Int.J.Curr.Microbiol.App.Sci (2017) *6*(11): 2947-2965

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 11 (2017) pp. 2947-2965**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.611.346

Occurrence of *Cucumber mosaic virus* on Potato and its Transmission to Muskmelon under Potato-Cucurbit Cropping Pattern Followed in Punjab, India

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Keywords

CMV, Potato, Muskmelon, RT-PCR

Article Info

Accepted:
24 September 2017
Available Online:
10 November 2017

A B S T R A C T

Survey was conducted over six major potato growing districts of Punjab during November to December, 2015 to investigate the occurrence of *Cucumber mosaic virus* on potato. Leaf samples of total 95 potato plants showing various viral diseases like symptoms were collected and exposed against antisera of 10 viruses in DAS/TAS-Enzyme Linked Immunosorbent Assay (ELISA).Ten potato samples (10.53 %) showed positive reactions with antisera of CMV in ELISA. The prevalence of CMV sub group I was more (8.42 %) as compared to CMV sub group II (6.32 %) over potato crop in Punjab. Furthermore, successful amplification of approximately 540 bp region of CMV coat protein by RT-PCR confirmed the presence of CMV in the potato samples collected. Variable symptoms including mosaic, yellowing, mild blistering, wavy leaf margin, malformation of leaves, curling, stunting and reduced leaf size were induced by CMV infection on potato. CMV was mechanically transmitted (60%) from infected potato to muskmelon. The aphids *viz. Myzus persicae* and *Aphis gossypii* transmitted CMV from infected potato to muskmelon with an efficiency of 40 per cent under controlled conditions. The aphid population recorded highest in potato (245 aphids/ 100 compound leaves) and in muskmelon (75 aphids/10 vines) during the second fortnight of February. In Punjab, the commonly followed cropping pattern is cultivation of potato from October to February followed by the cultivation of cucurbits from February to May. Hence, the aphids are acquiring *Cucumber mosaic virus* from infected potato plants and transmitting the virus into healthy muskmelon plants mainly during the months of February and March.

Introduction

Potato belongs to the Solanaceae family whose other members include tomato, eggplant and pepper. Potato is a Rabi crop in India and Punjab is the leading producer of both quality seed potato and table potato in India. The state mainly grows potato varieties viz. Kufri Pukhraj, Kufri Chandramukhi, Kufri Jyoti, Kufri Bahar, Kufri Sandhuri, Kufri Badshah, Kufri Chipsona 1 and Lady Rosetta (LR). *Cucumber mosaic virus* (CMV) belongs to the genus *Cucumovirus* in the family of *Bromoviridae*. CMV is a multipartite virus consisting of three genomic single-stranded RNAs and a sub-genomic RNA 4. Each genomic RNA is encapsidated individually in a 28 nm diameter icosahedral particle. The virus is having the largest host range among the plant viruses as it infects over 1200 plant species belonging to 100 plant families (Zitter and Murphy, 2009).

Incidence of CMV has been reported over potato in Europe (MacArthur 1958; Bode 1975, Agur 1975; Chrzanowska *et al.,* 2003), Japan (Matsunami *et al.,* 1972, Kano *et al.,* 1985, Sato *et al.,* 2001), India (Sanger and Agrawal, 1986), Central California (Somerville *et al.,* 1987), Saudi Arabia (Al-Shahwan *et al.,* 1997), Egypt and India (Jeffries, 1998) and from Syria (Ali *et al.,* 2012). Occurrence of CMV in potato seems to be rare (MacArthur 1958; Agur 1975; Bode 1975) and has no economic importance in production of potato (Jeffries, 1998). Celebi *et al.,* (2003) reported that most of the potato cultivars confers natural resistance to systemic infection of CMV at 24°C but becomes susceptible to systemic infection with increase in temperature to 30°C. Zitter and Murphy (2009) reported that more than 80 species including *M. persicae* and *A.gossypii* were capable of transmitting CMV in a non-persistent, stylet-borne manner. Thus in the present scenario of global warming, CMV can arise as a major pathogen of potato in future. Hence taking into consideration the importance of CMV infection on potato, we conducted the study to investigate the role of potato and aphids in survival and spread of CMV to cucurbits, under potato-cucurbit cropping pattern followed in Punjab.

Materials and Methods

Survey and collection of potato samples

Leaves of 50 muskmelon plants showing deviation from healthy leaves (blistering, curling, mosaic and malformation) were collected during February to March 2015 from Patiala, Barnala, Ludhiana, Kapurthala, Jalandhar and Hoshiarpur districts of Punjab. The samples were preserved at -20° C in deep freezer for further use.

Leaf samples of 95 potato plants showing various viral disease like symptoms were collected from potato growing fields of Patiala, Barnala, Ludhiana, Kapurthala, Jalandhar and Hoshiarpur districts of Punjab during October to December, 2015. Symptoms shown by the potato samples were observed and noted down. The leaf samples were preserved at -80° C for further use.

Serological test (DAS/TAS-ELISA)

The collected muskmelon leaf samples were exposed to antisera of CMV sub group I and CMV sub group II in TAS-ELISA. Potato leaf samples collected were exposed to antisera of CMV sub group I, CMV sub group II, $PVY^{o/c}$, PVY^{n}, PVX, PVA, PVM, PVS and PAMV in DAS/TAS-ELISA. The procedure for DAS/TAS-ELISA was followed as per Clark and Adam (1977). Commercial DAS/TAS-ELISA kits provided by Agdia were used according to the protocol of manufacturers. Leaf samples were grounded in extraction buffer of pH 7.4 in a ratio of 1:8 using pestle mortar. Healthy potato leaves provided commercially by Agdia was used as negative control. Readings of ELISA plates were taken by ELISA reader at OD of 405nm.

The degree of absorbance or extent of positiveness shown by the positive samples against respective antisera in ELISA was derived by dividing the absorbance value of the positive samples with the absorbance value of negative control used in respective plates.

$$\text{Degree of absorbance} = \frac{\text{Absorbance value of positive samples}}{\text{Absorbance value of negative control}}$$

Total RNA isolation and reverse transcription polymerase chain reaction (RT-PCR)

The presence of CMV in potato was further confirmed with RT-PCR. Total RNA was

extracted from the potato samples found positive with CMV infection in TAS-ELISA with the help of RNAeasy Mini (Qiagen). First strand cDNA was synthesised using Revert aid first strand cDNA synthesis kit of Thermo Scientific by setting up a reaction mixture of 10 µl consisting of 5 µl RNA, 0.5µl of M-MuLV Reverse Transcriptase (200u/µl), 1 µl of 10 X RT, 0.5µl of CMV PR (CMV coat protein gene specific reverse primer), 0.5µl ribolock, 1µl of 10 mM dNTPs mix and 1.5µl of double distilled sterile water. RT-PCR was conducted with the help of *Cucumber mosaic virus* coat protein gene specific primers CMV PF (5′-GCGCGAAACAAGCTTCTTATC-3′) and CMV PR (5′-GTAGACATCTGTGACGCG-3′) synthesized by De Blas *et al.,* (1994). A total of 25 µl RT-PCR reaction was set up which contained 2µl of cDNA, 5 µl of 5X PCR buffer, 1 µl each of 20 pm/ml forward and reverse primer, 1.5µl of 25 mM $MgCl_2$, 0.5µl of 10 mMdNTPs mix, 0.3 µl of 1 U Taq polymerase and 13.7 µl of double distilled sterile water. The amplification reaction was carried out in a thermocycler (Appendorf) with the following parameters: initial denaturation at 94°C for 3 min; 35 cycles consisting 3 sec of denaturation at 94°C, 1 min of primer annealing at 53°C, 3 min of extension at 72°C; and a final extension for 3 min at 72°C. The RT-PCR products were analysed by electrophoresis in 1 per cent agarose gel prepared in 1X TAE buffer.

