



INSECT VECTORS ASSOCIATED WITH VIRAL DISEASES OF KING CHILLI (*CAPSICUM CHINENSE* JACQ.) IN NORTH EAST INDIA

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ABSTRACT

A transmission study was conducted to identify and confirm the insect vectors of viral diseases of king chilli *Capsicum chinense* Jacquin, at the Virology Laboratory, Assam Agricultural University, Jorhat. The study reveals that Cucumber Mosaic Virus (CMV), Potato Virus Y (PVY) and Chilli Leaf Curl Virus (ChLCV) were the viral diseases transmitted by *Aphis gossypii* (Glover), *Myzus persicae* (Sulzer) and *Bemisia tabaci* (Gennadius), respectively. The detection of CMV and PVY were done through DAS-ELISA assay and ChLCV was done through PCR technique. The results showed that *A. gossypii* and *M. persicae* were successfully transmitting the CMV and PVY (40% each). DAS-ELISA revealed that 57.14% of the plant and 66.67% of *A. gossypii* samples were found to be positive to CMV. While, 42.17 and 60.00 % of PVY detection was observed from plant and *M. persicae* samples, respectively. *B. tabaci* also successfully transmitted ChLCV with 80% of the tested plant and the PCR results revealed that the primer pair ChLCV F1 and ChLCV R1 successfully yields 550bp at annealing temperature of 48°C from the infected plant as well as from *B. tabaci* samples and fail to detect from healthy plants, aphids, and leafhoppers samples.

Key words: *Capsicum chinense*, viral diseases, CMV, PVY, ChLCV, *Aphis gossypii*, *Myzus persicae*, *Bemisia tabaci*, vectors, transmission, DAS-ELISA, PCR, detection

North East India is one of the prominent hot spots for biodiversity, and king chilli *Capsicum chinense* Jacquin, one of the hottest chilli is extensively grown here with high benefit-cost ratio (Thangjam et al., 2021). However, one of the challenging problems in chilli cultivation is viral disease complex and chilli leaf curl disease is one of these (Shih et al., 2006; Meena et al., 2008). Kenganal et al. (2006) also reported that >20 viruses were found to infect chilli, of which mosaic and leaf curl diseases were the most devastating viral diseases. King chilli is highly susceptible to viral disease complex showing symptoms like stunted growth, leaf mosaic, leaf mottling, leaf curl, deformed leaf and deformed fruits which significantly hamper its production and productivity. Talukdar et al. (2015) identified 4 viral diseases based on symptomatology, transmission, host range and serological assays. These diseases are caused by Cucumber Mosaic Virus (CMV), Potato Virus Y (PVY), Tomato Spotted Wilt Virus and Chilli Leaf Curl Virus (ChLCV) from Assam. However, limited information was available on insect vectors aspects and therefore, the present study to identify the vectors and confirmation of these viruses through serological and molecular methods.

MATERIALS AND METHODS

To identify the vectors of viral diseases and confirmation of these viruses, transmission studies were carried out in the Virology Laboratory at the Department of Plant Pathology, Assam Agricultural University, Jorhat, during 2016-17. About 20 plants were grown in the plastic pot to study the transmission studies. The insect vectors viz., aphids (*Aphis gossypii* and *Myzus persicae*) and whitefly (*Bemisia tabaci*) were collected from the field using brush and aspirator, and then separately reared and maintained on healthy brinjal and king chilli plants in insect-proof cages for five generations and maintained non-viruliferous before transmission studies. The transmission study of CMV and PVY were conducted following standard procedure given in Garzo et al. (2004) and *A. gossypii* and *M. persicae* were used for transmission. For ChLCV, *B. tabaci* was used as vector following Butter and Rataul (1977) with little modification. After inoculation, the viruliferous vectors were killed by spraying with imidacloprid @ 0.5ml/ l of water. The plants were maintained for 30 days inside insect-proof cages and were inspected daily for symptom development. For

detection of viruses through serological and PCR methods, the samples were collected from experimental fields as well as from Laboratory. Double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) of both the plant samples as well as aphids were performed following methods described by Clark and Adams (1977) to detect CMV and PVY. The antibodies were obtained from Bioreba, AG, CH4153, Reinach BL1, Switzerland. After the addition of substrate, detection was performed after 60 minutes at 405nm in an ELISA reader (Bio-Rad Instruments Inc). The samples were considered infected with virus if ELISA reading was three times above the average reading of the control and the two blanks.

