Molecular Detection of Cucumber Mosaic Virus in Bell Pepper (*Capsicum annuum* L.)

Tanvi¹, Anil Handa¹* and Shelly Kapoor²

¹Department of Biotechnology, ²Department of Plant Pathology, Dr YS Parmar University of Horticulture and Forestry Nauni-173230, Solan, Himachal Pradesh, India

*Corresponding author

Abstract

Extensive surveys were conducted to determine the incidence and distribution of Cucumber mosaic virus (CMV) infecting bell pepper (*Capsicum annuum* L.) between 2017-2019 indifferent capsicum growing localities of Solan district in Himachal Pradesh. Symptoms on infected plants included mottling, mosaic, mid vein distortion, chlorotic spots, leaf deformation, puckering and stunting with disease incidence ranging between 9.3 to 71.3 percent. The identity of the causal virus was established on the basis of symptomatology and molecular assays. Primers designed against coat protein (CP) gene resulted in obtaining a desired amplicon of ~162 bp confirming the isolate to be of Cucumber mosaic virus.

Keywords

Bell pepper, Cucumber mosaic virus, incidence and RT-PCR

Introduction

*Capsicum* (*Capsicum annuum* L.), a member of *Solanaceae* family, is an important vegetable and spice crop grown throughout the world. Owing to its high nutritive value and multiple uses in food industry it has emerged as an important cash crop for farmers in major capsicum growing countries like China, India, Korea, Nigeria, Malaysia, Indonesia and Mexico. In India, it is cultivated over an area of 34,000 ha with an annual production of 4.87 lakh MT (Anonymous, 2018). The major capsicum producing states in India are Andhra Pradesh, Uttarakhand, Himachal Pradesh, West Bengal, Karnataka, Maharashtra, Tamil Nadu and Uttar Pradesh. In Himachal Pradesh, capsicum is the third most important vegetable crop grown after pea and tomato and occupies an area of 2.59 thousand ha with an annual production and productivity of 59.52 thousand MT and 23.10 MT/ha, respectively (Anonymous, 2018).

Capsicum has immense export potential in food processing sector, the crop is however a victim of several biotic and abiotic stresses that affect its productivity and is susceptible to a number of bacterial, fungal and viral
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Pathogens causing considerable economic losses. Among these, viral diseases attract attention since they result in considerable production constraints as they are difficult to control and also affect yield and quality of the crop (Nono-womdin, 2001). Around sixty eight viruses are known to infect capsicum naturally (Pernezny et al., 2003) and cucumber mosaic virus is most devastating among these as it has a wide host range including cucurbits, Leguminous and ornamental plants besides Solanaceous crops and is readily transmitted in a non-persistent manner by more than 75 species of aphids (Zitikaite and Staniulis, 2006; Berniak et al., 2009). Capsicum is grown on large scale in Solan district of Himachal Pradesh and routine surveys conducted in different capsicum growing localities revealed typical symptoms of viral etiology. Symptoms expressed on infected capsicum plants were mottling, mosaic, chlorotic spots, mid vein distortion, leaf deformation, vein banding, puckering and stunting of plants. Keeping in view the widespread occurrence and severity of symptoms, the investigations were conducted to establish the exact identity of the causal virus based on RT-PCR molecular assays.

Materials and Methods

Surveys for the presence of CMV

Surveys were conducted in major capsicum growing areas of Solan district of Himachal Pradesh during active cropping seasons of 2017, 2018 and 2019 to record the occurrence and distribution of viral diseases on the basis of visual symptoms. Percent disease incidence was calculated by choosing random locations in the fields and counting number of healthy and diseased plants after recording observations on symptoms as per the formula given below (McKinney, 1923):

\[
\text{Disease incidence (\%)} = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \times 100
\]

Molecular characterization

Total RNA was extracted from leaves of symptomatic bell pepper plants suspected to be infected with cucumber mosaic virus by employing Real Genomics Total RNA Extraction Kit (Real Biotech Corporation, Taiwan). After confirming the presence of total RNA on agarose gel, cDNA synthesis was performed by using Thermo Scientific / Fermentas first strand cDNA synthesis kit protocol using random hexamers. PCR assay was carried out for the amplification of cDNA strand according to the protocol of Sambrook and Russel (2001). CP gene primer pair CMV 1F (GGCTGCAGTGGTCTCCTT) and CMV 1R (GAGTCGAGTCATGGACAAATC) was used to amplify coat protein gene (Sharma et al., 2016) and a desired amplicon obtained was run on 1.0percent agarose gel.