Transmission of CMV from potato to cucurbits

CMV infected potato leaves were grinded in phosphate buffer of different molar concentration and the sap was used for mechanical inoculation on cotyledonary leaves of CMV susceptible muskmelon variety SM 2012-13. Similarly**,** muskmelon leaves found to be infected with CMV were grinded using phosphate buffer and the sap was used to inoculate six leaves staged healthy potato plants. Mechanical inoculation was carried on according to Celebi *et al.,* (2003). Leaves from both healthy and symptomatic plants were collected and exposed to antisera of CMV sub group I and CMV sub group II in TAS-ELISA for detection of virus.

Alate and apterous aphids were collected from potato and preserved in 70 per cent ethanol for identification according to aphid identification key of Blackman and Eastop (2006). The aphids were reared on non CMV host *viz.* cotton, radish and cabbage. Apterous aphids were used for insect transmission when they attained sufficient population. Camel hair brush was used for collecting non-viruliferous aphids from the host plants and were given a starvation period of 15 minutes in petriplate under dark conditions. Starved aphids were transferred on to petriplate containing CMV infected potato leaves (PK-3 and PH-2) by using camel hair brush for acquisition. Acquisition period of 10 minutes were provided to these pre-fasted non-viruliferous aphids. After the acquisition period, the viruliferous aphids were transferred on to 15 healthy muskmelon seedlings of the variety SM 2012-13 for 24 hours.

Groups of five aphids were allowed to settle per muskmelon seedling. The next day the seedlings were sprayed with Confidor (Imidacloprid 200 SL) and kept under insect proof cages. Symptoms were observed after inoculating the muskmelon seedlings with viruliferious aphids. Infected and healthy plants were exposed to TAS-ELISA for confirmation of virus transmission from infected potato to muskmelon. Transmission results were re-confirmed by conducting RT-PCR with RNA extracted from TAS-ELISA confirmed CMV infected muskmelon samples.

The transmission efficiency according to El-borollosy (2015) was calculated by using the formula:

$$\text{Transmission efficiency} = \frac{\text{Number of infected plants}}{\text{Number of plants inoculated by aphids}} \times 100$$

Aphid population dynamics was observed on potato and muskmelon during the periods of October 2015 to March 2016 in relation to weather conditions including mean temperature, rainfall and wind velocity. In potato, aphids were counted per hundred compound leaves while in case of muskmelon aphids were counted per ten vines. Counting of winged aphids was conducted by installing yellow sticky traps in muskmelon fields.

Results and Discussion

Serological detection

Eight muskmelon samples (ML-4, ML-6, MH-1, MH-5, MK-1, MK-2, MK-4 and MK-6) were found to be infected with CMV sub group I. Symptoms observed included variable degree of mosaic, and blistering (figure 1). Thus the occurrence of CMV was found to be 16 per cent on muskmelon in Punjab. No samples were found to be infected with CMV sub group II. Incidence of CMV in muskmelon was found to be highest in Kapurthala (40%) followed by Hoshairpur (20%) and Ludhiana (20%) as shown in the figure 3. Table 2 shows the muskmelon samples with symptoms for which CMV sub group I infection was found. Figure 2 depicts the degree of absorbance of the CMV sub group I positive samples.

Collected leaf samples of 95 potato plants were exposed to DAS/TAS-ELISA with antisera of CMV sub group I, CMV sub group II, $PVY^{o/c}$, PVY^{n}, PVX, PVA, PVM, PVS and PAMV. 10 potato samples (PK-3, PK-6, PK-7, PH-2, PH-5, PH-7, PJ-4, PJ-15, PJ-16 and PP-1) were found to be positive with CMV infection and thus the incidence of CMV on potato was 10.53 per cent (Table 3). Occurrence of CMV sub group I was more (8.42 %) as compared to CMV sub group II (6.32 %) over potato crop in Punjab. Among the ten CMV positive potato samples, four samples (PK-3, PK-7, PH-2 and PH-7) were positive for both CMV sub group I and CMV sub group II, four samples (PJ-4, PJ-15, PJ-16 and PH5) were CMV sub group I positive and two samples (PK-6 and PP-1) were CMV sub group II positive. The incidence of CMV was found on Kufri Pukhraj, Kufri Badshah and LR varieties of potato grown in Punjab.

Symptom variability

The potato samples PP 1, PJ-15 and PJ-16 were only CMV positive and exhibited variable symptoms which included mosaic, yellowing, mild blistering and wavy leaf margins. Mixed infections were observed in the potato samples PK-3, PK-6, PK-7, PH-2, PH-5, PH-7 and PJ-4 where CMV was associated with other potato viruses. Severe symptoms like malformation of leaves, blistering, stunting and reduced leaf size of potato were observed when CMV was present in potato in association with other potato viruses like PVX, PVY^{n}, $PVY^{o/c}$, PVA, PAMV and PVM. CMV infection in potato occurred mostly in association with PVX (60 %), PVY^{n} (60 %) followed by PVA (40 %), $PVY^{o/c}$(30%) and PVM (30%). Rarely it was associated with PAMV (10 %).

RT-PCR

Total RNA was extracted from the potato samples PH-2, PK-3, PK-6, PK-7andPP-1 which showed strong positive reactions with antisera of both CMV sub group I and CMV sub group II in ELISA. RT-PCR was conducted using the synthesized cDNA as

template and CMV coat protein gene specific primers designed by De Blas *et al.*, (1994). The RT-PCR products were separated on 1% agarose gel along with 100bp DNA marker. Presence of CMV in the potato samples (PK-3 and PH-2) was confirmed with observation of desired amplicon of approximately 540 bp in 1 per cent agarose gel electrophoresis (Figure 6).

Mechanical transmission of CMV from muskmelon to potato

Upon mechanically inoculating healthy muskmelon seedlings with CMV infected potato leaves (PH-2 & PK-3) various types of symptoms *viz*. severe mosaic, mosaic, mild mosaic, marginal chlorosis, curling and mild blistering were produced on muskmelon (figure 8).

Among 10 test muskmelon plants, 6 were found to be positively infected with CMV sub group I in TAS/ELISA (Table 4). Maximum transmission was observed using 0.05 M phosphate buffer. Hence, 60 per cent of muskmelon was found to be successfully infected by CMV after mechanical inoculation with CMV infected potato leaves.

The samples Mi-MMC-1, Mi-MMC-4, Mi-MMC-5, Mi-MMC-6, Mi-MMC-7 and Mi-MMC-9 which were found to be successfully infected with CMV sub group I were grinded in 0.05 M chilled phosphate buffer and the sap was used to inoculate two weeks old healthy potato plants of variety Kufri Pukhraj.

Local symptoms like mild chlorosis and malformation of leaves started appearing on leaves by the end of 4^{th} week. Among the three potato plants inoculated with CMV infected sap, two of them i.e. Mi-PC-2 and Mi-PC-3 gave positive reactions with antisera of both sub group I and sub group II in TAS-ELISA.

