ISSN NO: 2771-8840
Date of Publication: 22-12-2022

Determination of Potato X-Virus Reservoir Plants by Immunoenzyme Method

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Abstract: The importance of natural reservoir plants in the conservation and circulation of phytoviruses in nature is considered to be very high. These reservoir plants in most cases keep the virus at a low concentration and may not show symptoms of the disease, so a very sensitive diagnostic method is needed to detect such latent reservoirs. One of these methods is the enzyme immunoassay method. In this work, natural reservoir plants of Potato Virus X were identified by enzyme immunoassay. As a result of the research, it was found that out of thirty species of cultivated and wild plants, the natural reservoirs of the virus are Cucumis sativus L, Rumex crispus L, Brassica juncea (L) Czern, Althaea officinalis L, Malva neglesta Wall.

Keywords: Brassica juncea, Cucumis sativus L, Althaea officinalis L, Malva neglesta Wall.

More than 20 types of potato virus diseases have been identified around the world, which cause various degrees of disease in plants and cause great damage to agriculture by reducing productivity [3, 5, 6].

One of these viruses is potato X-virus which causes disease symptoms such as mottling and growth point necrosis in potatoes. In some varieties of potatoes, this disease can pass in a latent state without showing symptoms at all, so the X-virus is also called the healthy potato virus. The size of this filamentous virus can range from 450 x 10 nm to 600 x 12 nm [1, 2, 6, 8].

Potato X-virus is transmitted to a healthy plant by the contact of healthy plant organs with diseased plant organs, during agrotechnical processing of plants and by the Calarado beetle [1, 4], and reduces productivity up to 10-51% [5, 7, 8].

In studying the circulation of potato X-virus in nature, identification of its carrier insects and reservoir plants is of great practical and theoretical importance today. Therefore, we identified reservoirs of potato X-virus from wild and cultivated plants in the climatic conditions of Uzbekistan using one of the modern and most sensitive methods, immunofermet analysis.

Material and work style. The main materials for the research were potato virus antibodies, conjugate (IgG+enzyme), polystyrene plates and chemical reagents from the CIP organization "International Center of Potato".

The following are used to diagnose potato viruses using immunofermet analysis:

1. Specific antisera prepared for potato X-virus;
2. Polyethylene plates;
3. Conjugate (virus antiserum+Ig+enzyme);
4. Buffers (phosphate buffer, 1 l. composition for distilled water: 8 g. NaCl, 0.2 g. KH_2PO_4, 1.15 g. Na_2HPO_4, 0.2 g. KCI, 0.195 g. NaN_3), tween phosphate buffer (PBS-T), sample grinding buffer (0.4 g PVP-40.000, 2.0 g egg albumin), conjugate buffer (4.0 g PVP-40.000, 0.04 g egg albumin), substrate buffer;
5. Clean dishes;
6. Polyethylene bags for samples;

Samples for testing were prepared from the fields of the joint-stock farm of Uzbekistan, Zangiota district, Tashkent region, and from various organs (leaves, stems, roots) of wild and cultivated plants growing on the roadsides. Plants belonged to different families and samples were taken from symptomatic, non-symptomatic, dead or vegetative plants.

To detect plant viruses using IFA, plant organs (leaf, stem, root) with disease symptoms are placed separately in polyethylene bags and phosphate buffer in a ratio of 1:1 (contents per 1 liter: NaCl, KH_2PO_4, Na_2HPO_4, KCl, NaN_3, pH 7.4) and thoroughly crushed to prepare a homogenate. After that, viral antibodies

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were immobilized on polystyrene plates for 3-4 hours at 37 ^{0}C or 5-6 hours at room temperature, excess antibody was washed off with tween phosphate buffer (0.5 ml (20 drops) tween is added to 1 liter of prepared phosphate buffer, RVS-T), a homogenate prepared from plant organs to be determined was placed on it and kept in a thermostat at 37 ^{0}C for 3-4 hours. After a certain period of time, the plant sap was washed away using the twin phosphate buffer (RVS-T) for washing and after putting the conjugate (IgG+enzyme) into the microplastic cavity of the polystyrene plates, the plates were placed in polyethylene bags and kept at 37 ^{0}C for 3-4 hours or left at room temperature (+-25^{0}C) for 5-6 hours. After washing off the excess IgG with tween phosphate buffer (RVS-T), microplastic plates are filled with substrate (diethanolamine, HCl (37%), distilled water and substrate pellet (r-nitrophenylphosphate)) at 50 microlitre per well. The reaction appears for 30-60 minutes, that is, the reaction goes with a color change.

