Identification of some viruses of the medicinal plant *Physalis alkekengi L*

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Abstract. Determining the medical properties of Physalis alkekengi L. extracts, extracting active compounds with antibacterial activity and immunostimulatory properties, that is, physalins and flavonoids, requires the development of biotechnology for the creation of virus-free, healthy medicinal plants by microclonal propagation of plants using modern biotechnological approaches. In this article, TMV-Ph strain of tobacco mosaic virus was isolated from medicinal plant Physalis alkekengi L. and its biological, morphological and physical characteristics were studied. TMV-Ph strain was identified to be belong to the Virgaviridae family of the tobamovirus group. As a result of research on this topic, virus disease symptoms were identified in the Physalis alkekengi plant, and methods for isolating and biologically purifying the virus were developed. To achieve this, viral sap prepared from the leaves of infected plants was inoculated into the Nicotiana sylvestris plant to isolate the virus. For biological purification, infectious sap was prepared and inoculated into the Nicotiana glutinosa plant. The resulting necroses were excised, and the virus was passed through the plant two more times in a mononecrotic state, thereby biologically purifying it. After that, the virus was transferred to a host plant to propagate.

1 Introduction

Globally, scientific research is centered around the preservation of many therapeutic plants from phytopathogens, particularly tobamoviruses, which lead to severe harm to the members of the family of ituzums. To determine the effectiveness of microclonal propagation methods for plants, it is essential to select optimal nutrient media for the cultivation and multiplication of plant explants, obtain somatic hybrids through plant protoplast fusion, isolate plant cells for genetic transformation, and refine techniques for producing haploid and dihaploid plants. In the case of Physalis alkekengi L., it is also necessary to explore the medicinal properties of its extracts, focusing on the isolation of active compounds such as physalins and flavonoids, which exhibit antibacterial and immunostimulatory activities. Developing biotechnological approaches for virus-free, healthy medicinal plants through microclonal

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propagation is crucial in modern biotechnology. This will enable the production of high-quality plants with enhanced therapeutic value [1, 2].

In many research centers around the world, a number of scientists have conducted research into the preventive and therapeutic properties of Physalis species, which belong to the nightshade family (Physalis alkegengi L.). Work on Physalis species is being intensively carried out in the USA, France, China, Japan, India, Pakistan, and Iran by scientists. Laczkó-Zöld E [3]. The anti-inflammatory properties of alcoholic extracts of *Physalis alkekengi* L. were studied by scientists Kang H. And et al [4], Ji L., Yuan Y [5], Hong J.M., Kwon O.K [6], Shu Z., Xing N [7], Moniruzzaman M., Bose S [8], Montaserti A., Pourheydar M [9], Ji L [10]., the effective action of the fruit extract of P. alkekengi L. in the treatment of anemia by scientists Shahnaz Shekar-Foroosh et al [11].

The analysis of the literature shows that there is insufficient information on the in vitro microclonal propagation of the medicinal plant *Physalis alkekengi* L. and the creation of virus-free healthy plants. Therefore, creation of virus-free medicinal plant *Physalis alkekengi* by microclonal reproduction method and obtaining biologically active substances is of scientific and practical importance.

2 Materials and methods

In the course of research, the "indicator plants" method, solid-phase point method dot-IFA, extraction of flavonoids, sterilization, preparation of Murasige-Skuga nutrient medium, microclonal propagation of plants, polymerase chain reaction (PCR) and other microbiological and biotechnological methods are used to detect viral diseases of plants. methods used.

3 Results and Discussion

Tobamovirus disease symptoms detection, i.e. tobacco mosaic virus - physalis TMV-Ph strain results are presented. Based on the observation of viral diseases of Physalis alkekengi L., leaves infected with virus-specific symptoms in the form of a mosaic (change in the color of leaves, interlacing light-green colors with dark-green colors) were identified. Virus-specific symptoms were studied in the indicator plants of N. glutinosa, N. tabacum variety "Samsun" and in the plant Physalis alkekengi L. itself, and dark-green mosaic symptoms indicated the presence of the virus, but yellow spots on the leaf surface, symptoms of leaf deformation (Fig. 1, B, G) did not show virus-specific symptoms (Table 1).

