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DISTRIBUTION AND TRANSMISSION OF PLUM POX VIRUS IN UKRAINE

SUMMARY

The most sensitive viral disease of the stone crops is Sharka, the causative agent of which is the plum pox virus (PPV, Plum Pox virus). PPV worldwide has a quarantine status. PPV is widespread in many regions of Ukraine and poses a serious problem to horticulture stone crops of our country. The purpose of our research was to analyze the distribution and the harmfulness of this pathogen, to describe the ways of transmission, to carry out research on the identification of aphids that were carriers of the disease and to characterize its molecular features and strains diversity in the territory of Ukraine. The samples were visually selected from the central and northern regions of Ukraine. Modern methods of molecular diagnostics were used such as: polymerase chain reaction with reverse transcription, sequencing. The phylogenetic analysis confirmed the identity of the strains and helped us made a comparative characterization of the samples to the already known strains. Depending on the strain, different kinds and varieties of plants could be damaged and crop losses could significantly vary. Therefore, it is important to determine the diversity of PPV strains and their similarities with other isolates. The result revealed high level of damage to stone crops in the territory of Ukraine, especially in Odessa and the Kiev region. Harmfulness and distribution of this disease increases every year. These researches are needed to find ways to fight this pathogen and stop the spread of a dangerous virus in Ukraine.

Keywords: Plum pox virus, distribution, strain diversity, PCR, phylogenetic analysis, Ukraine.

INTRODUCTION

Plum pox virus (Potyviridae, Potyvirus) is a harmful agent that causes a dangerous disease of stone crops. PPV is a quarantine object in most countries of the world. Plum pox virus has a wide range of host plants.

For the first time Plum pox virus was discovered in Bulgaria in 1915. The disease is spread from Bulgaria to the north and east (Atanassov, 1932). The

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disease caused by PPV was first described in Ukraine in 1966 and since then it has been spreading all over the country. Then in 1969 a minor outbreaks seen in Chernivtsi, Lviv, Zakarpattia, Ivano-Frankivsk and Vinnitsa regions. PPV is widespread in almost all regions and is a serious threat to horticulture of our country. The main method of fighting the disease is destruction of infected plants, which leads to significant economic losses.Other prevention of disease is a breeding resistant crops that are not susceptible to the virus. Genetically modified plums carrying the gene of capsid protein PPV, showed high resistance to PPV infection (James, 2006). The rate of spread of the virus in the garden depends on the distance between healthy trees and source of infection and the PPV transmission effectiveness depends on the sensitivity of culture and population density of aphids (Ratushnyak, 2002). The main route of transmission of the virus - with the help of aphids. The virus is persistently transmitted by about twenty different species of aphids: Aphis cracciora, A. gossypii, A. spiraecola, A. hederae, Muzus persicae, M. varians, Phorodon humuli (Subr, 2013). During non-persistent transmission, viruses are not transported through the membranes of the carrier and do not enter the membrane. The maximum ability aphids to transmit of Plum pox virus was recorded at temperatures of 20-23 ° C. The infectivity of the virus in the body of insects persists for no more than 4 hours (Garcia, 2007). The rate of spread of the virus in gardens depends on the distance between healthy trees and the source of the infection, and the effectiveness of aphids in the transmission of PPV depends on the sensitivity of the culture, the density of the aphid population and the period of virus entry. To establish phylogenetic relationship used approaches and methods of molecular phylogeny (Chirkov, 2016). In general, describing the divergence of PPV isolates circulating in Ukraine can be noted their high homologic nucleotide sequences capsid protein, regardless of region distribution or host plant. To date, nine strains of the virus have been isolated: PPV-An, PPV-C, PPV-CR, PPV-D, PPV-EA, PPV-M, PPV-Rec, PPV-T and PPV-W (Garcia, 2014). According to the literature data, strains of PPV such as Winona, Marcus, Dideron were detected in Ukraine (Mavrodieva, 2013).

The aim of our work is to study the distribution and the harmfulness PPV, to describe the ways of transmission, to carry out research on the identification of aphids that were carriers of the disease and to characterize its molecular features and strains diversity in the territory of Ukraine.

MATERIAL AND METHODS

Materials for research were samples of stone crops from private garden farms of Kyiv, Cherkasy, Odessa, Kharkiv, Ivano-Frankivsk, Vinnytsia regions (Fig 1).

Samples of leaves and fruits of plums, apricots, peaches, cherry plums, cherries, sweet cherries were taken with visual symptoms. Namely: chlorotic spots or rings, deformation of leaves and small rings on the fruit, sometimes with brown or reddish necrotic lesion.