Identification of aphids

The microscopic examination of aphid sample A revealed dark blackish green body colour, weakly developed antennal tubercles, tongue shaped cauda and the cornicle was twice the length of cauda. On the basis of above mentioned key feature, species was identified as cotton aphid *A. gossypii*.

The microscopic examination of sample B revealed pale green body colour, convergent antennal tubercles, cornicle were much longer than cauda and swollen only on apical half. On the basis of these features the sample was identified as *M. persicae*.

Serological detection to estimate efficiency of aphid transmission

Symptoms like mosaic, yellowing, mild blistering, reduced leaf size and malformation started appearing on muskmelon 4 weeks post inoculation with aphids. Successful transfer of CMV from the CMV infected potato samples PK-3 and PH-2 was mediated by aphids into 6 muskmelon samples AT-MC-1, AT-MC -2, AT-MC -3, AT-MC -5, AT-MC -7 and AT-MC-12 among 15 muskmelon seedlings exposed to inoculation by the viruliferious aphids.

Three samples, AT-MC -1, AT-MC -2 and AT-MC-3 were found to be infected with both CMV sub group I and CMV sub group II.

RT-PCR re-confirming transmission of CMV from potato to muskmelon by aphids

Transmission of CMV from infected potato to muskmelon was reconfirmed by RT-PCR. Total RNA was extracted from two symptomatic leaf samples of muskmelon AT-MC-3 and AT-MC-3 which showed strong positive reactions with antisera of both CMV sub group I and CMV sub group II in ELISA.

Fig.1 Variability of symptoms produced by CMV on muskmelon (A: Mosaic and severe blistering, B: Mild mosaic, C: Blistering, D: Blistering and mild mosaic, E: Mosaic, F: Mosaic and blistering)