The reaction components and conditions of PCR were standardized and presented in Table 1 and 2. Size of bands on the gel was determined by simultaneously running 100 bp marker ladder (Real Biotech Corporation, Taiwan) with the PCR product. The electrophoresed gel was then analysed under Gel Documentation System (Gel Doc XR+, BIO-RAD, USA).

Results and Discussion

Survey and symptomatology

Incidence of CMV was recorded to unravel the occurrence and distribution of CMV infecting capsicum in Solan district. Naturally infected pepper plants under field conditions exhibited mottling, mosaic, mid vein distortion, leaf deformation, vein banding and puckering symptoms (Figure 1). Such type of symptoms have been reported to be associated
with cucumber mosaic virus infecting capsicum plants by a number of workers (Benchaim et al., 2001; Bhadramurthy et al., 2009; Iqbal et al., 2011; Rahman et al., 2016; Kapoor et al., 2018; Gunes and Gumus, 2019).

Virus incidence on capsicum plants in different localities surveyed in Solan district revealed significant variation in the intensity of occurrence. The incidence of CMV ranged from 9.3 to 71.3 during the period of survey (Table 3).

Maximum disease incidence was recorded at Salogra (71.3 percent) followed by Amber (62.8 percent) whereas minimum incidence of 9.3 percent was recorded at Experimental farm of the Department of Soil Science, YSP UHF Nauni.

**Molecular characterization**

Thirty virus isolates which tested positive in DAS-ELISA for cucumber mosaic virus were subjected to RT-PCR based molecular assays. Total RNA extracted was examined on 1.0 percent agarose gel (Figure 2) and cDNA was later synthesized from total RNA. The amplification of desired amplicon ~162 bp of CMV-CP gene was obtained at annealing temperature of 55°C for 40 seconds with 35 cycles. The PCR product was examined by electrophoresis on 1.0 percent agarose gel and compared with 100 bp marker ladder on lane M (Figure 3). The results were in conjunction with the findings of many workers on the use of RT-PCR for the detection of CMV in bell pepper (Eiras et al., 2004; Khan et al., 2006; Zitikaite and Samuitiene, 2009; Kapoor, 2012; Azizan et al., 2017; Ramesh and Sreenivasulu, 2018).

**Table 1** Components of PCR reaction mixture for amplification of cucumber mosaic virus (CMV) coat protein gene

| Components (stock concentration) | Reaction volume | Final concentration |
|----------------------------------|-----------------|---------------------|
| cDNA                             | 2.0 µl          | 2.0 µl              |
| Forward Primer (CMV-1F)          | 1.0 µl          | 10 mM               |
| Reverse Primer (CMV-1R)          | 1.0 µl          | 10 mM               |
| 10X Reaction Buffer              | 2.5 µl          | 1X                  |
| dNTP mix (10mM)                  | 2.5 µl          | 2.5 mM              |
| Taq DNA polymerase (5U/µl)       | 0.2 µl          | 1U/ µl              |
| Nuclease free water              | 10.8 µl         | -                   |
| Total                            | 20.0 µl         |                     |

**Table 2** PCR cycle set up for amplification of cucumber mosaic virus (CMV) coat protein gene

| Steps                | Temperature (°C) | Duration minutes) | No. of cycles |
|----------------------|------------------|--------------------|---------------|
| Initial Denaturation | 94               | 4:00               |               |
| Denaturation         | 94               | 0:15               |               |
| Annealing            | 55               | 0:40               | 35            |
| Extension            | 72               | 1:00               |               |
| Final Extension      | 72               | 5:00               |               |
### Table 3 Incidence of virus diseases on capsicum at different locations of Solan