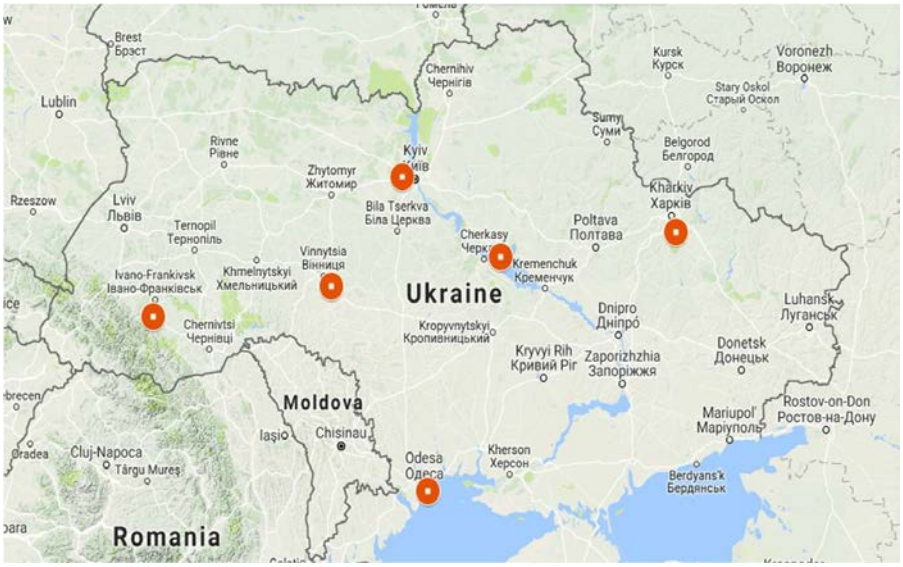

Fig. 1. Regions of monitoring distribution Plum pox virus in Ukraine

The following methods were used: visual diagnosis, total RNA purification, polymerase chain reaction with reverse transcription, sequencing, phylogenetic analysis (Cambra, 2006)

From the samples, total RNA was isolated using a set of RNeasy Plant Mini kit (Qiagen, UK) reagents. Subsequently, all specimens were diagnosed with RT-PCR, using the Thermo Scientific RevertAid Reverse Transcriptase reagents and primers P1, P2 (Wetzel, 1991) and primers for detecting D and M strains(Glasa, 2004). For detection of amplification products was used electrophoresis with 1.5% agarose gel (Sigma) in electrophoretic buffer TBE (89 mM TRIS borate and 2 mM EDTA, pH 8.3). The establishment of the nucleotide sequence of the capsid protein gene of Plum pox virus is carried out after amplification of this gene. The amplification products were cleansed using MinElute Gel Extraction Kit (Qiagen, UK). Amplified fragments were sequenced using Applied Biosistems 3730x1 DNA Analyzer, Big Dyeterminators, version 3.1. MEGA 6 software package was used for phylogenetic analysis.

RESULTS AND DISCUSSION

In the first stage samples of leaves and fruits of stone groups were selected in the spring-summer period from 2017 to 2019. Part of the specimens had characteristic symptoms of Plum pox virus, other part of it had asymptomatic infection or mixed. The classic symptoms of PPV on plums, apricots, cherries, peaches were very rare (Fig. 2,3).

The visual observation of symptoms of the lesion is a rather unreliable method of detecting and diagnosing viral infections, since the manifestation of the symptoms of viral lesion mainly depends on the interaction of the virus and the plant.

A B

Fig. 2. Symptoms caused by PPV: A - leaf deformation light green mosaic on plum, B - yellow rings and blotches, brown rings on fruit peach

A B

Fig. 3. Symptoms caused by PPV: A – aphid of Phorodon humuli; B - leaf deformation on plum and plant damage by aphids.

Often, the strains of the same virus can cause various symptoms on plants of the same species, as the symptoms of plants are influenced by the conditions of plant growth and the presence of a mixed infection, which is quite common in the case of viral disease. Therefore, the presence of a viral infection should be confirmed by specific methods of diagnosis of viral infections and the identification of viruses, in particular by methods of serological diagnostic. Further, all positive samples were diagnosed using molecular techniques RT-PCR (Fig.4).

