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# The occurrence and distribution of major viruses infecting cucurbits in Tamil Nadu state, India

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ABSTRACT

Field surveys were conducted during 2012–14 to document the occurrence and distribution of viruses infecting cucurbits in all the seven agroclimatic zones of Tamil Nadu state, south India. Samples collected from various types of cucurbits showing virus-like symptoms were tested for *Cucumber mosaic virus* (CMV), *Papaya ringspot virus* (PRSV), *Zucchini yellow mosaic virus* (ZYMV), *Cucumber green mottle mosaic virus* (CGMMV) and begomoviruses using serological (Dot Immuno Binding Assay, DIBA) and molecular (RT-PCR for RNA viruses and PCR for DNA viruses) methods. Results indicated higher incidence (98.6%) of begomoviruses, followed by PRSV (32.1%) and CGMMV (22.2%). ZYMV and CMV were detected at a lower frequency of 7.1% and 5%, respectively. Mixed infections with two or three of these viruses were observed in more than 50% of the samples analyzed. Sequencing of begomovirus-specific amplicons indicated the presence of sequences highly similar to *Tomato leaf curl New Delhi virus* (ToLCNDV) and *Squash leaf curl China virus* (SLCCNV).



## 1. Introduction

Global production of cucurbits is around 151 million tones. In India, the total production of cucurbits is around 6.5 million tones contributing about 74% of total production in South Asia. Cucurbitaceous vegetables contribute nearly 18% of total vegetable production in India. There is increasing demand in the production of cucurbits to meet nutritional security for an increasing population. Cultivation of cucurbits was advocated to enhance overall productivity and production of vegetables (Rai et al., 2008). Various types of cucurbits are grown in several regions of India. They include pumpkin (*Cucurbita maxima* Duchesne.), snake gourd (*Trichosanthes cucumerina* L.), bitter gourd (*Momordica charantia* L.), ash gourd (*Benincasa hispida* Thunb.), ridge gourd (*Luffa acutangula* Mill.), bottle gourd (*Lagenaria siceraria* (Molina) Standl.), cucumber (*Cucumis sativus* L.), gherkins (*Cucumis anguria* L.), ivy gourd (*Coccinia grandis* (L.) Voigt), musk melon (*Cucumis melo* L.), watermelon (*Citrullus lanatus* (Thunb.) Matsumura & Nakai), chayote (*Sechium edule* (Jacq.) Sw.) and smooth gourd (*Luffa aegyptiaca* Mill.).

Tamil Nadu is one the major states in India producing different types of cucurbits. Cucurbits are often grown in different agro-ecological zones of Tamil Nadu state by subsistence farmers in periurban and rural areas to meet the increased demand for vegetable consumption in urban areas. Consequently, production of cucurbits has become a profitable enterprise for household income to resource poor farmers. In addition, increased vegetable cultivation has generated continuous employment opportunities for field workers.

More than 59 different viruses were reported to cause a wide variety of diseases in cucurbits (Lecoq and Desbiez, 2012). These viruses produce different types of symptoms, including mild to severe forms of yellow and green mosaic, mottling, puckering and blistering of leaves, reduced leaf size, various types of leaf

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deformations, malformation of fruits and stunting of plants. Viruses belonging to the genera *Begomovirus, Potyvirus, Cucumovirus, Tobamovirus, Tymovirus, Nepovirus, Polerovirus* have been reported world-wide (Mansilla et al., 2013; Johnson et al., 2013; Sobh et al., 2012; Dreher et al., 2012; Abdalla et al., 2012; Zitter and Murphy, 2009; Liu et al., 2009). Viruses belonging to these genera are known to be transmitted by different types of vectors. Some of these viruses, especially in the genera *Potyvirus, Cucumovirus, Tobamovirus, Tymovirus, Nepovirus,* are also transmitted via seed (Reingold et al., 2014; Simmons et al., 2011; Tobias et al., 2008).

Although several viruses infecting cucurbits have been reported in India (Raj et al., 2012), detailed information is not available on the occurrence of viruses in cucurbit crops. In view of the increased acreage of cucurbit cultivation, a statewide survey was conducted to document predominant viruses infecting cucurbitaceous crops in Tamil Nadu.

## 2. Materials and methods

### 2.1. Survey area and sample collections

Surveys of cucurbit crops (pumpkin, snake gourd, bitter gourd, ash gourd, ridge gourd, bottle gourd, cucumber, gherkins, ivy gourd, musk melon, watermelon, chayote and smooth gourd) were conducted during the growing seasons between 2012 and 2014 in seven agro climatic zones of Tamil Nadu, India. These included High Rainfall Zone, Southern Zone, Cauvery Delta Zone, Western Zone, North-Western Zone, North-Eastern Zone and High Altitude Zone (Supplementary Fig. 1). Surveys were conducted during different growing seasons and leaf samples showing virus like symptoms such as mild to severe mosaic, mottling of leaves, reduction in plant height and leaf size, leaf narrowing, leaf filiformity, rolling and upward cupping of leaves, vein banding, vein clearing, yellowing of leaves, reduction of fruit size, blistering and deformations of fruits were collected randomly from different types of cucurbits.