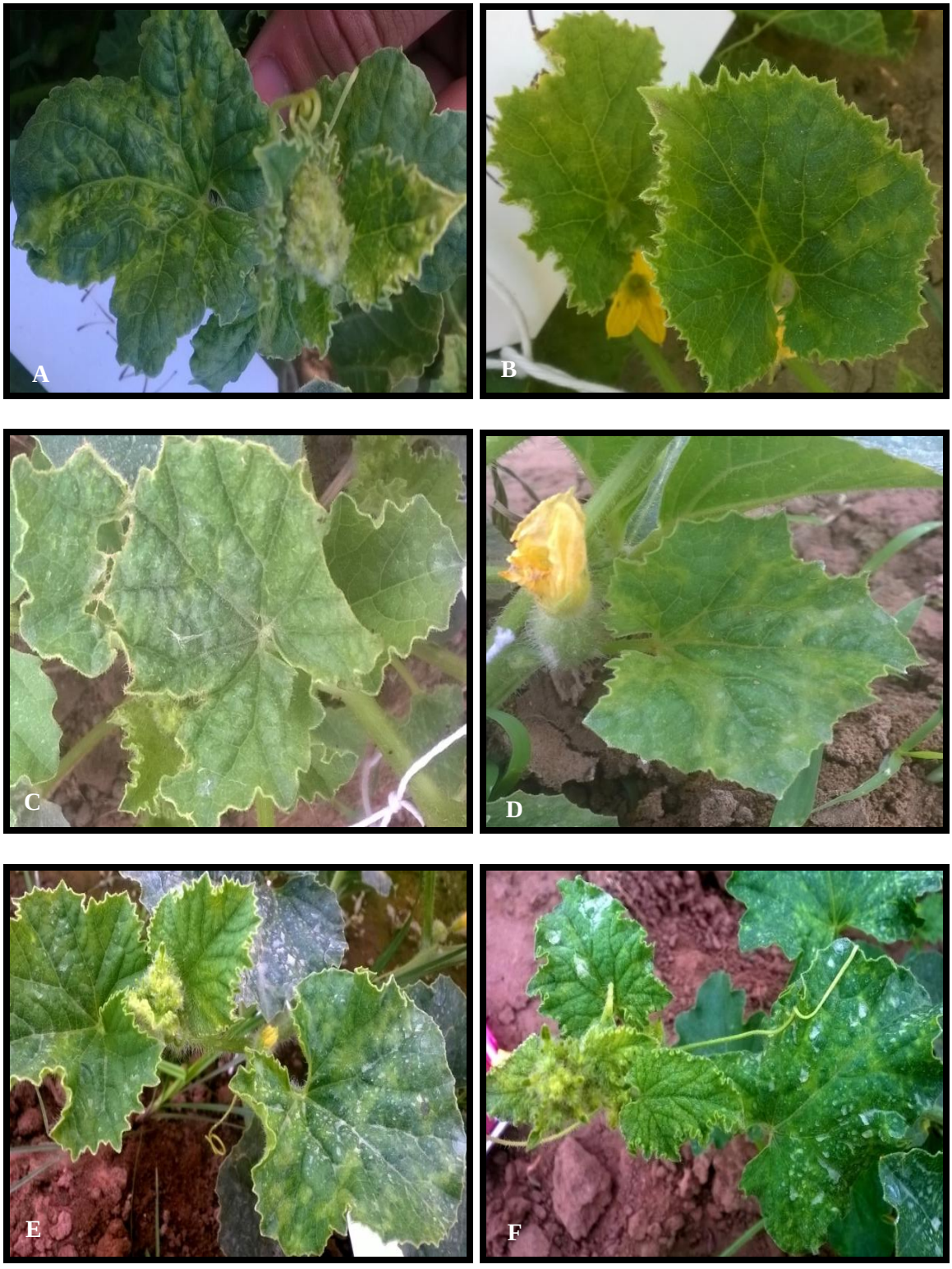


Fig.2 Degree of absorbance of CMV positive muskmelon samples

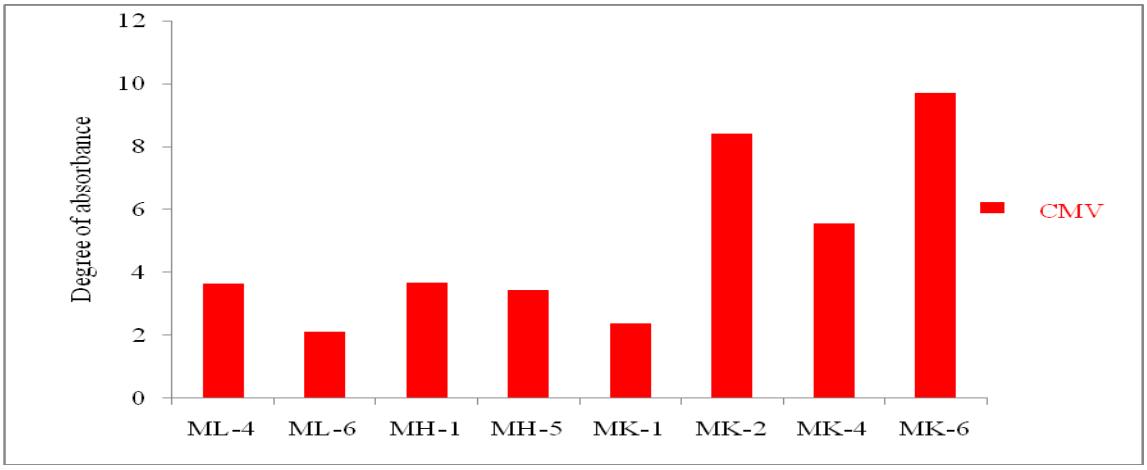


Fig.3 Per cent viral incidence on potato in different districts

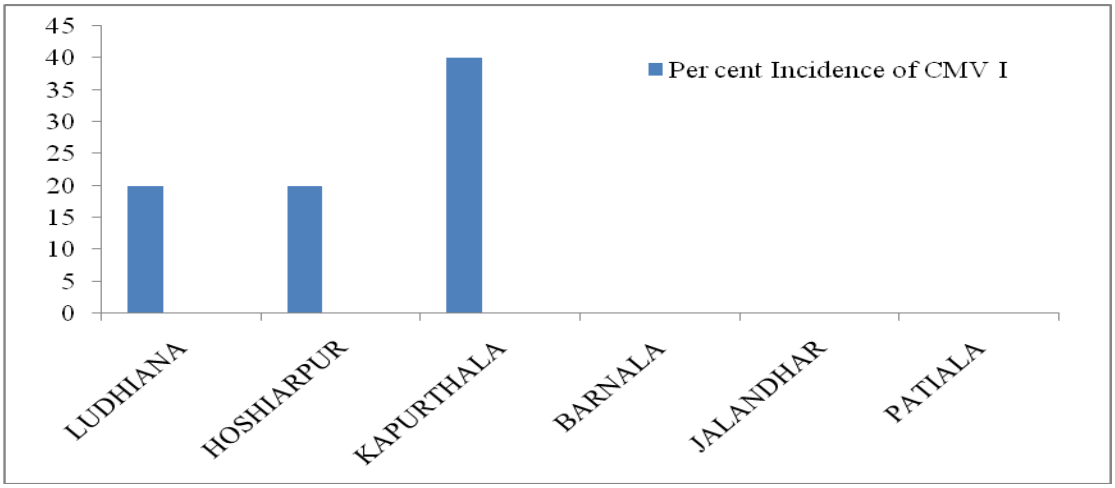


Fig.4 Degree of absorbance of CMV positive potato samples

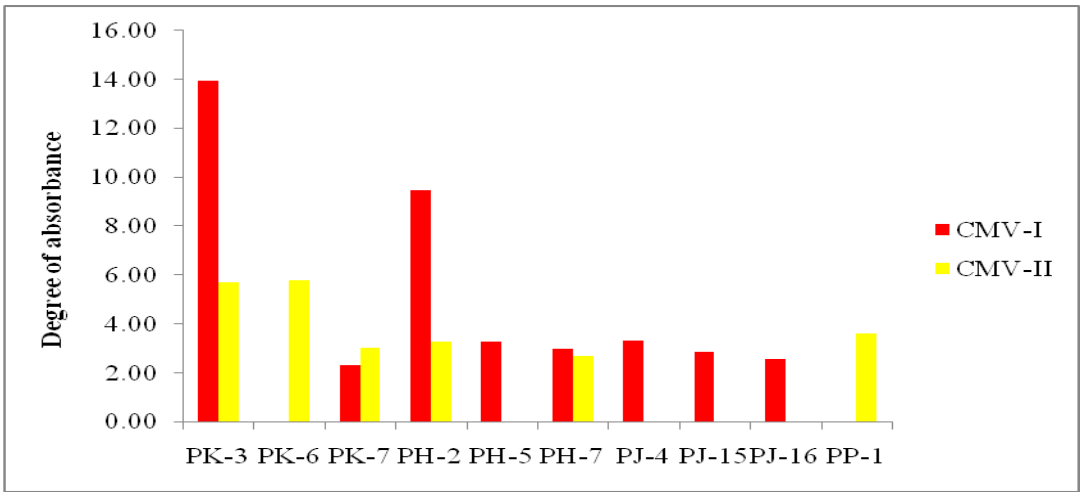
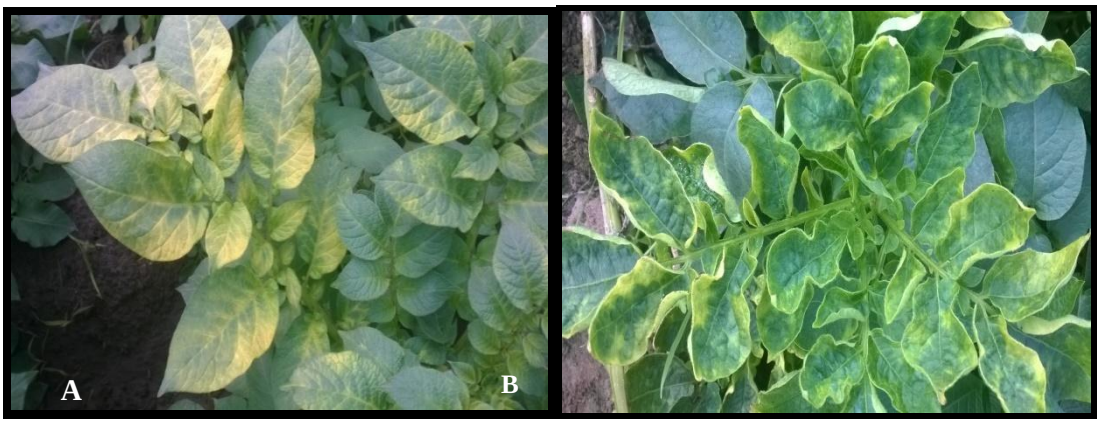


Fig.5 Symptom variability produced by CMV on potato



Variability of symptoms produced by CMV on potato (A: Yellowing and mosaic, B: Mosaic, blistering and wavy leaf margin)

Variability of symptoms produced by CMV associated with PVX, PVY^{n}, $PVY^{o/c}$, PVA PAMV and PVM on potato (A: Yellowing, mosaic and wavy leaf margin, B: Mosaic and yellowing, C: Moderate blistering and mosaic, D: Mosaic, wavy leaf margin and stunting, E: Malformation and mosaic and F: Sever curling and stunting)

Fig.6 Single ~ 540 bp resulting from RT-PCR amplification using CMV coat protein gene specific primer CMV PF/ CMV PR (Lane C = Control, 1 = PH-2, 2 = PK-3, 3 = PK-6, 4 = PK-7, 5= PP-1 & L = 100 bp ladder)