For detecting ChLCV from both plant samples and insect vectors, PCR technique was followed. CTAB (Cetyl Trimethyl Ammonium Bromide) method of Kollar et al. (1990) was performed to isolate genomic DNA from plants samples and Phenol-Chloroform extraction protocol of Barr et al. (2009) was followed for insect vectors with little modification. A pair of primers (ChLCV1) specific to the coat protein region of PepLCV genome sequence (GenBank accession no. EF 190217) was used for PCR to amplify the ChLCV coat protein gene as described by Sinha et al. (2011). The forward primer (5' AGAATTATGTCCAAGCGACCA 3') and the reverse primer (5' AAGCGTTGGGGATACACAAA 3') having an amplicon size of 550 base pair was used for amplification of king chilli leaf curl virus. PCR was performed in 25 µl volume using primer pair ChLCV1. The reaction mixture consisted of 2.5 µl 10X PCR buffer (with 17.5 mM MgCl₂), 2.0 µl of dNTPs, 2.0 µl of forward and reverse primers, 0.9 µl Taq DNA polymerase (3U/ µl), 2.0 µl template (50ng/ µl), 13.6 µl of nuclease free water. For primer pair, ChLCVF1 – ChLCVR1 DNA amplification parameters were 40 cycles of initial denaturation for 3 min at 94°C, denaturation for 1 min at 94°C, annealing for

1 min at 48°C, extension for 1 min at 72°C and final extension at 72°C for 10 min. Amplification products were hold at 4°C for infinity prior to gel electrophoresis. The annealing temperature for primer ChLCV was optimized by using gradient PCR. The gradient PCR experiment was performed at 46- 48°C viz., 46, 47 and 48°C with a reaction mixture of 25 µl. The PCR products were electrophoresis at 50 mAmp in BIO-RAD electrophoretic apparatus with 1.2% agarose gel stained with ethidium bromide and visualized under UV trans-illuminator and captured the gel images using the geldoc (Alpha Innotech, USA) for documentation.

RESULTS AND DISCUSSION

The transmission studies of viral diseases in king chilli reveals that the aphids; *A. gossypii* and *M. persicae* could successfully transmit CMV and PVY respectively, showing symptoms of stunted growth, yellow mosaic, mottling, deformed leaves and fruits. *A. gossypii* and *M. persicae* were transmitting the viruses up to 40 % of the plants. The initial symptoms expression of CMV takes about 14 to 15 days after inoculation, whereas in case of PVY, it takes about 9 to 10 days. The finding was in conformity with Talukdar et al. (2015), who observed successful transmission of CMV and PVY through *A. gossypii* and *M. persicae* in *Bhut Jolokia*. However, Baruah et al. (2016) observed that the aphids could not transmit PVY in *Bhut Jolokia* plant, whereas CMV was successfully transmitted through mechanical method after 14 days of incubation period in Assam. A similar result was also reported by Biswas et al. (2013). The whitefly (*B. tabaci*) successfully transmitted the ChLCV to king chilli up to the extent of 80 % during the transmission study. The leaves were deformed, elongated, and curled upward and downward. The fruits that bear were also small and deformed. The symptoms were started to be expressed to the inoculated plant after 18 to 23 days (Table 1). Similar results were also

Table 1. Transmission efficiency of insect vectors on CMV, PVY and ChLCV, respectively on king chilli

Insect vector	Virus	No. of vector released per plant	Acquisition access period	Inoculation access period	No. of plants		% Infection	Days to symptoms (days)	Average symptom appearance (days)
					Inoculated	Symptomatic			
<i>Aphis gossypii</i>	CMV	10	5 min	2 hr	5	2	40.00	14-15	14.50
<i>Myzus persicae</i>	PVY	10	5 min	2 hr	5	2	40.00	9-10	9.50
<i>Bemisia tabaci</i>	ChLCV	10	24 hrs	5 hr	5	4	80.00	18-23	20.50

**CMV= Cucumber mosaic virus, PVY= Potato virus Y and ChLCV= Chilli leaf curl virus

reported by Senanayake et al. (2006), who observed 100% successful transmission on the release of 8 or more whiteflies in chilli, and Baruah et al. (2016) also found 50% successful transmission with whitefly in Bhut Jolokia. Pandey et al. (2010) also reported that chilli leaf curl is transmitted by whitefly (*B. tabaci*) and inoculated chilli plants showed typical leaf curl symptoms after 2-6 weeks.