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Table 1. Detection of virus-specific symptoms in Physalis alkekengi.

Ph. alkekengi plant viral diseases	**Symptoms in indicator plants**		
	N. glutinosa	**N. tabacum «Samsun»нави**	**Ph. alkekengi**
Control (healthy leaf)	-	-	-
Dark green mosaic	N	M	M
Yellow spots on the surface of the leaves	-	-	-
Deformation of leaves	-	-	-

Note: M-mosaic, N-necrosis, (-)-necrosis did not appearю

Physalis alkekengi L. plant food mosaic virus diseases on the basis of comparative treatment with other TMV strains within host plants Physalis strain-TMV-Ph to other TMV strains TMV-V "Vostryavovo" VII TMV-K (IV (IV), TMVL- TMV "Leningrad" VIII, TMV "Krymsky" XII, Tomato "Lenungton" III "Leningrad tomatnyy zelenyy IX", Ogurechnyy shtamm "zelenyy", Ph. in some strains, 6-, 10-, 12-, etc. days are arranged in Table 2.

Table 2. Comparative characterization of *Ph. alkekengi* plant TMV with other TMV strains.

№	**Name of strains**	**N.tabacum «Samsun»**	**N.tabacum «Sylvestris»**	**N. tabacum «Debney»**	**N. tabacum copr Barley**	**N. glutinosa**	**L.esculentum**	**Ch.quinoa**	**Ch. amaranticolor**	**D. stramonium**	**P.hybrida**	**Ph. alkekengi**	**Plantain (P. lanceolata)**	**G.globosa**	**Cucumber (Cucumis sativus)**
1	O-TMB	6M	M	N	N	N	12M	N	N	N	M	M	M	M	N
2	V-TMV "Vostryakovo" VII	6Md	M	N	N	N	-	N	N	N	Mc	-	M	M	N
3	K-TMV (IV)	10M	10N	N	N	N	25M	N	N	N	M	N	-	M	N
4	L-TMV (VI)	6Md	M	N	N	N	-	N	N	N	M	-	M	M	Md
5	TMV "Leningrad"VIII	7Md	10Md	N	N	N	14Md	N	N	N	M	-	M	M	-
6	TMV "Krymsky"XII	10M	M	N	N	N	Mc	N	N	N	M	N	-	M	-
7	Tomato "Lenungton"	6Md	6H	N	N	N	14M	N	N	N	M	M	-	M	-
8	Tomato "Ontario" III	10M	M	N	N	N	M	N	N	N	15Ml	-	-	M	-
9	Leningrad tomato green IX	6M	M	N	N	N	M	N	N	N	M	L	-	M	-
10	Cucumber strain "green"	6Mc	M	N	N	N	Mc	N	N	N	M	-	-	M	-
11	Ph. alkekengi (Ph-TMB)	10M	3 N	5 N	3 N	2 N	17C M	10H	10H	6 N	17M	15M	25M	21M	3 N

Note: **M**-mosaic, **SM** – systematic mosaic, **Ms** – weak mosaic, **N**-necrosis, **L**-local spots, **D**- leaf deformation, (-) – symptoms did not appear, numbers indicate the day of disease symptoms. (R≤0.05).

The results showed that *Ph. alkekengi* plant virus showed symptoms typical of the tobamovirus group and was named "physalis strain - TMV-Ph" of tobacco mosaic virus.

Gibbs A., Harrison B. (1978) and V.M. According to the systematics of Zhdanova (1990), the family Virgaviridae belongs to the "tobamoviruses" group. The cryptogram consists of R/1: 2/5: E/E: S/0:, that is, "hard" virions are 300 nm long and 18-20 nm in diameter; heat inactivation point (ITIN) – 95-98 ºC; structured on the basis of spiral symmetry (spiral pitch 2.3 nm); single-stranded RNA (5% RNA, 95% protein) has a molecular mass of 2x106 D; The ITIN of the TMV-Ph strain in the study virus juice is -98 ºC; degree of dilution - 10-7; isoelectric point - 3.5, that is, close to the tomato strain of TMV. A passport was obtained for the tobacco mosaic virus physalis strain (TMV-Ph). The isolated TMV-Ph virus strain was registered in the World Data Center for Microorganism (WDCM) National Collection of Phytopathogenic Microorganisms (NCAM) database under the number WDCM #862 (http://new.wfcc .info/ccinfo/i ndex.php/ collection/by_id/862).