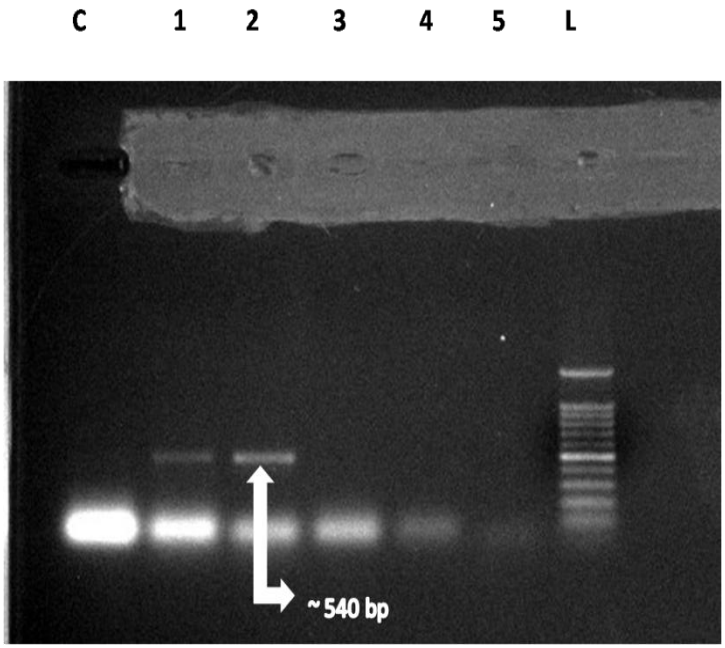
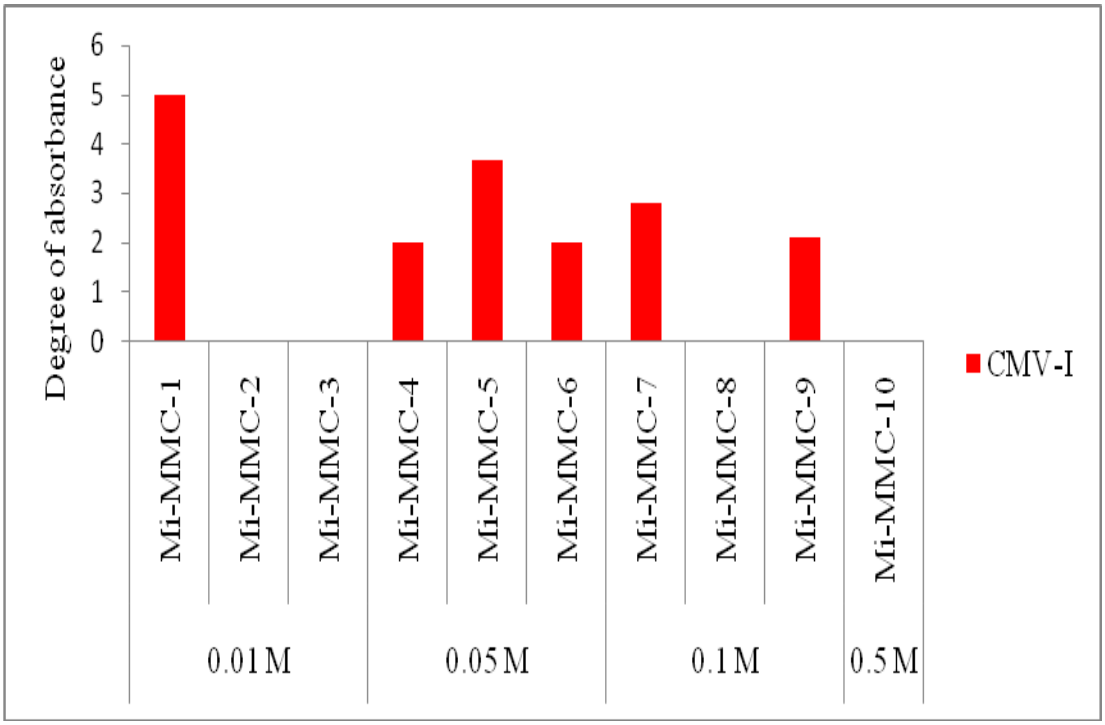


Fig.7 Degree of absorbance of CMV positive muskmelon samples inoculated with CMV infected potato leaves



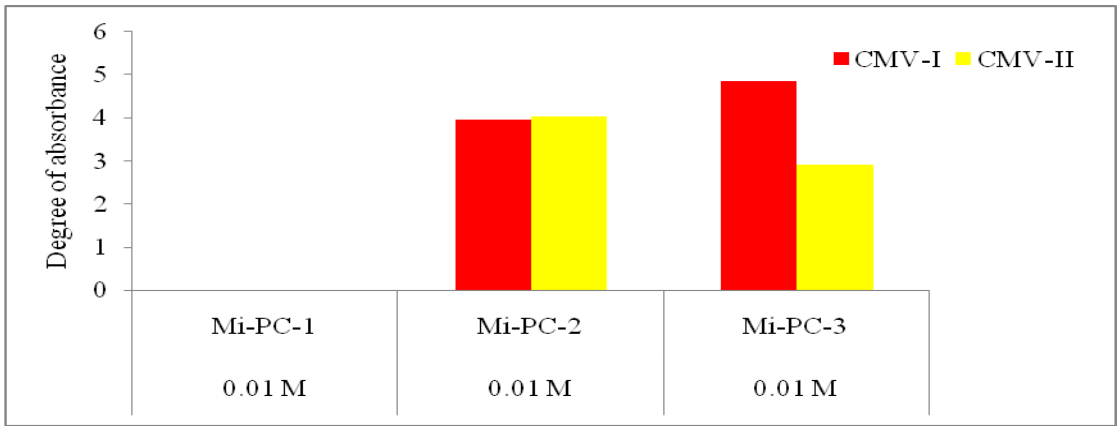
*Mi-MMC signifies the mechanical inoculation in muskmelon by CMV

Fig.8 Symptoms produced by CMV on muskmelon after mechanical inoculation from CMV infected potato leaves

A. Marginal chlorosis and curling (Mi-

B. Severe mosaic (Mi-MMC-5)

Fig.9 Degree of absorbance of CMV positive potato samples inoculated with CMVinfected muskmelon samples



*Mi-PC signifies the mechanical inoculation in potato by CMV

Fig.10 Symptoms produced by CMV on potato (Kufri Pukhraj) after mechanical inoculation from CMV infected muskmelon leaves

A. Local chlorosis (Mi-

B. Local chlorosis and malformation

Fig.11 (a) Aphids on chinese cabbage (b) Pre-acquisition fasting (c & d) Access to acquisition of viruses

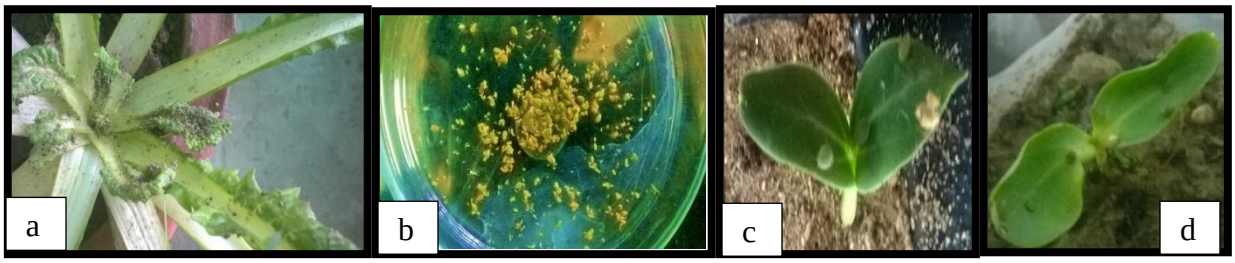
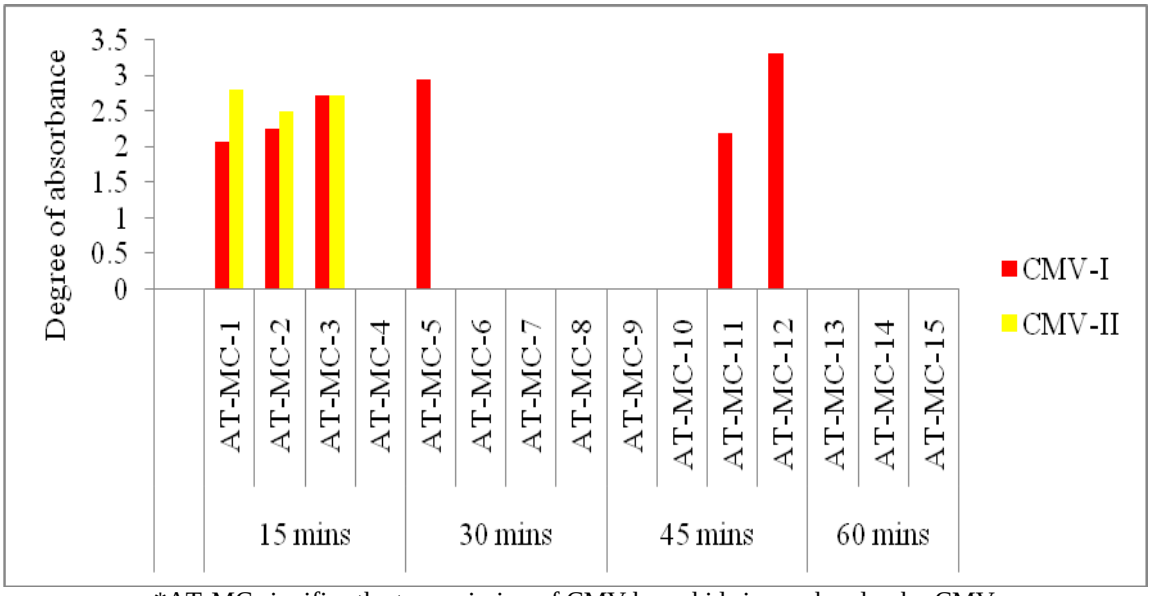


