

## Serological and Molecular Characterization and Detection of *Capsicum chlorosis* Virus (CaCV) in Chilli

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***Capsicum chlorosis virus*(CaCV) affecting chilli belongs to genus *Tospovirus* of the *Bunyaviridae*. This is economically important disease affecting chilli crop. Virus causes, stem necrosis and chlorotic and necrotic spots on leaves. Serological detection showed CaCV positively reacted with GBNV Polyclonal antibodies in affected plants. Reverse transcription-polymerase chain reaction (RT-PCR) was used for studying the presence of CaCV in affected chilli plants from field sample in Northern Karnataka. Amplification of ~900 bp by RT-PCR with degenerate primers of L genes of *Tospovirus* revealed the positive reaction for CaCV infection in leaves of chilli.**

**Key words:** *CaCV*; *Tospovirus*, RT-PCR; Detection; Serology, chilli.

Chilli suffers from a large number of viral, bacterial, nematode and phytoplasma diseases. Chilli is highly susceptible to a large number of viruses through natural infection and in addition to artificial infection. Chilli is known to be affected by 42 viruses. Among, 22 are found to occur naturally, while the rest are known to infect on artificial infection (Raju, 2010). In the Indian subcontinent, tomato and chilli are the major vegetable crops that are widely infected by tospoviruses. The diseases caused by these viruses can cause 100% yield loss and often have severe economic and social consequences reported by Varma (2007). In October of 2006, symptoms indicative of tospovirus infection were noticed in several commercial fields of chili pepper near Bangalore in Karnataka State. Chlorotic and

necrotic spots and rings on leaves, apical necrosis, and leaf distortion were observed. Disease incidence was more than 20% (Krishnareddy *et al.*, 2008).

Of the five tospoviruses that have been reported so far from India, *Peanut bud necrosis virus* (PBNV) (Reddy *et al.*, 1992), *Watermelon bud necrosis virus* (WBNV) (Jain *et al.*, 1998) and *Capsicum chlorosis virus* (CaCV) (McMichael *et al.*, 2002) belong to the *Watermelon silver mottle virus* (WSMoV) serogroup (Yeh and Chang, 1995) and infect solanaceous and leguminaceous crops, whereas *Iris yellow spot virus* (IYSV) (Ravi *et al.*, 2006) and *Peanut yellow spot virus* (PYSV) (Satyanarayana *et al.*, 1998) are confined to onion and peanut, respectively.

The known vectors for CaCV are *Thrips palmi* and *Frankniella schultzei* in Australia (Persley *et al.*, 2006) and *Ceratothripoides calartris* in Thailand (Premachandra *et al.*, 2005). In India, Kunkaliker *et al.* (2010) who characterized CaCV suggested aphid as possible vector for its transmission and need for its determination.

During 2010-11 chilli plants showing typical symptoms of stem necrosis, chlorotic spots and necrotic spots on leaves (Figure 1) were

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collected and taken for serological and molecular detection CaCV using reverse transcription-polymerase chain reaction (RT-PCR) is presented below.

## MATERIALS AND METHODS

### Source of samples

Tospovirus infected chilli samples showing symptoms of chlorotic and necrotic spots and rings on leaves and apical necrosis, were collected from different parts of Northern Karnataka. Then the leaf samples were subjected to serological studies and molecular characterization with RT-PCR detection.

### Serological detection

A DAC-ELISA technique (Hobbs *et al.*, 1987) was employed for serological detection of the virus associated with the above symptoms in diseases chilli samples. This Immunoassay was performed for the detection of suspected *Capsicum chlorosis virus* (a tospovirus) using commercially purchased polyclonal antibodies raised against GBNV (Groundnut bud necrosis virus). The assay was carried out in 96-well polystyrene microtitre plate. For detection of Tospovirus, the polyclonal antiserum directed against nucleocapsid (N) protein of PBNV was used in 1:10,000 dilution against 1:10 dilution of sample with conjugate (Alkaline Phosphatase labeled-goat anti-rabbit IgG, Sigma-Aldrich) in 1:2,000 dilutions.

### Molecular detection

#### Total RNA isolation

Leaves of CaCV-infected chilli plants were taken for RNA isolation. RNA from corresponding healthy sample was also extracted to be used as

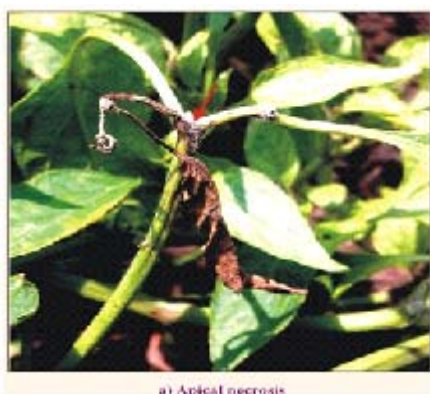
negative control. Total RNA was extracted using RNeasy Plant Mini Kit (Qiagen Inc., Chatsworth, CA, USA).