The results of DAS-ELISA assay revealed that 57.14 % of the plant and 66.67% of *A. gossypii* samples were found to be positive to CMV, while in the case of PVY, 42.17 and 60.00% of detection was recorded from plant and *M. persicae* samples, respectively (Table 2). Similar results were reported by Talukdar et al. (2017) who reported that 87.5% of Potyvirus and 75.0% of cucumovirus was found to be positive in Bhut Jolokia through DAS-ELISA and Baruah et al. (2016) also found 55.0% infection of CMV and 36.0% of PVY in Bhut Jolokia samples through DAS-ELISA assay in Assam. The PCR results revealed that the primer pair ChLCVF1 and ChLCVR1 successfully yielded a 550bp PCR product from 35.56% of the plant samples tested and 33.33% from vector *B. tabaci* out of three samples tested but failed to detect from healthy plants, aphids, and leafhoppers samples using the same primer (Table 2; Fig. 1). The results were in conformity with Adluri et al. (2017) that detect the leaf curl virus from the infected plants of *Bhut Jolokia* by using the primer pair ChLCV1, and a similar result was also reported by Sinha et al. (2011) from the pepper. The king chilli is highly susceptible to viral disease complex which is one of the significant constraints that hamper its production and productivity which are transmitted by sucking pests. From the present study, it is found that the Cucumber

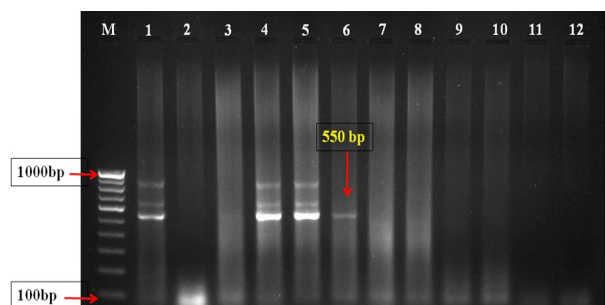


Fig. 1. ChLCV amplified product on agarose gel using ChLCV specific primer. M: 100bp Marker, 1: +ve Sample, 2: -ve Sample, 3: Healthy plant, 4-5: Infected Plant, 6: Whitefly, 7-8: Aphids, 9-12: Leaf hoppers

Mosaic Virus (CMV) and Potato Virus Y (PVY) are transmitted by aphids (*A. gossypii* and *M. persicae*) and it is confirmed by conducting DAS-ELISA assay of both the vectors and plants samples. Similarly, the whitefly (*B. tabaci*) is a vector of ChLCV in king chilli and it is also confirmed through PCR techniques. Hence, these techniques are very accurate and helpful in detecting and identifying the vectors of viral diseases and managing the viral diseases to a considerable limit.

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AUTHOR CONTRIBUTION STATEMENT

All authors equally contributed.

CONFLICT OF INTEREST

No conflict of interest.

Table 2. Detection of viruses from king chilli and its vectors through DAS –ELISA and PCR technique

Detection	Samples	Number of samples tested	Number of positive samples	Per cent detection	Range of ELISA value (OD ₄₀₅)	Mean positive OD ₄₀₅
CMV	King chilli	126	72	57.14	0.102 - 0.563	0.431
	<i>A. gossypii</i>	6	4	66.67		
PVY	King chilli	83	35	42.17	0.113 - 0.368	0.294
	<i>M. persicae</i>	5	3	60.00		
ChLCV	King chilli	180	64	35.56	-	-
	<i>Bemisia tabaci</i>	3	1	33.33		
	Aphids	3	0	0.00		
	Leafhoppers	3	0	0.00		

**Cut off value for ELISA positive sample for CMV = 0.420 and PVY =0.194

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