Fig.12 Degree of absorbance of CMV positive muskmelon samples infected byaphid transmission



*AT-MC signifies the transmission of CMV by aphids in muskmelon by CMV

Fig.13 Single ~ 540 bp resulting from RT-PCR amplification using CMV coat protein gene specific primer CMV PF/ CMV PR (Lane 1 = AT-MC-3, 2 = AT-MC-5, L = 100 bp ladder and C = control)

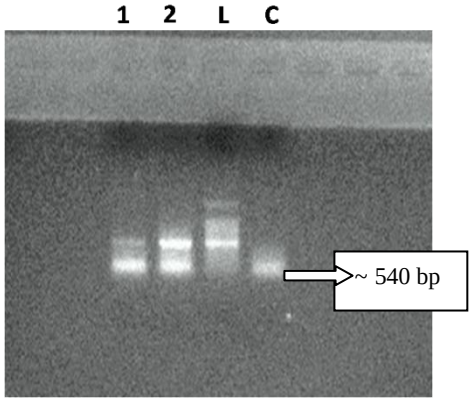
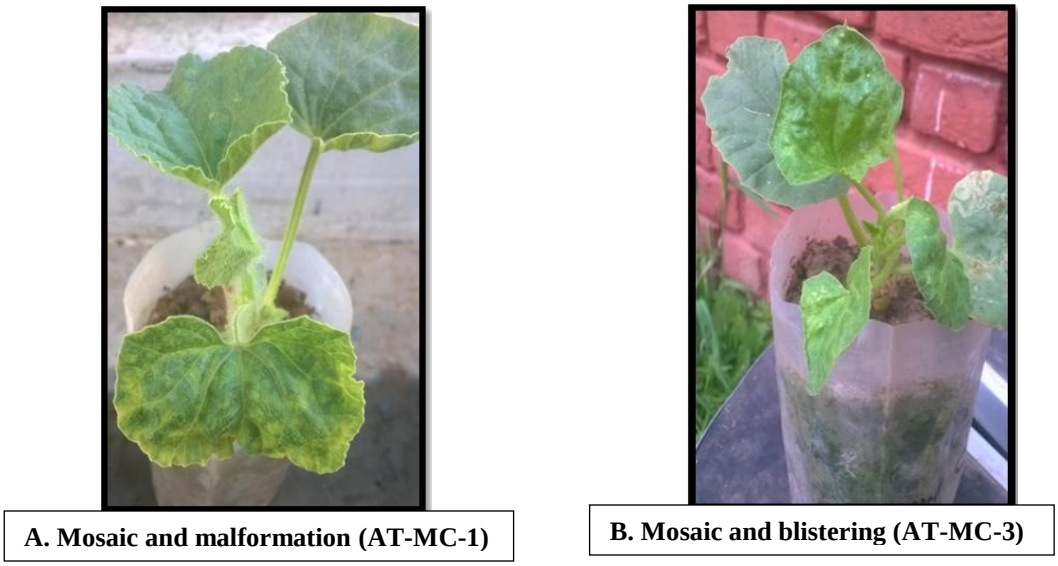


Fig.14 Symptoms produced by CMV on muskmelon after transmitted by aphid from CMV infected potato



A. Mosaic and malformation (AT-MC-1)

B. Mosaic and blistering (AT-MC-3)

Fig.15 Dynamics of aphid population in relation to temperature and rainfall during 2015-2016

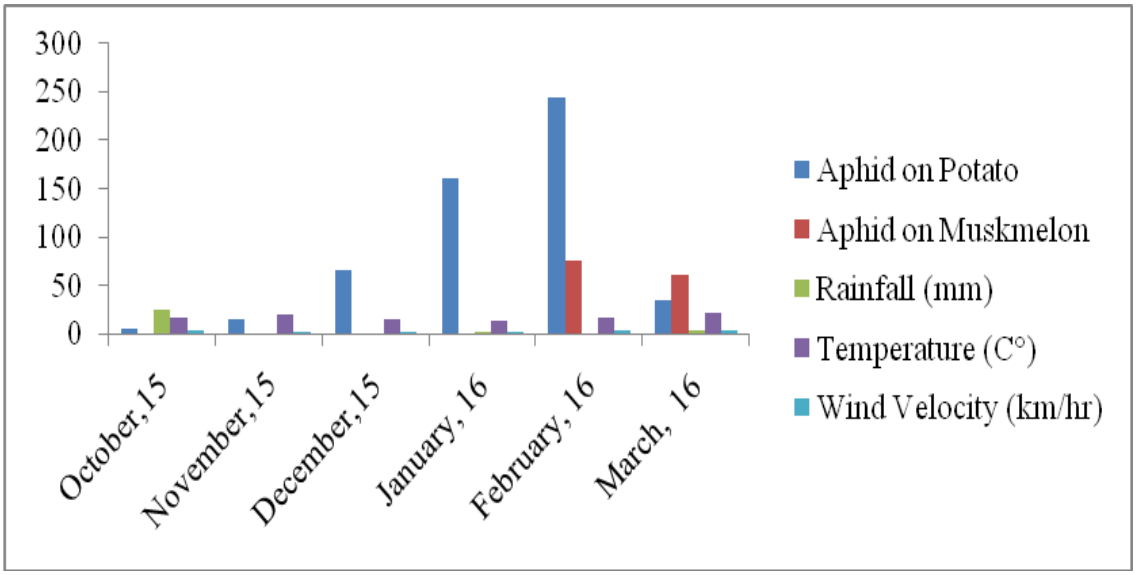


Table.1 Scale used for depicting ELISA results

OD at 405 nm	Response	Symbol
Same as negative control	Negative	-
2-5 times higher than negative control	Mild positive	+
>5 and <10 times the negative control	Positive	++
>10 than negative control	Strong positive	+++