Research results and their discussion. In order to identify reservoir plants of potato X-virus, a number of plants belonging to different families were examined using immunoenzyme analysis. The results are presented in Table 1.

Table 1
Identification of host plants of potato X-virus using immunoenzymatic analysis

Name of the plant	Reaction indicator
Cynodon daktulon (L) Pers	-
Cyperus rodundus	+-
Cucumis sativus L.	+ + + +
Xanthium strumarium	-
Atriplex micrantha C.A.Mey	+-
Sorghum helepense	-
Solanum nigrum L.	+
Artemisia vulgaris L.	-
Chenopodium amaranticolor	+-
Rumex crispus L.	+ + +
Datura stramonium	+
Solanum melon-gana L.	+ +
Capsicum annum L.	-
Petunia hybrida	+
Datura metel	+-
Brassica juncea (L) Czern	+
Mentha asiatica Boriss	-
Solanum tuberosum	+
Solanum tuberosum	+ + +
Solanum tuberosum	+ + + +
Chenopodium quenoa	+-
Convolvulus arvensis L.	+ + +
Ocumus basilicum L.	-
Amaranthus retroffexus L.	+-
Sinapsis arvensis L.	+-
Althaea officinalis L.	+ + + +
Lycopersicum esculentum Mill	+ + + +
Alhagi adans	-
Malva neglecta Wall	+ + +
Artemisia vulgaris L.	-

Note: "-"-no reaction at all ;
"+-"-the presence or absence of a reaction is abstract ;
"+" –the course of the reaction is very pale yellow;
"+ +" – reaction progress is yellow;

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"+ + +"- the transition of the reaction is orange ;

"+ + + +"- the reaction progress is very orange ;

As can be seen from the table, except for potatoes, X-virus can be found in *Cucumis sativus, Solanum nigrum* L, *Rumex crispus* L, *Datura stramonium, Solanum melon-gana* L, *Petunia hybrida*, It was found that *Brassica juncea* (L) Czern, *Convolvulus arvensis* L, medicinal *Althaea officinalis* L, tomato (*Lycopersicum esculentum* Mill), *Malva neglesta* Wall are preserved. In addition, it is not known whether there is a virus in plants such as Atriplex micrantha C.A. May, *Chenopodium quenoa, Cyperus rodundus, Datura metel, Amaranthus retroffexus* L, *Sinapsis arvensis* L, i.e. reaction indicator it showed "+-". *Cynodon daktulon* (L) *Pers, Xanthium strumarium, Sorghum helepense, Capsicum annum* L, *Ocumus basilicum* L, *Artemisia vulgaris* L *Artemisia vulgaris Lwere* found be virus free. Based on the above information, it should be noted that potato X-virus infects annual and perennial plants belonging to the *Solanaceae, Malvaceae, Cruciferae, Amaranthaceae, Compositae* families and is present in them in different amounts (3+,4+). Therefore, these plants undoubtedly serve as reservoir plants of potato X-virus.

Potato virus diseases have been studied since 1916 by Kvainer, Botes, Shultsem, Folsom, Kassanis, Martin, Yora, Morel, and Ambrosov in countries such as England, Holland, USA, Germany, Russia, and Estonia. However, in the conditions of Uzbekistan, the sensitivity of the work was carried out only at the sensitivity level of indicator plants, drop method or ABV-test (0.2 micg/ml). A small number of viruses in many host plants, potato cultivars, remained outside the style sensitivity level. In this study, we used highly specific, high-titer antisera with a sensitivity level of 0.01 ng.

Many plants are asymptomatic and are being analyzed for the first time. For example, the plants analyzed for the first time are: *Cucumis sativus L, Rumex crispus L, Brassica juncea (L) Czern, Althaea officinalis* L, *Malva neglecta* Wall (table 1). They were found to have potato X virus (3+)-(4+). So, there is no doubt that these plants are being included as reservoirs for the first time in phytoviruslogy.

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