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DETECTION AND IDENTIFICATION OF SOME BIOLOGICAL CHARACTERISTICS OF POTATO L-VIRUS USING REAL-TIME PCR METHOD

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Abstract. Nowadays, several phytopathogenic viruses infect the potato plant and cause great economic damage. Early detection of the virus from sowing material and natural reservoir plants is the main reason to prevent its spread, so that measures can be taken to control these viruses. This article presents the results of research on detection and identification of potato L-virus (PLV) from natural host plants and different disease symptoms, using real-time polymerase chain reaction (PCR-real-time) method.

Key words: PCR, RNA, virus, identification, potato, amplification, variety, reservoir.

ОПРЕДЕЛЕНИЕ НЕКОТОРЫХ БИОЛОГИЧЕСКИХ СВОЙСТВ И ИДЕНТИФИКАЦИЯ Л ВИРУСА КАРТОФЕЛЯ С ПОМОЩЬЮ МЕТОДА ПЦР

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Аннотация. В настоящее время некоторые фитопатогенные вирусы поражают растения картофеля и наносят большой экономический ущерб. Раннее обнаружение вируса из эквивалентного материала и природных растений-резервуаров является основной причиной предотвращения его распространения, чтобы можно было принять меры против этих вирусов. В этой статье проведены исследования по обнаружению и идентификации L-вируса картофеля (KLV) из природных растений-хозяев и различных симптомов заболевания с использованием метода полимеразной цепной реакции в реальном времени (ПЦР в реальном времени), а результаты представлены эти исследования.

Ключевые слова: ПЦР, РНК, вирус, идентификация, картофель, амплификация, сорт, резервуар.

INTRODUCTION

Agricultural products are of great importance in human life. Potatoes, one of these products, play an important role in the diet. In developed countries, potatoes are used as raw materials for food, fodder, starch and alcohol production. As in other plants, there are phytopathogenic diseases in potatoes, which have a serious negative impact on its growth, yield and product quality. In most cases, during the growth period of the plant, under the influence of various infectious diseases,

most of the crop is lost and its quality decreases sharply. As a result of the lack of regular control measures and other preventive measures, diseases are often found in crop sown fields and private farms and cause great damage.

Potatoes are the fourth major food crop in the world and feed a large part of the world's population. In recent years, many diseases and pests have negatively affected the productivity of agricultural crops. Among agricultural crops, potato is one of the main crops, and the productivity of this plant is also decreasing due to various diseases. Viruses are the most dangerous pathogens that damage potato plants in agriculture. More than 50 viruses naturally infect the potato plant worldwide. These viruses can reduce the yield of potato plants by up to 80%. Of the 400 phytoviruses known to date, 52 cause damage to the potato plant, 36 of which are widespread, and the rest are found only in South American countries. Of these, 16 species are very common, and 6 species are found in the Republic of Uzbekistan. They are: PVX, PVY, PVM, PVS, PLV, PVA [6,7].

Despite the improvement of breeding efforts to introduce disease-resistant and high-yielding varieties, potato production in cultivated fields is low due to various biotic and abiotic factors, including viruses. Viral diseases play a major role in the drastic reduction of plant productivity, insects (beetles, leafworms and whiteflies), fungi and tubers serve as a source of virus infection and a vector of virus, as well as, a reservoir of infection. Low phytopathogenic isolates of potato L-virus alone can reduce yield by 15-35%, strong pathogenic isolates by 65-80%. Studies have shown that infected potato tubers replanted over many years reduce yield by almost 50% [6, 10, 12].

L- virus belongs to the Luteovirus family, and its virions are spherical in shape, 23-25 nm in size. The protein (m.m) of the virus is 28,3×106 D, virion consists of 28% RNA and 72% protein. The RNA m.m. 2,0×106 D, protein m.m. 26,3×103 D, sedimentation coefficient 127 C, E260 0,1% 1cm≈5. The degree of the temperature resistance of the virus is 80°C, the virus is stored at 42-43°C for 1-5 days, and at 20°C for more than a month.