Table.2 Reaction of Muskmelon plants to ELISA

Sample ID	Symptoms	CMV-I	CMV-II
ML-1	Blistering with small green islands	-	-
ML-2	Vein banding	-	-
ML-3	Blistering	-	-
ML-4	Mosaic and severe blistering	+	-
ML-5	Yellowing and mild blistering	-	-
ML-6	Mosaic	+	-
ML-7	Vein banding, mosaic and blistering	-	-
ML-8	Vein clearing and blistering	-	-
ML-9	Vein banding and yellowing	-	-
ML-10	Mosaic, mottling and vein clearing	-	-
MH-1	Mosaic	+	-
MH-2	Mild mosaic	-	-
MH-3	Mosaic and yellowing	-	-
MH-4	Blistering and mosaic	-	-
MH-5	Blistering	+	-
MH-6	Yellowing and mild blistering	-	-
MH-7	Vein banding	-	-
MH-8	Pale green leaves	-	-
MH-9	Yellowing and reduced fruit size	-	-
MH-10	Yellowing and mosaic	-	-
MK-1	Blistering and mild mosaic	+	-
MK-2	Blistering	++	-
MK-3	Malformation of leaves	-	-
MK-4	Mosaic	+	-
MK-5	Symptoms of insect feeding and blistering	-	-
MK-6	Mosaic and blistering	++	-
MK-7	Blistering, mosaic and malformation	-	-
MK-8	Severe blistering and reduced leaf size	-	-
MK-9	Mosaic and retarded growth	-	-
MK-10	Mosaic and blistering	-	-
MJ-1	Malformation of leaves	-	-
MJ- 2	Leathery hard and brittle leaves	-	-
MJ-3	Malformation of leaves	-	-
MJ-4	Blistering, puckering and mosaic	-	-
MJ-5	Malformation of leaves	-	-
MB-1	Yellowing and blistering	-	-
MB-2	Mosaic and malformation	-	-
MB-3	Yellowing	-	-
MB-4	Blistering and yellowing	-	-
MB-5	Mosaic and retarded growth	-	-
MP-1	Yellowing and mild blistering	-	-
MP-2	Pale green leaves	-	-
MP-3	Vein banding	-	-
MP-4	Vein banding and blistering	-	-
MP-5	Vein clearing	-	-
MP-6	Vein banding and yellowing	-	-
MP-7	Mosaic and mottling	-	-
MP-8	Blistering and puckering	-	-
MP-9	Vein clearing	-	-
MP-10	Palegreen leaves	-	-

ML - Muskmelon Ludhiana, MH - Muskmelon Hoshiarpur, MK - Muskmelon Kapurthala
MJ - Muskmelon Jalandhar, MB - Muskmelon Barnala, MP - Muskmelon Patiala

Table.3 ELISA reaction of potato samples

Sample	Symptoms	CMV-I	CMV-II	ZYMV	PAMV	PVY^{n}	$PVY^{o/c}$	PV-X	PV-S	PV-A	PV-M
PK-3	Malformation, mosaic and stunting	+++	++	-	-	+	+++	++	-	++	+
PK-6	Yellowing, mosaic, wavy leaf margin and reduced leaf size	-	++	-	-	-	-	++	-	-	-
PK-7	Moderate blistering and mosaic	+	+	-	-	+	-	++	-	+	-
PH-2	Mosaic and wavy leaf margin	++	+	-	-	++	+	++	-	+	+++
PH-5	Mosaic and yellowing	+	-	-	-	+	+	++	-	-	-
PH-7	Mosaic and small leaves	+	+	-	+	+	-	++	-	-	-
PJ-4	Mosaic, severe curling, reduced leaf size and stunting	+	-	-	-	++	-	-	-	+	++
PJ-15	Mosaic and yellowing	+	-	-	-	-	-	-	-	-	-
PJ-16	Mosaic, mild blistering and wavy leaf margin	+	-	-	-	-	-	-	-	-	-
PP-1	Mosaic	-	+	-	-	-	-	-	-	-	-

Table.4 Muskmelon samples infected and confirmed by ELISA after mechanical inoculation

Phosphate buffer	Muskmelon Sample ID	Symptoms	CMV-I	CMV-II
0.01	Mi-MMC-1	Mosaic and mild blistering	++	-
	Mi-MMC-2	Mild mosaic	-	-
	Mi-MMC-3	Healthy	-	-
0.05	Mi-MMC-4	Marginal chlorosis and curling	+	-
	Mi-MMC-5	Severe mosaic	+	-
	Mi-MMC-6	Mild mosaic and blistering	+	-
0.1	Mi-MMC-7	Mild mosaic	+	-
	Mi-MMC-8	Healthy	-	-
	Mi-MMC-9	Mild mosaic	+	-
0.5	Mi-MMC-10	Healthy	-	-

RT-PCR was conducted using the synthesized cDNA as template and CMV coat protein gene specific primers designed by De Blas*et al.,* (1994). Observation of approximately 540 bp amplicon in 1 per cent gel electrophoresis (figure 12) further confirmed the transmission of CMV from infected potato to muskmelon. Thus CMV was transmitted with an efficiency of 40 per cent by *Myzus persicae* and *Aphis gossypii* from infected potato to muskmelon.

Under Punjab conditions, the aphid population starts building up by middle of October and is maximum during February. Aphid population was recorded maximum over potato (245 aphids/100 compound leaves) in February, whereas, minimum (5 aphids/100 compound leaves) in the month of October (Figure 15). The aphid population was maximum on muskmelon (75 aphids/10 vines) during the month of February (Figure 15). 940 winged aphids were counted from muskmelon with the help of yellow sticky traps during the month of February. Thus the temperature, rainfall and wind velocity conditions during February and March were optimum for thriving and shift of aphid population from potato to muskmelon.

Aphid population dynamics

The aphid population study revealed that the population started building up on potato from October and crossed the critical level (20

aphids/ 100 compound leaves) by month of December. The aphid population was found be to maximum during February (245/ 100 compound leaves) and then reduced with the increase of daily mean temperature during March**.** In case of muskmelon, the highest aphid population was also observed during the month of February (75/ 10 vines).

TAS-ELISA results revealed that occurrence of CMV sub group I was 16 per cent on muskmelon grown in Punjab. Similarly, Sharma, (2014) also reported maximum incidence of CMV sub group I on muskmelon in Punjab while found only single muskmelon plant infected with CMV sub group II in their experiments. Likewise Eiras *et al.,* (2004) reported incidence of only CMV-I sub group on passion fruit, sweet pepper, black pepper, melon squash, tomato, pea, water-cress, zingiber and banana in Brazil.

DAS/TAS-ELISA conducted on potato samples collected from the survey over Punjab showed 10 per cent incidence of CMV on potato. Similarly, Al-Shahwan *et al.,* (1997) surveyed over potato growing fields of Tabuk, Saudi Arabia and conducted ELISA on collected potato samples. They reported 5.7 per cent and 2.8 per cent incidence of CMV on potato during autumn and spring season of the year 1990, respectively. Murphy *et al.,* (2002) detected CMV in two potato plants by conducting ELISA on potato grown over Jackson, Alabama. In The Same Way Chikh *et al.,* (2007) surveyed over major potato growing fields in Syria and reported 3.7% incidence of CMV on potato by conducting ELISA. They further observed that CMV mostly occurred in association with PVY in Syria.