Among 436 samples collected, 9.2% samples were from the High Rainfall Zone, 12.8% samples were from the Southern zone, 3.4% samples were from the Cauvery Delta zone, 40.6% samples were from the Western zone, 13.8% samples were from the North-Western zone, 18.1% samples were from the North-Eastern zone and 2.1% samples were from the High altitude zone. About two to three young symptomatic leaves were collected per plant and placed in separate plastic bags and labeled. Samples were carried to the laboratory in a cool box and processed within 24 h of collection.

### 2.2. Preparation of samples for dot-immunobinding assay

Samples were processed and tested by dot-immunobinding assay (DIBA) as described previously by Ali et al. (2012). Each sample was spotted on nitrocellulose membranes in three replications. In addition, negative and positive controls were spotted on each membrane. Sap from apparently healthy cucurbit leaves from seedlings grown in an insect proof net was used as a negative control. Positive controls for each virus used were obtained from DSMZ, Germany. All samples were tested against polyclonal antisera specific to CMV, PRSV, ZYMV, CGMMV and SLCV (DSMZ, Germany).

### 2.3. Reverse-transcription polymerase chain reaction

Total RNA was extracted from symtomatic leaves using Trizol Reagent (Sigma Aldrich, USA) according to the manufacturer's instructions. Total RNA was used in reverse-transcription polymerase chain reaction (RT-PCR) using the RevertAid First Strand cDNA synthesis kit (Thermo Scientific, USA) according to the manufacturer's instructions. The cDNA was used in PCR for amplification of a portion of the CMV, PRSV, ZYMV and CGMMV genome using virus specific primers.

### 2.4. DNA extraction and PCR

For the detection of begomoviruses, total DNA was extracted from symptomatic leaves by the GEM-CTAB method (Rouhibakhsh et al., 2008) using 2% β - mercapto ethanol. A positive control for each virus was used for comparision (Courtesy: DSMZ, Germany).

### 2.5. Design of primers for polymerase chain reaction

Primers for the nucleocapsid genes of CMV, PRSV, ZYMV, CGMMV and ToLCNDV were designed from the published sequences in the National Center for Biotechnology Information (NCBI) database. Details of virus-secific primers, annealing temperatures and the size of amplicons are provided in Table 1.

### 2.6. Cloning, sequencing and sequence analysis

Amplified PCR products were purified using the QIAquick Gel Extraction Kit (Qiagen) and cloned in the pGEM-T Easy Vector System (Promega Corp.) (Sambrook et al., 1989). Plasmid DNA preparations were obtained using Wizard® Plus Minipreps DNA Purification (Promega Corp). Two clones were selected for each sample for sequencing. Sequencing was performed at the Xcelris Pvt. Ltd., Ahmadabad. Sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) for the identification of virus at species level. The top three to five hits against each database were included in the analysis for each sequence.

## 3. Results

### 3.1. Symptomatology

During the survey of viruses on cucurbitaceous crops in Tamil Nadu, virus like symptoms as given in Fig. 1 were observed and collected. Due to the frequent occurrence of mixed infections, it was difficult to describe the virus using symptomatology under field conditions. Based on symptomatology, average virus disease incidence of 72% was recorded on different cucurbits grown in Tamil Nadu. Among the agroclimatic zones, maximum incidence was observed in the High altitude zone (85%) followed by the North Eastern zone (81%). The lowest disease incidence was observed on musk melon from the Western zone (12%) followed by snake gourd from North Western zone (30%). Also 100% disease incidence was observed on snake gourd, ridge gourd and bottle gourd from the North Eastern zone and chayote from the High altitude zone.

### 3.2. Occurrence and detection of virus by DIBA and PCR/RT-PCR

All samples collected were subjected to DIBA and nucleic acid based detection using PCR for Begomoviruses and RT-PCR for RNA viruses such as CMV, PRSV, ZYMV and CGMMV. Most of the samples (98.6%) were found infected with Begomoviruses. PRSV, CGMMV and *Begomovirus* were detected in all the agro-climatic zones of Tamil Nadu, but CMV was present only in the Western zone, High rainfall zone and Southern zone. ZYMV distribution was minimal and was only detected in a few samples collected from the Western zone, High rainfall zone, Southern zone and North-Eastern zone (Table 2).