



Study of Watermelon Mosaic Virus in Cucurbit Fields of Jahrom Area, Iran

Kavous Ayazpour* and Maryam Vahidian

Abstract

Watermelon mosaic virus (WMV, Potyvirus) is one of the most important viruses and is responsible for important losses in most major crops worldwide. To study the importance of WMV in cucurbit fields of Jahrom area, an ELISA survey was done during 2013 and 2014 growth seasons. Sampling was done from cucurbits with symptoms of mosaic, deformation, blistering of leaves and fruits. Results of DAS-ELISA showed that 68 of 135 collected samples were infected with WMV. Results of DAS-ELISA verified by reverse transcriptase polymerase chain reaction (RT-PCR) method using a pair specific primer of coat protein gene of a positive sample. Comparisons of nucleotide sequences of CP gene of this isolate with those from gene bank (NCBI) showed more than 98% nucleotide sequence homology. According to the results of this study, the most infection of WMV was found in melon (78%) and then squash (40%) and watermelon (35%), respectively.

Keywords

Watermelon mosaic virus; Viral losses; Plant viral importance; Jahrom Area; Das-ELISA; RT-PCR

Introduction

About 35 viruses are associated to cucurbit disease and among them are at least 10 potyviruses [1]. Watermelon mosaic virus belongs to the genus *Potyvirus*, family *Potyviridae* [2] with mostly distribution in temperate and Mediterranean regions and with broader host range than most potyviruses. WMV causes disease in important crops such as cucurbits, pea, orchids, vanilla and *Habenaria radiat* [2]. WMV occurs in many cucurbits growing regions of Iran [3].

The genus *Potyvirus* comprises rod shape, flexuous, positive sense, single stranded RNA viruses [4]. Genome of this virus has a single open reading frame (ORF) encoding a single polyprotein with 3217 amino acids. The polyprotein is broken by protease enzymes encoded by the virus in particular spots and each small protein plays a specific role [5].

The most important symptoms of WMV include: mild to severe mosaic with blistering of the leaves, moderate to severe stunting of plants, deformation of leaves and fruits and pale color of fruits [5]. WMV transmitted by 38 aphid species in a non-persistent manner [6].

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WMV was reported from cucurbit plants of many areas of Iran but there was not any research about importance of that in Jahrom area and because several viruses on cucurbits cause mosaic this study was performed to assess the distribution and importance of watermelon mosaic virus among cucurbits with mosaic symptoms in Jahrom area.

Material and Methods

Sampling

In order to identify and detect WMV, from the beginning of June 2013 until the end of July 2014 different fields and cucumber greenhouses of Jahrom area were visited. Cucurbit plants including cucumber, watermelon, squash and melon with symptoms of mosaic, deformation, blistering of leaves and fruits collected and in a cooling chamber transferred to laboratory.

ELISA determination

To diagnose cucurbit plants infected by WMV, direct double antibody sandwich (DAS) ELISA was performed [7]. In this study a polyclonal antiserum (Bioreba) was used. Extractions were prepared from 0.5 g of shoot and leaves in 5 ml of 1 × PBST buffer (0.15 M NaCl; 0.015 M NaH₂PO₄; 0.05% Tween 20, pH 7.0). Positive reactions were defined as an OD 405 nm two times higher than negative control.

RT-PCR

Total RNA (tRNA) extraction and cDNA synthesis was done as Ayazpour et al. method [8]. PCR was performed using cDNA as a template in a total volume of 20 µl reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.05 mM each of the four dNTPs, 2 mM MgCl₂, 0.3 µM of each primer (sense primer: 5'-CGGTATTGTAATGGTTCTCCCTG-3' and antisense primer: 5'-CGGTATTGTAATGGTTCTCCCTG-3'), 1.25 U Taq DNA polymerase (iNtRON Biotechnology) and 1 to 4 µl of cDNA mixture. The PCR cycling profile consisted of one cycle at 94°C for 5 min., followed by 30 cycles of 94°C for 45 s, 54°C for 45 s, and 72°C for 1 min, with a final extension step at 72°C for 10 min. PCR amplified fragments were separated in 1% agarose gels in Trisborate (TBE) buffer (89 mM Tris; 89 mM boric acid; two mM EDTA, pH 8.3). After electrophoresis, the gels were stained in ethidium bromide and analyzed using BIO imaging system (Bio Rad). A 100 bp DNA Ladder (Fermentas) was used as a nucleic acid marker. One of the positive samples was sent for sequencing to Korea.

Results

During visiting of farms and greenhouses of Jahrom area, WMV symptoms depending on the host, plant age and time of infection were seen in all types of cucurbits. The most common symptoms observed were mild to severe mosaic, leaf deformation and blistering. Most infections were observed in various species of melon. Other symptoms can be partly darkening of the leaves, stunting of the plant and leaf chlorosis in watermelon. The most common symptoms observed in greenhouse cucumber were mosaic with blistering of leaves from mild to severe, short and deformation of fruits (Figure 1).

