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

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A B S T R A C T

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.

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

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 1.0percentagarose 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 (Ben chaim *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 to71.3 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 1.0percentagarose 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	35
Annealing	55	0:40	
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.	Koti	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.	Koti	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.	Naganji	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

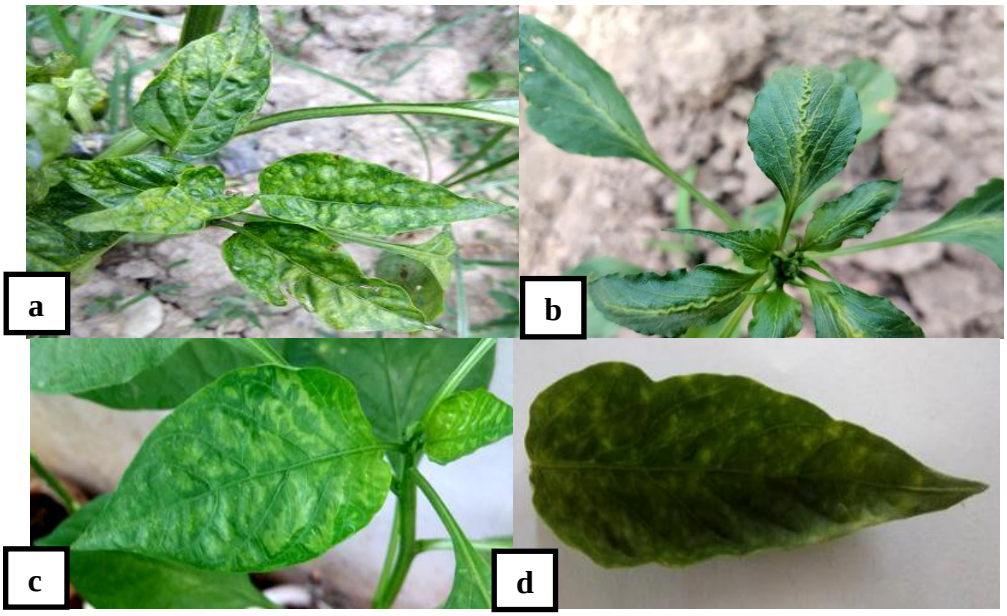


Fig.2 RNA bands fractionated on 1.0 agarose gel

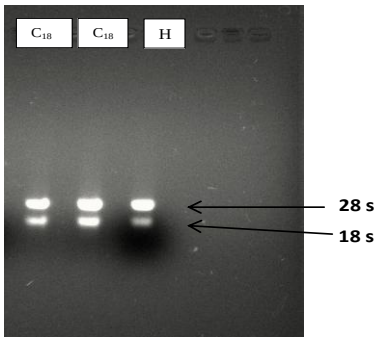
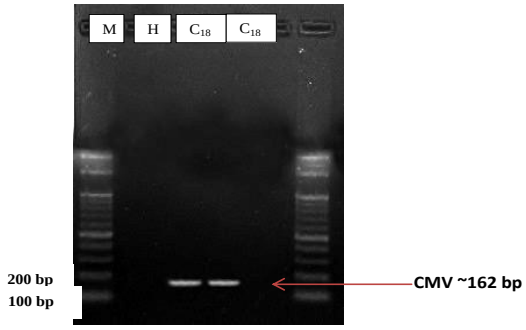


Fig.3 PCR product on 1.0 percent agarose gel



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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