Summit (2017, Rome, Italy);
- at the International Conference “SmartBio” (2017, Kaunas, Lithuania);
- at the International Conference “International virology conference” (2017, Toronto, Canada);
- at the 5th International Farabi Readings (2018, Almaty, Kazakhstan).

According to the materials of the research published 19 publications, including 3 articles and 1 thesis in journals included in the Scopus database, 5 articles in republican scientific journals from the list of the Committee, 10 theses in materials of international conferences and summits, 2 patents and 1 patent application, 2 copyright certificates and 2 acts of implementation in the production of scientific and technical development.