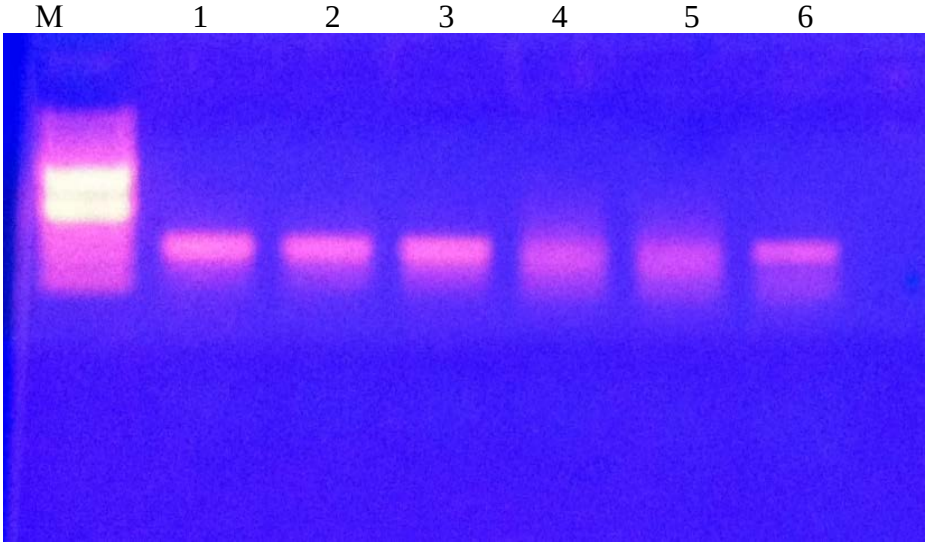

Fig. 4. Electrophoresis of products (243 bp) of RT-PCR of PPV in agarose gel with primers: M - markers, 1-2 - plum samples from Odessa region, 3- 4 peach sample from Odessa region. 5- sample apricots of the Kiev region; 6- positive control

Currently, polymerase chain reaction (PCR) is a promising and accurate method for detecting viral infection. The advantages of this diagnostic method are high sensitivity and ability to determine variety of strains of viruses. In the studied regions we detected strains and found that the most common was D strain (apricot, peach, plum), but M strain (apricot, peach) was rarely found, and in some cases we observed a co-infection. As a result, samples of the capsid protein gene of the Ukrainian isolates were sequenced. The genetic diversity of PPV has been established in different regions of Ukraine.

We comparatived Ukrainian isolates with the isolates of neighboring countries from the GenBank database (http://www.ncbi.nlm.nih.gov). According to the topology of Neighbor-Joining tree it was based on sequences of CP gene, Ukrainian isolates of PPV belonged to M and D strains. These nucleotide sequences of Ukrainian isolates of PPV together with the sequences of other PPV strains were used when constructing the phylogenetic tree. Partial nucleotide sequence of the CP gene of potato virus Y was used as the out group. (Fig.5). From this dendrograms, it can be argued that all Ukrainian isolates have high similarities, regardless of the region or the plants distribution within the host (Tab.1,2).

Methods and approaches for molecular phylogeny are used to establish the phylogenetic affinity of isolates of plum pox virus. To determine the phylogenetic relationships between different strains and isolates, the nucleotide sequences of the capsid protein genes are compared using methods for determining evolutionary distances.