| S.No. | Locations                        | Incidence (%) | 2017 | 2018 | 2019 |
|-------|----------------------------------|---------------|------|------|------|
| 1.    | Wakna                            | 29.8          | 31.8 | 30.1 |
| 2.    | Kot                              | 35.0          | 33.1 | 32.9 |
| 3.    | Kiari                            | 30.2          | 29.5 | 36.2 |
| 4.    | Chail                            | 49.4          | 50.2 | 48.1 |
| 5.    | Salogra                          | 69.7          | 71.3 | 70.0 |
| 6.    | Guthan                           | 13.4          | 15.9 | 15.7 |
| 7.    | Panrot                           | 41.4          | 38.3 | 40.0 |
| 8.    | Shamber                          | 39.0          | 42.0 | 41.8 |
| 9.    | Dhar                             | 25.9          | 30.1 | 28.4 |
| 10.   | Jaunaji                          | 40.2          | 34.9 | 37.8 |
| 11.   | Ashwinikhadd                     | 44.9          | 41.5 | 42.6 |
| 12.   | Saproon                          | 10.5          | 13.4 | 16.0 |
| 13.   | Lavighat                         | 47.0          | 39.6 | 46.8 |
| 14.   | Kaylar                           | 35.2          | 29.4 | 33.3 |
| 15.   | Deothi                           | 40.2          | 41.9 | 37.0 |
| 16.   | Subathu                          | 30.1          | 26.8 | 26.7 |
| 17.   | Kanda                            | 17.4          | 19.9 | 19.6 |
| 18.   | Basal                            | 32.8          | 36.5 | 35.0 |
| 19.   | Kariyali                         | 24.8          | 25.1 | 23.9 |
| 20.   | Amber                            | 62.8          | 61.3 | 62.0 |
| 21.   | Kot                              | 58.9          | 60.1 | 60.2 |
| 22.   | Dharja                           | 48.4          | 39.6 | 46.6 |
| 23.   | Shamrod                          | 52.8          | 49.2 | 51.2 |
| 24.   | Oachghat                         | 31.1          | 33.9 | 31.3 |
| 25.   | Gadhog                           | 39.6          | 44.8 | 47.1 |
| 26.   | Galyan                           | 33.5          | 32.4 | 32.9 |
| 27.   | Molo                             | 25.8          | 29.5 | 27.6 |
| 28.   | Kot                              | 21.0          | 20.5 | 19.0 |
| 29.   | Jatoli                           | 40.6          | 42.1 | 39.1 |
| 30.   | Tatool                           | 15.6          | 11.9 | 12.2 |
| 31.   | Bhajol                           | 11.8          | 10.6 | 10.8 |
| 32.   | Sultanpur                        | 27.3          | 28.6 | 29.8 |
| 33.   | Diggal                           | 31.9          | 29.5 | 34.5 |
| 34.   | Ramshehar                        | 21.3          | 22.7 | 21.1 |
| 35.   | Manpura                          | 26.5          | 28.5 | 32.9 |
| 36.   | Sandholi                         | 10.8          | 11.9 | 11.7 |
| 37.   | Nanganji                         | 55.9          | 53.4 | 53.1 |
| 38.   | Experimental Farm of department of Vegetable Science, Nauni | 29.1 | 34.1 | 33.8 |
| 39.   | Experimental Farm of department of Seed Science and Technology, Nauni | 29.6 | 28.7 | 27.9 |
| 40.   | Experimental Farm of department of Soil Science, Nauni | 11.5 | 10.5 | 9.3 |
**Fig.1** Symptoms on infected capsicum plants a) Mottling and puckering b) mid vein distortion c) Mosaic d) Diffused chlorotic spots

**Fig.2** RNA bands fractionated on 1.0 agarose gel

**Fig.3** PCR product on 1.0 percent agarose gel
In conclusion the cucumber mosaic virus is fast emerging as a potential threat to the Solanaceous vegetable crops in Himachal Pradesh and the present investigations have identified the virus on the basis of RT-PCR molecular assays. The studies have opened a channel for conducting future studies on cucumber mosaic virus with the objective of understanding the dynamics of this devastating virus in Solanaceous crops.

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