Purification of TMV-Ph virus and obtaining antibodies. Information on virus purification by polyethylene glycol (PEG) "precipitation" is provided. The method of mechanical grinding and homogenization was used to obtain the viral drug from *Physalis alkekengi* L. plant cell. For this, 300 g of *Physalis alkekengi* L. plant leaves were homogenized in the presence of 0.05M phosphate buffer (rN 7.5). Then filter and 6000 rpm. was centrifuged and precipitated by incubating in 4% mM 6000 PEG at 4°C for 1 hour. Then 6000 km/h. The virus was centrifuged and partially purified virus was isolated in the amount of 33-35 mg/ml.

Antibodies specific to TMV-Ph virus were obtained by immunizing "Chinchilla" rabbits by injecting TMV-Ph immunogen in a mixture of Freund's adjuvant. In this case, the concentration of TMV-Ph was from 0.5 mg/ml to 1.5 mg/ml. Ten days after the last injection, rabbit blood was collected and antibodies specific for TMV-Ph were isolated. These specific antibodies were used to perform the dot-IFA analysis for the detection of TMV-Ph virus.

Identification of TMV-Ph virus by dot-IFA method. The results for the detection of TMV-Ph strain of *Physalis alkekengi* L. in field conditions using dot-IFA immunoenzyme analysis (dot-Elisa- dot solid-phase immunoenzyme analysis) method are presented. The results of diagnostic studies were carried out on nitrocellulose membrane filters, and calculations were visually determined by changes in the color of the substrate. Figure 2.

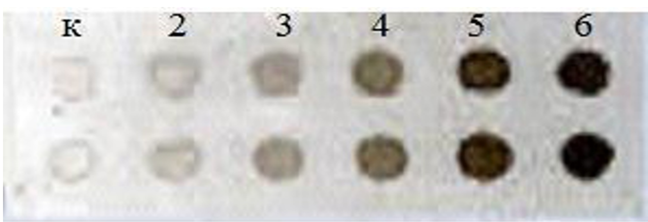

Fig. 2. Dot-IFA analysis of TMV-Ph virus on nitrocellulose membrane. **Note:** 1- control, 2, 3, 4, 5, 6 - virus samples with different concentrations.

As can be seen from Figure 2, dark brown spots appeared as the virus concentration increased. This information indicates the presence of the virus, that is, the detected antigen, in high concentration. The simplicity and efficiency of the used dot-IFA analysis compared to other methods allows to simultaneously detect many selected samples as infected with the virus and obtain relevant results without the use of complex and expensive instruments.

Preparation and sterilization of plant explants for microclonal propagation. Optimal conditions for sterilization of *Physalis alkekengi* L. and selection of plant explant size are presented. Strict adherence to sterility is essential when working with isolated tissue culture. Therefore, step-by-step methods of sterilization of plant explants in ethanol, chloroform, distilled water, etc. have been implemented. The prepared sterile explants were placed in the nutrient medium and placed in a cold incubator at a temperature of 22°C for 5 days to acclimatize the explants to the nutrient medium, then they were observed in a light room at a temperature of 24-25°C during the day and at night, with 5-6 kLx light. The results of the study showed that 2 out of 6 Petri dishes were infected with fungus (33%) on 4-5 days of growing explants. No fungal growth was observed in 4 cups (66%). Plant stems and root explants began to develop 10 days after planting in the nutrient medium and were fully formed in 18-20 days. In order to achieve 100% sterilization efficiency, we decided to sterilize plant explants at different concentrations of hypochlorite. The results are presented in Table 3. According to the results, when plant explants were sterilized in 20% hypochlorite solution, 9 out of 10 explants, and 7 out of 10 explants in 15% hypochlorite were not affected by fungi and bacteria. An increase in the number of infected explants was observed in 5% and 10% hypochlorite solution, that is, 6-8 out of 10 explants were infected. Plant stems and roots formed after 18-20 days.