Variability of symptoms produced by CMV on potato observed in our study are in line with the findings of previous works. Sanger and Agrawal, (1986) detected CMV in potato by slide agglutination technique and observed blistering, chlorosis and wavy leaf margin type of symptoms. In addition to these, Somerville *et al.,* (1987) reported reduced internodes and knobby tubers type of symptoms produced by CMV on potato in California. Chikh *et al.,* (2012) similarly observed mosaic, yellowing and stunting types of symptoms produced by CMV on potato. According to Hull, (2000) mixed infections intensify symptoms and increase viral titers in plant tissues, known as synergism. This explains the reason behind enhanced symptoms produced on potato plants when CMV infects potato in association with other viruses like PVX, PVY^{n}, $PVY^{o/c}$, PVA, PAMV and PVM. Hence the potato sample PK-3 infected with CMV along with five other viruses had the highest titre of CMV, while PP-1, PJ-15 and PJ-16 infected only with CMV had the virus in low titers.

The ELISA results were further confirmed with RT-PCR using primers designed by De Blas *et al.,* (1994). They designed these primers which are complementary to conserved sequences of RNA 3 of CMV and directly performed RT-PCR with crude sap extracts of CMV infected plants. They reported an amplicon of approximately 540 bp confirming the presence of CMV in the infected plants. Similarly, amplification of approximately 540 bp coat protein gene in our experiment confirmed the presence of CMV in potato plants of our experiment. Similarly Biswas *et al.,* (2013) detected CMV from chilli-pepper by RT-PCR using the De Blas *et al.,* (1994) designed CMV specific primers. The polymerization reaction produced approximately 540 bp of amplicon which confirmed the presence of CMV in the chilli-pepper samples. With references to these previous studies we finally confirmed the presence of CMV in potato samples collected from survey over Punjab.

Only CMV sub group I was detected in the muskmelon samples inoculated with potato leaf samples infected with both CMV sub group I and CMV sub group II (PH-2 and PK-3). This may be due the presence of sub group II in the inoculated muskmelon in a very low concentration which is not detectable by ELISA. In our study and previous survey conducted over Punjab also significantly reveals more incidence of CMV sub group I as compared to CMV sub group II on muskmelon (Sharma, 2016). The reason behind limited occurrence of CMV sub group II may be due to the physiological and biochemical characteristics of the muskmelon genotypes grown here in Punjab which in most cases restricts establishment and replication of CMV sub group II.

Symptoms produced on muskmelon upon mechanical inoculation with CMV infected potato leaves are in line with previous findings. Sanger and Agarwal, (1986) observed severe mosaic type of symptoms on muskmelon (*Cucumis melo*) and cucumber (*Cucumis sativus*) due to mechanical inoculation with sap from CMV infected potato plants. Somerville *et al.,* (1987) observed local chlorotic lesions on cotyledonary leaves and epinasty on young true leaves of cucurbit hosts after mechanical inoculation with CMV-Py potato isolate and CMV-C pepper isolate of CMV. Srivastava *et al.,* (1992) reported mosaic, vein clearing and necrotic local lesions type of symptoms on *Cucumis sativus* (Long Green) as a result of mechanical inoculation with a Chrysanthemum strain of CMV. All these findings along with our experimental outcome suggest that CMV can be mechanically transferred from infected potato to cucurbits.

On back inoculating healthy potato with CMV infected muskmelon, both the sub groups sub group I and CMV sub group II was detectable by ELISA in potato. This infers that CMV sub group II is mostly present in undetectable form in muskmelon but is able to express and replicate itself when transmitted to other crops *viz.* potato. Back inoculation of CMV to potato (Kufri Pukhraj) induced symptoms like local chlorosis and malformation on leaves. Similarly, Celebi *et al.,* (1998) observed localised symptoms produced on all 32 potato cultivars that were mechanically inoculated with CMV strain Fny-CMV obtained from muskmelon. Only 10 cultivars showed systemic symptoms produced by CMV. Chikh *et al.,* (2012) mechanically inoculated two potato cultivars with PoCNV7-5 potato isolate of CMV and observed local necrotic lesion type of symptoms. Again, mixed infection of CMV along with PVY produced various types of local necrotic spots and rings over sap inoculation on healthy potato. The reason behind localised symptoms may be mainly due to restricted movement of CMV within potato.

In our present study we observed that *Muzus persicae* and *Aphis gossypii* were able to transmit CMV from infected potato to cucurbits with an efficiency of 40 per cent. In support, Gildow *et al.,* (2008) conducted transmission studies with *Aphis gossypii* in Snap melon and found that 76 per cent of the test plants were infected with CMV. Interestingly, transmission of CMV sub group I was found to be higher as compared to that of CMV sub group II by aphids in the present study. Studies made earlier concludes that the transmission efficiency of CMV by aphids varies with coat protein structure of different strains of CMV (Zitter and Gonsalves,1991), the interaction of coat protein with binding sites of vector stylet (Nault, 1997) and on the titre of the virus concentration in the plant tissues. In a case study, Abdullah *et al.,* (1978) reported 90 per cent transmission efficiency of CMV-T and only 10 per cent transmission efficiency of CMV-6 by *Aphis*

gossypii. But exchanging the coat protein of the two virus strains in-vitro led to increased transmission of CMV-6, while there was a decrease in transmission rate of CMV-T. Furthermore, intrinsic property of virus particles or host-virus relationship affects the ability of the aphids to acquire and transmit different strains of CMV when same aphid species, virus source plant and test plants are used (Normand and Pirone, 1968). All these findings also well explain the relatively more incidence of CMV sub group I as compared to CMV sub group II on potato in Punjab.

Previous studies done by Gracia-Arenal *et al.*, (2000) and Sacristan *et al.*, (2004) revealed that CMV is transmitted in nature by many aphid species in a non-persistent manner. Again CMV is the major virus infecting large number of cucurbits every year grown in Punjab (Kang *et al.*, 2010). In our study we noted that the aphid population was maximum on both potato and muskmelon during the month of February. This maximum prevalence of aphid population was also been reported by CPRI in north western part of India. Furthermore, counts of winged aphids were more than that of wingless aphids. Volunteer infected potato plants were also found to be present in the fields of muskmelon. Hence the results of our investigation and the aphid population dynamics infers that, during February and onwards, the aphid population shifts from matured potato crop towards new and abundant available canopy of transplanted muskmelon in the adjacent fields. Hence by this time the aphids transmits CMV from infected potato to muskmelon.

Finally we concluded that potato is serving as an overwintering host of CMV during October to February in Punjab when preferred host plants are not available. The aphid populations while shifting from potato to cucurbits during February to May are transmitting CMV from infected potato plants to cucurbits under the cropping pattern followed in Punjab.

Acknowledgement

We are profoundly grateful to Head, Department of Plant Pathology, Punjab Agricultural University, Ludhiana for providing us with advice and all the necessary facilities at proper time.

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How to cite this article:

Ankit Kumar Ghorai, Santokh Singh Kang, Abhishek Sharma and Shikha Sharma. 2017. Occurrence of *Cucumber mosaic virus* on Potato and Its Transmission to Muskmelon under Potato-Cucurbit Cropping Pattern Followed in Punjab, India. *Int.J.Curr.Microbiol.App.Sci.* 6(11): 2947-2965. doi: https://doi.org/10.20546/ijcmas.2017.611.346