PLRV is not mechanically transmitted, but is very easily transmitted by stem and tuber-core grafting. In natural conditions, it is transmitted by a number of aphids and plant lice, such as *Myzus persicae Sulz., Myzus ascalonium, Myzus circum flexus Buckt., Myzus ornofus, Myzus convolvuli Kalt., Macrosiphum solanifolii Ashm.*

PLRV is transmitted by sap in a circulation way: from the phloem they move to the hemocoel and through the hemolymph to the salivary glands. Its localization in plants is in the phloem, it causes tissue necrosis, stunted growth, loss of chlorophyll and yellowing of leaves. *Myzus persicae* is its main vector. Studies have shown that PLRV can be transmitted to infected plants by aphids and exert pathogenicity on infected plants. However, the molecular mechanisms mediating these relationships are still poorly understood.

A plant infected with the PLRV virus through aphids will retain the virus for the next year. The seeds of an infected potato plant in the first year are a reservoir for the virus, resulting in unhealthy plants. Infected potato seeds serve as a systemic translocation of PLRV to all plant tissues. The degree of

temperature resistance of this virus is 80%, and the virus can survive at 42-43°C for 1-5 days, at 20°C for more than a month [6, 7, 9].

I.T. Ergashev detected natural reservoirs of potato L – virus in several wild plants and weeds: including, field ivy (*Convolvulus arvensis*), sorrel (*Rumex confertus*), plantain (*Plantago lanceolata*), jimsonweed (*D. stramonium*), black nightshade (*Solanum nigrum*), etc.. In the research work of V.B. Fayziev it was reported that the plants, such as eggplant *(S. melongena L.),* rough cocklebur (*Xanthium strumarium L),* wormwood *(Artemisia annua L.),* black nightshade *(Solanum nigrum L.),* thornapple *(Datura metel L.),* water foxtail *(Alopecurus geniculatus L.),* mugwort *(Artemisia vulgaris L.),* alfalfa *(Medicago sativa L.),* tomato *(Lycopersicum esculentum Mill.),* goosefoot (*Ch. amaranticolor*), cucumber *(Cucumis sativus L.),* dodder *(Cuscuta approximata Babing.),* common marshmallow *(Althaea officinalis L.),* common mallow *(Malva neglecta Wall.),* velvetleaf *(Abutilon theophrasti Medic.),* curly dock (Rumex crispus L.) are natural reservoirs of L-virus [7, 9].

The cryptogram of this virus is R/1:2/28:S/S:S/Ap; S/Ap. The protein of the virus (m.m) is 26,3×103 D, the diameter of the virion is 25-30 nm, each nucleocapsid contains 32 capsomeres. About 28% of the virion is RNA, 78% is protein. According to the classification of luteoviruses, it belongs to IV group of viruses. Each virion contains a single strand of (+) RNA. The genome consists of a sequence of 5300 to 5900 nucleotides [2, 3, 4, 9, 14].

Potato L-virus causes disease symptoms such as yellowing and coarsening of the leaf color and crinkled or wavy leaf plate in the plant. The abovementioned disease symptoms are differentiated from other potato virus diseases.

The PCR method was chosen for accurate and precise diagnosis of the disease and identification of the virus.

1. Polymerase chain reaction (PCR) is considered the most acute, accurate and fast method of molecular diagnosis of phytopathogenic viruses. When analyzed by real-time PCR method in a biological sample taken from the stem, leaf or tuber of a potato plant, it allows to determine the genetic molecule (DNA/RNA) of the pathogen. PCR diagnostics can detect even the lowest stage of viral infection in plant samples. The difference between the molecular diagnostic method and other methods is that the causative agent (pathogen) of the disease is diagnosed not by the connection between the antigen and the antibody, but by the direct recognition of the genetic molecule of the infection [10,11,16].