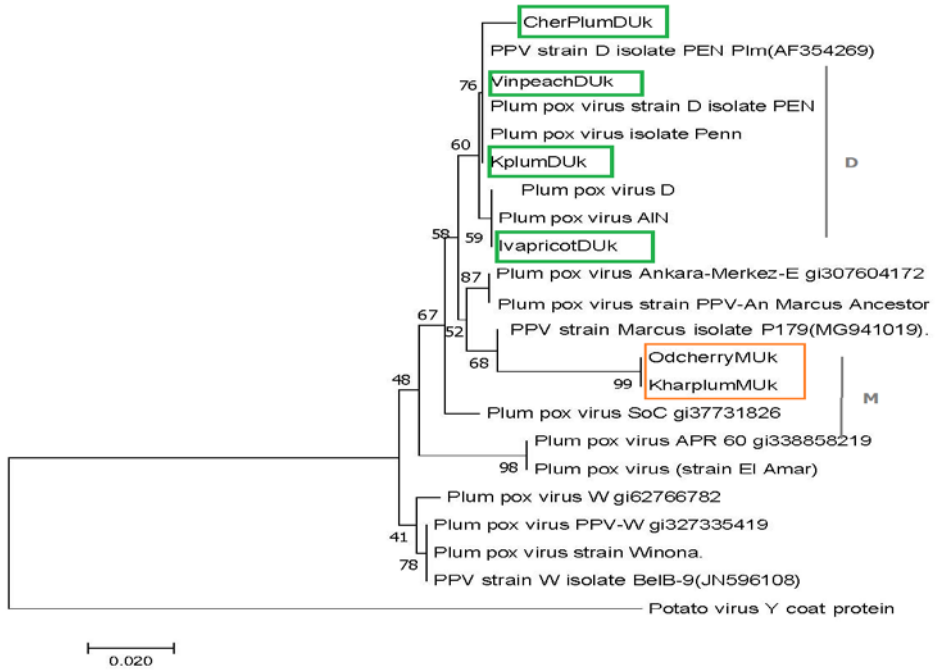

Fig. 5. Phylogenetic tree showing phylogenetic relationships among previously known strains/isolates of PPV and six Ukrainian isolates based on their partial nucleotide sequences of the coat protein gene. Hasegawa-Kishino-Yano model + Gamma distribution (HKY + G). Bootstrap values are shown next to the nodes.

To this end, the MEGA 6.0 program compared the PPV sequences obtained directly from our research and the GenBank international portal. Not only Ukrainian isolates were taken, but also isolated strains from other countries and continents. The aim was to calculate percentages of similarity (distance between sequences) of the sequences studied.

Using incomplete sequence of coat protein gene for the phylogenetic analysis of the Ukrainian isolates, we installed that PPV circulating in all regions of monitoring distribution, D strain of plum pox virus was the predominant one in all regions, whereas its M strain was rarely found and infected only in two regions. Ukrainian PPV isolates were highly 95-99% similar to previously described isolates of PPV from GenBank.

From this dendrograms, it can be argued that all Ukrainian isolates have high similarities, regardless of the region or the plants distribution within the host strains. Analyzing dendrograms we found that all samples belonged to D and M strains. In Cherkasy, Kharkiv, Ivano-Frankivsk Vinnytsia regions we found only D strain, and in Kyiv, Odessa regions, the presence M and D strains. Comparing PPV isolates from Ukraine, can be noted their high (99%) homogeneity of capsid protein nucleotide sequences, regardless of region distribution or host plant.

Table1 Comparison of Ukrainian PPV isolates by partial sequences of their CP gene, %

The name of the isolates	Odcherry MUk	Kharplum MUk	Vinpeach DUk	KplumD Uk	IVapricot DUk	Cherplum DUk
Odcherry MUk	-	98	95	96	95	97
Kharplum MUk	98	-	95	96	94	95
Vinpeach DUk	95	95	-	98	99	98
Kplum DUk	96	96	98	-	99	98
IVapricot DUk	95	94	99	99	-	99
Cherplum DUk	97	95	98	98	99	-

Table2 Comparison of Ukrainian PPV isolates with isolates from the GenBank database for nucleotide sequences of the part of CP gene, %

The name of the isolates	Plum pox virus strain D isolate PPVNJ MK208990 (Chine) Unpublished	Plum pox virus strain M isolate CY2 EF626558 (Cyprus) Unpublished	Plum pox virus PPV-D Ya1 LC375126 (Japan) (Maejia, 2020)	Plum pox virus isolate Pl45 EU734801 (Turkey) (Serçe, 2009)	Plum pox virus ElAmar DQ431465 (Turkey) (Glasa, 2006)
Odcherry MUk	97	99	96	96	97
Kharplum MUk	96	98	97	97	96
Vinpeach DUk	99	96	99	98	97
Kplum DUk	98,9	95	98,8	98	98,6
IVapricot DUk	99,5	96	99,7	98	97
Cherplum DUk	98	95	98	98	99

Analyzing the results of the research, it can be stated that the level of identity does not depend on the place of distribution and the host plant. For instance, apricot isolate of PPV collected in Ivano-Frankivsk region was most identical to Plum pox virus strain D isolate PPV NJ (Accession number MK208990) from Chine, Plum pox virus PPV-D Ya1 (Accession number LC375126) from Japan.

Analyzing the percent similarity sequences of the coat protein gene of Ukrainian isolates and isolates from GenBank, we can assume that due to

evolutionary processes, some strains may be reversed to related strains without changing their own strain.

CONCLUSIONS

In summary, Plum pox virus was detected in all investigated regions, the circulation of M and D strains. Results show high level of identity of coat protein gene sequences of Ukrainian isolates of PPV regardless of their sampling site or the host plant, region of distribution. The distribution and harmfulness of the disease increases, therefore it is important to determine genetic diversity of PPV strains, so that analyzing the development of spread of the virus in different regions of Ukraine and neighboring countries, to establish the origin and to predict the development of possible epidemics.

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