



ORIGINAL ARTICLE

Distribution of some viral disease on pepper (*Capsicum annuum*) plants in Dezful fields from Iran

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ABSTRACT

Pepper (*Capsicum annuum* L.) as one of the main crops in Dezful, Iran is treated by several viruses. In this survey for detection of viruses, around 500 leaf samples of sweet pepper plants from 23 fields of Dezful with symptoms including mosaic, mottle, crinkling and dark green vein banding, deformation and stunting were collected during 2012 and 2013 growing seasons. Collected samples were analyzed by DAS-ELISA using several polyclonal antibodies. Results showed 55, 23, 17, 15, 21, 18 and 13 samples were only infected with Pepper veinal mottle virus (PVMV), Pepper mild mottle virus (PMMV), Pepper mottle virus (PMV), Potato virus Y (PVY), Alfalfa mosaic virus (AMV), Cucumber mosaic virus (CMV) and Tomato spotted wilt virus (TSWV), respectively. Several samples were infected by more than one of aforementioned viruses. The incidence of infections with two was seen in 28.8%, with three viruses in 9.6% of collected samples. Multiple infection was detected in 8.6% of samples. The high incidence of PVMV revealed that this virus is the dominant constraint to cultivation of pepper crops in Dezful. To our knowledge this is the first report of pepper veinal mottle virus, Pepper mild mottle virus, Pepper mottle virus and Tomato spotted wilt virus in pepper fields of Iran.

Key words: PVMV, PMMV, PMV, PVY, AMV, CMV, TSWV and ELISA

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INTRODUCTION

Capsicum (*Capsicum annuum*) and chilli (*Capsicum frutescens*) originate from South and Central America and are members of Solanaceae family [1]. Virus diseases annually reduce yield and quality of all kind of pepper [2]. Symptoms of virus infection widely vary in expression and severity including mild mottle, mosaic, vein banding, ring spots, necrosis, leaf discoloration, deformation and blistering and severe stunting of the whole plant. Viruses could not just identified based on symptoms, because symptoms could vary with respect to the strain of the virus, the host cultivar, the age of the host, environmental conditions and co-infection with other viruses. Different viruses may cause similar symptoms, as well as insect damage, particularly by thrips and mites, may mimic virus symptoms. Certain herbicides, such as 2,4 D, and growth hormones may also cause plant reactions similar to virus symptoms [3]. To date, about 50 viruses have been reported to infect peppers [4]. The genus *Potyvirus* (family *Potyviridae*) containing about 200 species account for almost 25% of known plant viruses [5]. Species belonging to this genus can share many common properties [6]. Many potyviruses cause economically significant yield losses in pepper (*Capsicum spp.*) crops throughout the world [7, 8, 9]. *Potato virus Y* (PVY) is the most common *Potyvirus* infecting pepper [10,11]. Although this virus occurs worldwide, but it mostly appears in warmer climates [12]. In some areas, disease incidence may be as high as 100%, resulting in considerable crop loss [3]. *Pepper veinal mottle virus* (PVMV) is another member of *Potyvirus* genus which infects pepper plant and has been frequently reported in western Africa [13, 3].

Tobamoviruses including *Pepper mottle virus* (PeMV) and *pepper mild mottle virus* (PMMV) are stable and highly infectious which easily spread from plant to plant by mechanical contact. These viruses have been mentioned responsible for significant economic losses on pepper across the world [14, 2].

Pepper crop is strongly affected by *Cucumber mosaic virus* (CMV) from *Cucumovirus* genus, *Alfalfa mosaic virus* (AMV) from *Alfavirus* genus and *Tomato spotted wilt virus* (TSWV) from *Tospovirus* genus. These viruses are estimated to cause up to 50% losses in potential production of pepper varieties [15,14].

This study was undertaken to assess the occurrence and distribution of viruses in pepper fields of Dezful in south-west of Iran, which are useful to design control strategies.

MATERIALS AND METHODS

Survey

In Dezful, pepper (*Capsicum annuum* L.) is cultivated from september to december. A total of 500 samples were collected during the 2012 and 2013 growing seasons from 23 pepper fields in Dezful. Infected plants had symptoms like leaf mottle, crinkling and dark green vein banding, leaf and fruit deformation, and stunting. Young leaves of some symptomatic plants were arbitrarily collected. Plant samples were separately put in a plastic bag and were kept at 4 °C until analyzed.