MATERIALS AND METHODS

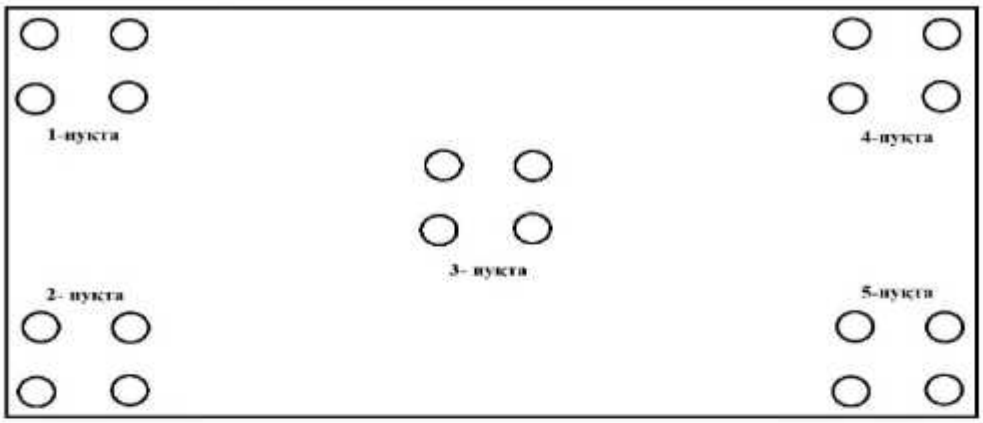
Conditions for research

The research work was carried out in the "Molecular Biology and Bioinformatics" scientific research laboratory of the "Biology" department of the faculty of Chirchik State Pedagogical University and in the IFT and PCR laboratory of the State Center for the Diagnosis of Animal Diseases and Food Safety of the Republic, as well as a potato growing farms in the Tashkent and Qibray districts of the Tashkent region.

The methods of research

Sampling for PCR analysis. For conducting analysis, potato leaves by 4 pieces from each site of the potato planting area, a total of 80 plant leaves from 20 sites, including plant stems and leaves of weeds growing among the

potato plants, were collected and placed in separate polyethylene bags and then put in a thermos with special ice packs (+4°C) and brought to the laboratory for PCR analysis



(*Fig.1*).

Figure-1. Scheme of envelope method of sampling from field crop area

The infestation level of plants was determined by using PCR based on the collected samples. During scientific research, monitoring was carried out by RV-PCR method for symptomatic differentiation of PVL with other viruses [1, 2].

Sequencing was performed according to the following algorithm to determine PVL by polymerase chain reaction method:

1. Isolation of RNA of PVL from the sample;

The set of reagents "PhytoSorb" RN-520 manufactured by the "SINTOL" scientific production company (Russia) was used for the isolation of RNA of virus from the plant sample.

2. Mixing isolated RNA with specific primers and enzymes (TAQ-polymerase, revertase, etc.);

"Reagent set for detection of RNA of PVL by real-time-polymerase chain reaction-repetitive transcription (RV-PCR-QT) method" (SINTOL PV-002) manufactured by "SINTOL" scientific production company (Russia) was used for the research. This test set can detect the RNA of the pathogen of PVL in any vegetation period of plants.