### RT-PCR for amplification of CP genes

cDNA was synthesized using Oligo (dT) as downstream primer (Hiskias *et al.*, 1998) from three microlitres of extracted RNA following a modified protocol for *Moloney Murine Leukemia Virus* reverse transcriptase (M-MLVRT, Pomega). The forward and reverse degenerate primer pairs gL3637 (5'-CCTTTAACAGT(A/T/G)GAAACAT-3') and gL4435c (5'-CAT(A/T/O)GC(A/G)CAAG A(A/G)TG(A/G)TA(A/G)ACAGA-3') (Chu *et al.*, 2001) were used for amplification of the conserved region of the L genes of tospoviruses. As the degenerate primers also complimentary to WSMoV and PBNV L RNA sequences (Chu *et al.*, 2001), ground nut and tomato samples infected with respective viruses were used as positive controls. Amplification was performed in an automated Thermocycler (JH, BIO, Germany) programmed for one cycle 2 min as initial denaturation at 94°C and 40 cycles involving 1 min of denaturation at 94°C, 1 min annealing at 55°C, 1.5 min for extension at 72°C, followed by one cycle of final extension for 10 min at 72°C. RT-PCR amplified products were analyzed by electrophoresis in 1% agarose gel at 60V for 1 h and staining with ethidium bromide.

## RESULTS AND DISCUSSION

Symptomatology : Chilli plants with yellow spots on younger leaves, which later coalesced to produce a mosaic pattern, chlorotic and necrotic spots and rings on leaves, apical

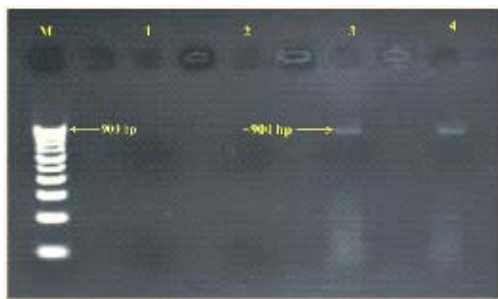


a) Apical necrosis



b) Chlorotic and necrotic spots

**Fig. 1.** Chilli plants affected with CaCV showing a) stem necrosis and b) chlorotic and necrotic spots



lane M: 100 bp Marker Lane 1= Healthy chilli samples, lane 2= Chilli plants affected with murda complex, lane 3= CaCV affected chilli leaf samples, lane 4= GBNV affected chilli samples

**Fig. 2.** Molecular detection of CaCV in chilli affected leaves

necrosis, and leaf distortion. The result are in confirmation with earlier findings in chilli crop by Krishnareddy *et al.* (2008). Kunkalika *et al.* (2010)

#### Serology

Chilli samples responded positively with PBNV antiserum (OD value: 1.25, Table1) along with PBNV infected groundnut samples (OD value: 1.30) in positive controls. These results confirmed from Kunkalika *et al.* (2010) who opined PBNV-N MAbS react with CaCV infected chilli samples and Krishnareddy *et al.* (2008) who also reported *Capsicum chlorosis virus* (CaCV) affected chilli samples reacted with antisera specific to *Groundnut bud necrosis virus* (GBNV).

**Table 1.** DAC-ELISA absorbance values

Sl. No.	Samples	Location	OD value (405 nm)	Reaction + / -
1	Chilli	UAS Campus (Dharwad district)	1.25	+
2	Positive Control, Ground nut (PBNV)	UAS Campus (Dharwad district)	1.30	+
3	Positive Control, Tomato (WSMoV)	UAS Campus (Dharwad district)	0.58	+
4	Healthy control, Chilli	UAS Campus (Dharwad district)	0.24	-
5	Buffer control (PBS-T)	—	0.16	-

RT-PCR: Leaves of CaCV-infected chilli plants (Figure. 2) gave positive results in RT-PCR. These samples yielded 900 bp PCR product. An expected ~820 bp DNA fragment amplified both in diseased ground nut (PBNV) and tomato (WSMoV) samples.

CaCV in India was identified by amplifying the N gene using degenerate primers to the N gene of PBNV, WBNV and CaCV by Krishnareddy *et al.* (2008) and Kunkalika *et al.* (2007). Whereas, Chu *et al.* (2001) designed degenerate primers and amplified the conserved region of L gene of tospoviruses for the detection of five distinct members of tospoviruses including thrips transmitted TSWV. Hence in the present study, the predicted amplicon size of nearly 820 bp was observed in PBNV and WSMV infected ground nut and tomato samples respectively and an amplicon of same size was produced on CaCV suspected samples. These findings are in

conformity with Chu *et al.* (2001) and results from CaCV suspected samples are in line with diagnosis carried on CaCV by Krishnareddy *et al.* (2006) and Kunkalika *et al.* (2007). From the present study and previous review it can be concluded that the technique is of great significance in detection of CaCV on leaves of infected chilli plants using RT-PCR.

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