Virus Identification

Plant samples were tested for presence of *Potato virus Y* (PVY), *Pepper veinal mottle potyvirus* (PVMV), *Pepper mottle potyvirus* (PMV), *Pepper mild mottle tobamovirus* (PMMV), *Cucumber mosaic virus* (CMV), *Alfalfa mosaic virus* and *Tomato spotted wilt virus* (TSWV) using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). DAS-ELISA test was performed in accordance with the Clark and Adams method 1977 [16], using PVY, PVMV, PMV, PMMV, CMV, AIMV and TSWV specific polyclonal antisera from DSMZ (Braunschweig, Germany).

For DAS-ELISA, wells of polystyrene micro plates (Greiner Bio-One, Frickenhausen, Germany) were coated with the corresponding antiserum diluted to 1000-fold in coating buffer (0.05 M sodium carbonate, pH 9.6). Samples were extracted in PBST buffer (phosphate-buffered saline, pH 7.4, containing 0.05% Tween 20), followed by addition of alkaline phosphatase (AP) conjugated IgG at 1:1,000 dilution in conjugate buffer (phosphate-buffered saline, pH 7.4, containing 0.05% Tween 20 and 0.2% egg albumin). The AP substrate tablets (Sigma-Aldrich, St. Louis, MO, USA) were prepared in the substrate buffer (9.7% di-ethanolamine, pH 9.8) to a final concentration of 1 mg ml⁻¹. Plant extracts, viruses antiserum and IgG alkaline phosphatase conjugate were incubated for approximately 2 h, and *p*-nitrophenyl phosphate substrate was incubated for 30 min at 37°C. Absorbance values were read at 405 nm using a microplate reader (BioTek ELX-808, USA). Extraction buffers were used as negative controls. Samples were considered to be positive when the absorbance values at 405 nm (A_{405}) values exceeded the mean of the negative controls (healthy plant or buffer) by least a factor of three.

RESULTS AND DISCUSSION

In this survey collected pepper samples were tested by seven virus polyclonal antibodies using DAS-ELISA. The results have been summarized in tables 1 and 2. All the mentioned viruses were detected in the samples from Dezful pepper fields.

The results revealed that single infection rates of PVMV, PMMV, PMV, PVY, AMV, CMV and TSWV were, 11%, 4.6%, 3.4%, 3%, 4.2%, 3.6% and 2.6% of collected samples respectively. The incidence of PVMV was more than other viruses. The proportion of double infections with PVMV, PMMV, PMV, PVY, AMV, CMV and TSWV were 37.8%, 7%, 6.2%, 5.4%, 8.4%, 7.2% and 6.4% respectively. PVMV+PMV was the most frequent double infection.

To sum up, in 2012 and 2013 growing seasons, almost 28.8% of symptomatic samples showed double infections as against 9.6% for multiple infections. PVMV+PMMV+ PVY, PVMV+PMMV+ PMV+PVY and PVMV+PMMV+PMV+PVY+AMV were the most frequent multiple infections.

Table 1. Number of pepper samples collected in 2012 and 2013 with single infections

Virus name	Number of infected plant
<i>Pepper veinal mottle virus</i> (PVMV)	55
<i>Pepper mild mottle virus</i> (PMMV)	23
<i>Pepper mottle virus</i> (PMV)	17
<i>Potato virus Y</i> (PVY)	15
<i>Alfalfa mosaic virus</i> (AMV)	21
<i>Cucumber mosaic virus</i> (CMV)	18
<i>Tomato spotted wilt virus</i> (TSWV)	13
Total	162

Table 2. Number of pepper samples collected in 2012 and 2013 with mixed infections