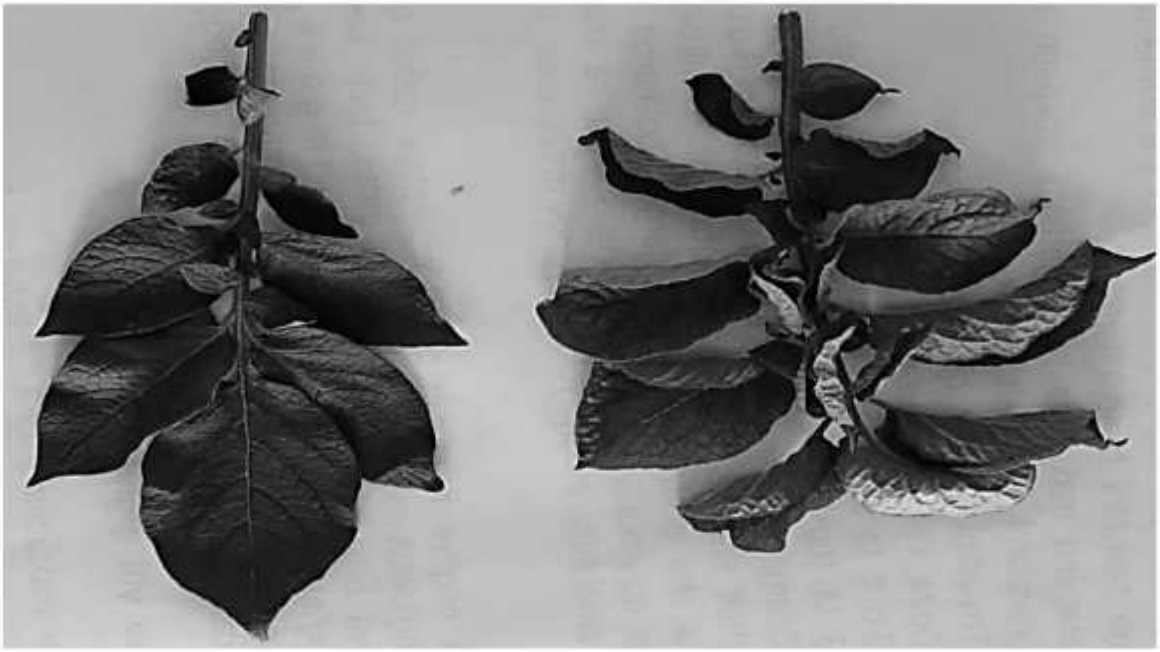
3. Amplification of the RNA storage mixture placed in microtubes in PCR amplifiers and interpretation of the result.

The amplification process was carried out in qTOWER^3/G amplifier equipment. The first 15 minutes of amplification were reverse transcribed at 45°C. After that, 50 cycles of denaturation, annealing and elongation processes were carried out sequentially.

RESULTS AND THEIR DICUSSION

4 samples of potato plant leaves of 4 varieties from 5 sites were taken from the fields of potato farms in Kibray district. During the sampling, plants with typical PVL symptoms shown in the literature were selected. Selected samples were sorted to differentiate PVL from other viral diseases of potato (PVY, PVX). Also, in order to study the preservation of the virus from one season to another in the fields planted with potatoes, samples of wild plants were taken and monitored.

Samples were homogenized using extracting solution in autoclaved sterile porcelain flasks. RNA of PVL was isolated from the homogenized sample using sequencing nucleic acid isolation reagents. Using a special PCR mixture, the isolated RNA of PVL was mixed with primers. The prepared mixture was placed in the equipment for amplification. At the end of the amplification, the following result was obtained (Table 1).



1-Figure. A) healthy leaf B) PVL infected leaf

Table-1

Expertise conclusion on PVL detection by PCR amplifier.

Tested samples	Sample name	Dye	Ct	mean Ct
A1	Santa variety 1 site	FAM	38,83	38,83
B1	Santa variety 2 sites	FAM	35,54	35,54
C1	Santa variety 3 sites	FAM	32,55	32,55
D1	Santa variety 4 sites	FAM	33,35	33,35
E1	Azara variety 1site	FAM	42,17	42,17
F1	Azara variety 2 sites	FAM	37,78	37,78
G1	Azara variety 3 sites	FAM	31,15	31,15
H1	Azara variety 4 sites	FAM	39,56	39,56
A2	Pikasso variety 1 site	FAM	32,66	32,66
B2	Pikasso variety 2 sites	FAM	30,56	30,56
C2	Pikasso variety 3 sites	FAM	21,65	21,65
D2	Pikasso variety 4 sites	FAM	26,36	26,36
E2	Kizil kuz variety 1 site	FAM	15,53	15,53
F2	Kizil kuz variety 2 sites	FAM	17,59	17,59
G2	Kizil kuz variety 3 sites	FAM	19,56	19,56
H2	Kizil kuz variety 4 sites	FAM	21,20	21,20
A3	PKO (K+)	FAM	30,74	30,74
B3	OKO (K-)	FAM	no Ct	

According to the results of the research, Santa, Azara, Picasso and Kizil kuz potatoes were infected with PVL. From all 4 samples, it was determined from the results of PCR that the infestation of Kizil kuz variety potato with the virus was higher.