The results of DAS-ELISA showed that the all of sampled farms and greenhouses were WMV infected. A total of 135 samples collected

from farms and greenhouses where symptoms of WMV virus were seen (Figure 2). The highest rate of WMV infection related to melon was approximately 78% and squash and watermelon were followed respectively by 40% and 35% infection. RT-PCR reaction using a pair of specific primers of coat protein gene produced a fragment of DNA with about 1055 bp nucleotides. Nucleotide sequences of a sample determined. Comparisons of nucleotide sequences of CP gene of this isolate with those from gene bank (NCBI) showed more than 98% nucleotide sequence homology (Figure 3).

Discussion

Cucurbit products such as cucumber, watermelon, pumpkin, squash and melon have very wide cultivation in the world and generally are destroyed by Potyviruses in narrow or wide range. Most viruses that cause damage to the cucurbits have a wide host range including of many plant genera belonging to different families [9].

During Visiting of farms and greenhouses of Jahrom area, the most common symptoms were seen include mild to severe mosaic, leaf deformation and blistering of leaves and fruits. Many of the symptoms observed in the fields were similar to symptoms reported

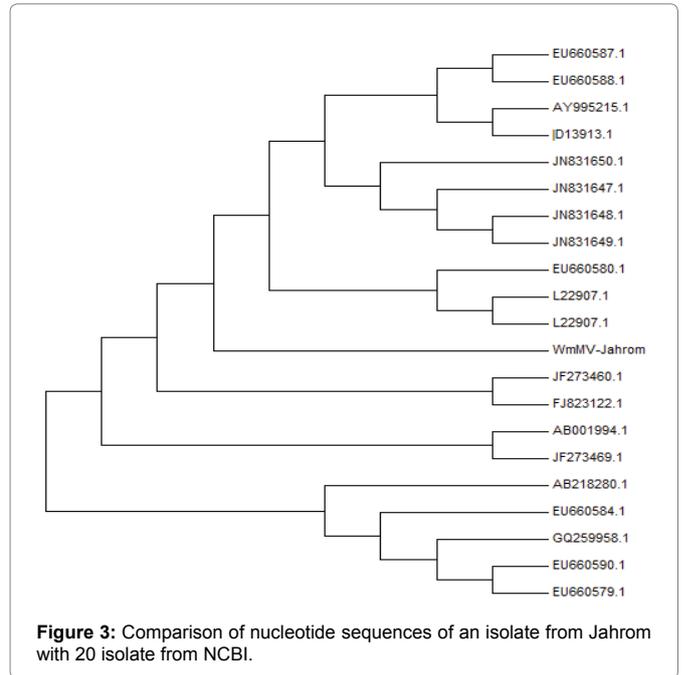


Figure 3: Comparison of nucleotide sequences of an isolate from Jahrom with 20 isolate from NCBI.



Figure 1: Symptoms of Watermelon Mosaic Virus on positive samples of cucurbits.

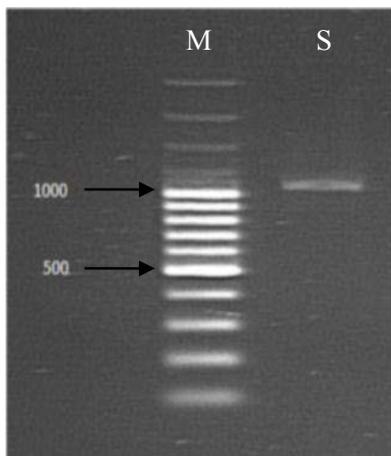


Figure 2: PCR product results in a 1% agarose gel, M: DNA marker, S: Positive sample.

by Sharifi et al. [10], Shoeibi et al. [11] and Moradi et al [5]. Other symptoms were partly darkening of the leaves, plant stunting and chlorosis of leaf in watermelon. These symptoms were the same as Hsu [12] and Lovisolo [13] reports.

The highest rate of WMV infection was observed on melon with approximately 78%. The results of the study of Shoeibi et al. [11] also confirmed these results.

Squash and watermelon were followed by 40% and 35% infection, respectively, and the lowest percentage of infection was observed on cucumbers cultivated in greenhouses. This study results confirmed with the results of Shoeibi et al. [11]. Since in greenhouse cucumbers prevent the spreading of the virus by aphids than in open fields, infection of them is very low [14,15].

After total RNA extraction and cDNA synthesis, PCR experiment was performed and produced a DNA segment with about 1055 bp length as Shoeibi et al. [16] study. The nucleotide sequences of coat protein gene of an isolate of WMV from Jahrom was compared with the 20 reported sequences of this gene in Iran and other parts of the world using Mega 6 program with Maximum Likelihood Tree method and was drawn phylogenetic tree. Results showed relationship between Jahrom isolate with isolates of Taga (Africa), Chile, America, and France, and were placed in the same group [17].

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