MOLECULAR IDENTIFICATION AND PHYLOGENETIC ANALYSIS OF PLUM POWDER VIRUS

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Abstract

The damage caused by plum pox virus to agriculture in pome fruit plants is one of the pressing issues, with an annual cost of $600 million worldwide. As a result of molecular genetic comparison of the UZ1 Uzbekistan isolate with other strains in the world, similarities and differences were identified with isolates from other countries of the world. Phylogenetic tree analysis showed that the MT038048.1 UZ1 isolate, distributed in the Tashkent region, is located in a close phylogenetic subcluster with the KP198594.1 isolate isolated in Russia, and in the same phylogenetic cluster with the isolates isolated in Colombia and Slovakia.

Keywords: HCV D isolate, ELISA Reagent set (Agdia), NCBI, Polyprotein-CP, molecular identification.

OLXO‘RI CHECHAGI VIRUSINING MOLEKULYAR IDENTIFIKATSIYASI VA FILOGENETIK ANALIZI

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Annotatsiya:

Olxo‘ri chechagi virusining qishloq xo‘jaligiga keltirayotgan zarari danak mevali o‘simliklarda dunyo bo‘yicha yiliga 600 mln dollarni tashkil qilayotgani dolzarb masalalardan biri bo‘lmoqda. UZ1 Uzbekistan izolyati dunyodagi boshqa shtammlari bilan molekulyar genetik jihatdan taqqoslash natijasida dunyoning boshqa mamlakatlari izolyatlariga o‘xshashlik va farqlari aniqlandi. Filogenetik shajara tahlilidan ko‘rinadiki, Toshkent viloyatida tarqalgan MT038048.1 UZ1 izolyati kelib chiqishi jihatdan Rossiyada ajratilgan KP198594.1 izolyat bilan yaqin filogenetik subklasterda joylashganligi, Kolumdiya va Slovakiyada ajratilgan izolyatlar bilan bitta filogenetik klasterda joylashganligi aniqlandi.

Kalit so‘zlar: OCHV D izolyati, ELISA Reagent set (Agdia), NCBI, Polyprotein-CP, molekulyar identifikatsiya.

Introduction

A number of phytopathogenic viruses that infect agricultural plants have been identified in the world, which negatively affect the quantity and quality of cultivated products and cause significant economic damage. As the development of plants in agriculture continues to increase, the impact of various factors, including microorganisms, on plants is also increasing. The damage caused by the plum pox virus to agriculture in pome fruit plants is one of the pressing issues, with the global annual cost of $600 million. Strain D of this virus is also spreading in the Republic of Uzbekistan.
For immunosorption of the virus particle, 100 µl of the antibody prepared from the ELISA Reagent set (Agdia) for AChV diluted 1:200 in 0.05 M carbonate-bicarbonate buffer (pH 9.6) was added to a microcentrifuge tube (Multiply-Pro cup 0.5 mL, cat. no. 72.735.002, Sarstedt, Germany) and incubated overnight at +4°C. The next day, the tube was washed three times with PBS buffer containing 0.05% Tween 20.

To prepare the plant leaf tissue extract, 50 mg of lyophilized or 100 mg of green leaf mass were taken and extracted in a porcelain mortar with 2 ml of extraction buffer (PBS, 0.2% sodium diethyldithiocarbamate (Merck, Germany), 2% polyvinylpyrrolidone (mol. m. 10 kDa) (Sigma). 100 µl of the extract centrifuged 3 times (1 minute each) using a MiniSpin centrifuge was added to the washed tubes. Incubation was carried out for 1 hour at 37□C in a table thermostat "Termit" (DNK-Technology, Russia). After the incubation, the tubes were washed three times with PBS buffer containing 0.05% Tween 20.

Based on the sequence results, the nucleotide sequence of the polyprotein (coat protein) gene of the isolate of OHV D isolate UZ1 isolated from plum plant was determined and deposited in the national database – NCBI under the name UZ1 under the number MT030848.1. The nucleotide sequence of the identified Polyprotein-CP gene of the virus was compared with the same gene of the isolates available in NCBI using the BLAST program, bioinformatic analysis was performed and their degree of kinship was determined. As a result of the analysis conducted using the BLAST program, the MT038048.1 isolate number UZ1 distributed in our country has 99.23% identity with the LT600780.1 isolate (Colombia), 99.00% with the GU461890.1 isolate (Slovakia), 98.70% with the LC375115.1 isolate (Japan), 98.31% with the MK208990.1 isolate (China), 98.31% with the MK372983.1 isolate (Turkey), 98.31% with the KP198594.1 isolate (Russia), 99.29% with the FN179152.1 isolate (Hungary), 98.23% with the AY912057.1 isolate (Canada), 98.23% with the HQ452362.1 isolate (Slovenia), 97.25% with the The similarity with isolate AM260937.1 (Bulgaria) was 88.14%, isolate HQ452359.1 (Slovenia) 87.76%, isolate AY690609.1 (Serbia) 87.59%, isolate JX013532.1 (Croatia) 87.53%. Based on the bioinformatic analyses, a phylogenetic tree of the OCHV UZ1 isolate was constructed using the MEGA11 program.