The samples provided for the identification of potato L-virus reservoirs were examined by PCR method and the following results were obtained (Table 2).

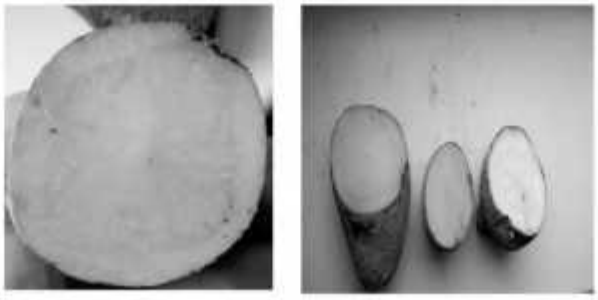
2-Figure. PVL reservoirs: a- *Malva L.*, b- *Brossica campestris* L.

Table-2

Expertise conclusion on the identification of PVL reservoirs carried out by the PCR amplifier

Tested samples	Sample name	Dye	Ct	mean Ct
A1	Fizalis *(Physalis)*	FAM	no Ct	
B1	Potato *(S.tuberosum) Kayroki variety tuber*	FAM	34,1	34,1
C1	Goosefoot *Chenopodiumalbum*	FAM	no Ct	
D1	Henbane *(Hyoscyamus L.)*	FAM	no Ct	
E1	Field mustard *(Brossicacampestris L.)*	FAM	no Ct	
F1	Purslane *(Portulacaoleracea L.)*	FAM	no Ct	
G1	Clover *(Trifolium)*	FAM	no Ct	
H1	Hedge bindweed *(Convolvulussepium)*	FAM	no Ct	
A2	Black nightshade *(Solanumnigrum)*	FAM	no Ct	
B2	Potato *(S.tuberosum) tuber of red potato*	FAM	29,79	29,79
C2	Tomato *(Solanumlycopersicum)*	FAM	no Ct	
D2	Malva *(Malva L.) mallow*	FAM	no Ct	
E2	Sweet pepper *(Capsicum annuum L)*	FAM	no Ct	
F2	Thistle *(Cirsiumarvense)*	FAM	30,37	30,37
G2	Potato *(S.tuberosum) local Gala variety tuber*	FAM	no Ct	
H2	PKO (K+)	FAM	no Ct	
A3	OKO (K-)	FAM	28,86	28,86

According to the results of the analysis conducted to identify the reservoirs of PVL in the weeds grown in the potato fields: it was found that the body of the wild and cultivated potato plants shown in the literature has a high titer of the virus. (Fig. 1. a, b).



3-Figure. PVL reservoir: a-*(S.tuberosum) Kayroki variety tuber,* b-*(S.tuberosum) local Gala variety tuber.*

Taking into account that wild and cultivated potato plants grow in autumn, their tubers overwinter under the soil, and continue to grow in spring, the storage of PVL in reservoir plants causes the periodic circulation of the virus and its spread to a wider area.

CONCLUSION

One of the main conditions for ensuring a high and quality harvest from plants is timely detection of the disease, correct and prompt diagnosis, and proper protection of crops from diseases. Therefore, it is necessary to have information about the identification of the causative agent, its development, spread, and how it is preserved from one season to another.

In this study, disease symptoms such as leaf discoloration or yellowing and coarsening, as well as crinkling of the leaf plate were studied by PCR monitoring.

From the studied literature and conducted experiments, it was found that: PVL is found mainly in representatives of the nightshade family. It was also found that these plants, which are found in potato fields as weeds, keep PVL in their bodies and participate in the periodic cycle.

Identifying natural reservoir plants and avoiding their growth in fields is very important in preventing the spread of this virus.

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