Double infection	No. infected	Triple infection	No. infected	Multiple infection	No. infected
PVMV+PMMV	15	PVMV+PMMV+TSWV	1	PVMV+PMMV+ PMV+PVY	5
PVMV+PMV	20	PVMV+PMMV+ PVY	7	PVMV+PMMV+ PVY+TSWV	3
PVMV+PVY	9	PVMV+PMMV+AMV	3	PVMV+PMMV+ PMV+CMV	1
PVMV+AMV	17	PVMV+PMV+CMV	4	PVMV+PMMV+ PMV+TSWV	2
PVMV+CMV	15	PVMV+PMV+TSWV	5	PVMV+PMMV+AMV+CMV	1
PVMV+TSWV	15	PVMV+PVY+CMV	3	PVMV+PMMV+AMV+TSWV	3
CMV+ TSWV	5	PVMV+PVY+ TSWV	1	PVMV+PMMV+CMV+TSWV	1
PMMV PVY	7	PMMV+ PMV+ PVY	5	PVMV+PMV+PVY+AMV	1
PMMV+ AMV	5	PMMV+ PMV+ TSWV	3	PVMV+PMV+PVY+TSWV	2
PMMV+ CMV	5	PMMV+ PVY+ CMV	2	PVMV+PMV+AMV+CMV	1
PMMV+ TSWV	3	PMMV+ AMV+CMV	1	PVMV+PVY+AMV+CMV	4
PMV+PVY	4	PMV+PVY+ CMV	1	PMMV+ PMV+ PVY+AMV	2
AMV+ TSWV	8	PMV+AMV+ TSWV	5	PMMV+ PMV+ AMV+CMV	2
AMV+ CMV	8	PMV+ CMV+ TSWV	2	PMMV+ PVY+ AMV+ CMV	1
PMV+ TSWV	1	PVY+ AMV+ TSWV	2	PMMV+ AMV+CMV+TSWV	2
PVY+ AMV	4	PVY+ CMV+ TSWV	1	PMV+PVY+ CMV+TSWV	3
PVY+ CMV	3	AMV+ CMV+ TSWV	2	PMV+AMV+ CMV+TSWV	1
				PVMV+PMMV+PMV+PVY+AMV	4
				PVMV+PMMV+ PVY+AMV+TSWV	1
				PMMV+ PMV+ PVY+ TSWV+AMV	2
				PMMV+ PVY+ CMV+TSWV+AMV	1
Total	144	Total	48	Total	43

Plant virus diseases cause significant losses to agricultural crops all around the world. Chemical agents like fungicides and bactericides are not effective in controlling virus diseases. Strategies for virus management in plants are mostly aimed at eradicating the source of infection. However, the most effective virus diseases management method is through cultivating the virus-resistant varieties. Generally, viral infection causes visible symptoms such as various forms of mosaic and distortions, resulting in reduction in crop growth and yield [17]. In nature, plants are commonly co-infected with multiple viruses and a number of disease syndromes are caused by interaction of two independent viruses. The accumulation dynamics of the interacting viruses in such mixed infection often change, drastically [18]. Besides, mixed infections with two unrelated viruses often produce a more severe disease than that caused by a single virus [19].

Sweet pepper (*Capsicum annuum*) is an important horticultural crop in Dezful, Iran. Viral diseases naturally impose significant production constraints, affecting yield and quality and are difficult to control [20]. About 68 viruses have been reported to infect pepper from various part of the world [9]. More than half of the pepper viruses are transmitted by aphids, some are transmitted by nematodes, thrips, leafhopper, beetle and fungi or by contact and through the soil [3, 21]. There is no data on the prevalence and infection rate of viruses in pepper growing region of Dezful. The aim of this study was to establish a base line data for which can be used to manage the diseases and reduce yield loss and increase production and quality.

In this survey we collected 500 pepper leaf samples having viral symptoms. Our results showed that PVMV, PMMV, PMV, PVY, AMV, CMV, and TSWV was found in 37.8, 20.6, 18.6, 17.6, 18.4, 17.6 and 16.4 percent of samples. Therefore, PVMV is the most widespread pepper virus in Dezful. Up to know CMV, Tomato mosaic virus, Tomato yellow fruit ring virus, PVY and Alfalfa mosaic virus have been reported from pepper fields of Iran. To our knowledge this is the first report of *pepper vein mottle virus*, *Pepper mild mottle virus*, *Pepper mottle virus* and *Tomato spotted wilt virus* in *C. annuum* fields from Iran [22].

The high prevalence of viruses in pepper might be due to a number of factors such as climatic condition, alternate host, vector activity and also the fact that pepper (*Capsicum annuum*) is the primary host of PVMV [23]. The high incidence of viruses on pepper could be the result of numerous alternative host species surrounding the pepper field such as tomato, potato and cucurbits.

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