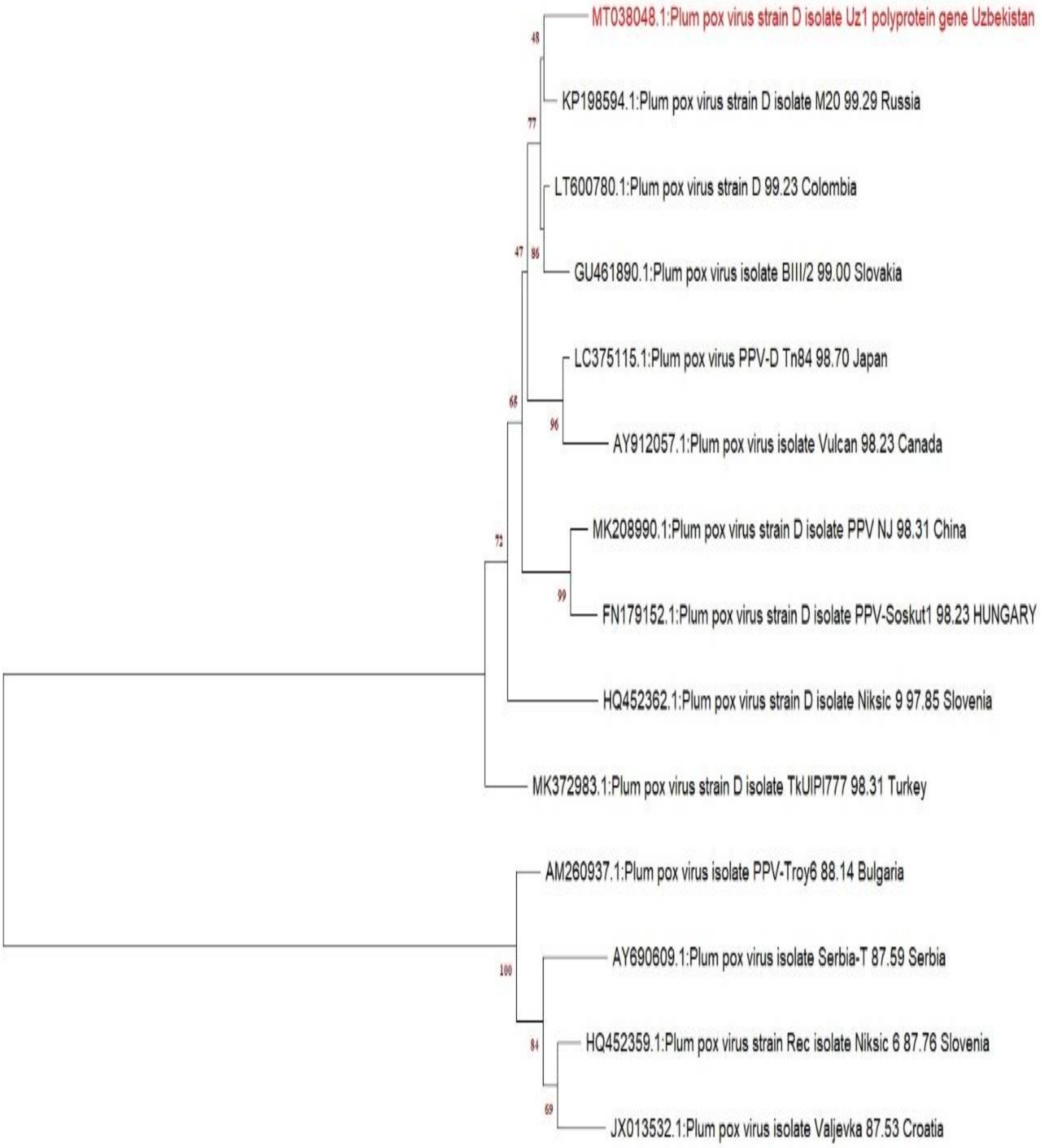

Figure 1. Phylogenetic tree of the UZ1 isolate of AChVi. Neighbor Joining statistical analysis, bootstrap replications 1000, Kimura-2 model.

As a result of molecular genetic comparison of the UZ1 Uzbekistan isolate with other strains in the world, similarities and differences with isolates from other countries of the world were revealed. Phylogenetic tree analysis showed that the MT038048.1 UZ1 isolate distributed in the Tashkent region is located in a phylogenetic subcluster close in origin to the KP198594.1 isolate isolated in Russia, and in the same phylogenetic cluster with isolates isolated in Colombia and Slovakia (Figure 1).

Thus, it can be concluded from the results of the studies that the MT038048.1 UZ1 isolate identified in our country has a 99.23% similarity with the LT600780.1 Colombian isolate in the polyprotein-cp gene nucleotide sequence, but in the phylogenetic tree it is located in the same subcluster with the KP198594.1 isolate isolated from Russia.

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