Table 3. Selection of optimal nutrient medium for growing explants of *Physalis alkekengi.*

№	**Number of sterile explants**	**Hypochlorite concentration, %**	**Sterilization time (second)**	**infected number of explants**	**Damaged number of explants**
1	10	20	20	9±1	1
2	10	15	20	7±2	3
3	10	10	20	4±1	6
4	10	5	20	2±1	8

Note: infected and uninfected number of explants (M±m; n=5), P≤0,05.

Thus, from the obtained results, it became clear that sterilization of plant explants in 20% hypochlorite solution has a positive effect on the growth and formation of explants in microclonal propagation of *Physalis alkekengi* plant. Selection of optimal nutrient medium and explant size for microclonal plant propagation. Results of research on in vitro microclonal propagation of *Physalis alkekengi* plant explants are presented. Explant size (stem and root explant lengths 0.4, 0.6, 0.8, 1.0 cm), the composition of the nutrient medium and the antifungal drug nystatin (from 500,000 ed/l to 1,500,000 ed/l) of the plant influence on growth and development is studied.

The results showed that no growth was observed in the explant of 0.2 cm roots. Regeneration of the root explant with a size of 0.6-1.0 cm was observed on the 8-9th day. Explants with stem lengths of 0.8-1.0 cm started to grow and develop faster, that is, from the 8th day. The best results were observed in stem explants with dimensions of 0.8-1.0 cm (Table 4, nutrient media #2 and #3). In addition, the effect of the optimal concentration of the antifungal drug nystatin on the growth and development of *Physalis alkekengi* plant explants was determined. According to the obtained data, after 8 days, when the concentration of nystatin was 500,000 ed/l, the growth of the stems was 3.0-6.0 cm, the size of the leaves was 0.5 cm to 2.5 cm in length (Fig. 3, A, B, C, D).

Nutrient medium №. 3 with a concentration of 1,000,000 ed/l of nystatin had a positive effect on the growth and development of plant explants. After 10 days, the stem length was 8.0 cm and the leaf length was 3.5 cm. When the obtained results were compared with the growth and development of explants in the control (the length of the roots was 0.3-0.5 cm, and the leaves were 0.5-1.0 cm in the control), it was found that these parameters increased several times in nutrient medium No. 3. Therefore, plant explants were planted in nutrient medium #4 with a nystatin concentration of 1,500,000 ed/l. According to the obtained results, the increase in the concentration of nystatin had a negative effect on the growth and development of stems and leaves. Nutrient medium with nystatin concentration of 1,500,000 ed/l had an inhibitory effect on plant growth and development.

Fig. 3. The effect of nystatin on the growth and development of Physalis alkekengi plant (A-control, B- nutrient medium № 2 (with 500,000 ed./l nystatin), C- nutrient medium № 3 (with 1,000,000 ed/l nystatin), D- nutrient medium no. 4 (with 1 500 000 ed/l nystatin), E – Growth of Physalis alkekengi plant in sterile soil.

4 Conclusion

TMV-Ph strain of tobacco mosaic virus was isolated from the medicinal plant *Physalis alkekengi* L. and its biological, morphological and physical properties were studied. The TMV-Ph strain was found to belong to the Virgaviridae family of the tobamovirus group. A passport was obtained for this strain of the virus. The TMV-Ph virus strain is listed in the database of the World Data Center for Microorganism (WDCM) National Collection of Phytopathogenic Microorganisms (NCAM) under the number WDCM #862 (http://new.wfcc.info /ccinfo/i ndex.php/ collection/by_id/862).

The TMV-Ph strain was identified using a sensitive solid-phase immunoenzymatic method based on antibodies immobilized on a nitrocellulose membrane, which allowed simultaneous analysis of a large number of samples in field conditions.

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