# Survey of Cassava Mosaic Disease in Kerala 

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#### Abstract

A survey was conducted on the incidence of cassava mosaic disease (CMD) in Kerala. Cassava mosaic disease occurred throughout the state at low to high incidences (44.5-96.75\%) in the 35 regions surveyed. The disease incidence was higher in Thiruvananthapuram and Kollam districts and lower incidence was noticed in Wayanad district. Maximum white fly population was also observed in Thiruvananthapuram district of Kerala followed by Kollam. Survey results also indicate that Sri Lankan Cassava Mosaic Virus (SLCMV) is wide spread in Kerala.


Key words: Cassava mosaic disease (CMD), incidence, severity, cassava mosaic begomovirus, whitefly vector populations, Bemisia tabaci, survey methods

## Introduction

Cassava (Manihot esculenta Crantz), popularly known as tapioca, is cultivated in 102 countries. It is an important staple food for more than 500 million people in the tropics, apart from being an industrial crop. It ranks $4^{\text {th }}$ as a carbohydrate yielding crop in the world. In India, it is grown in an area of 2.424 lakh ha with an annual production of 76.202 lakh tonnes (FAO, 2008) for domestic and industrial uses.

In India, this crop is mostly grown in Kerala, Tamil Nadu, Andhra Pradesh and Karnataka and also in a few NorthEastern states. Cassava is the staple or subsidiary food and it is the raw material for many value added products. Cassava also constitutes a basic daily source of dietary energy (Kenneth et al., 2000). Roots are processed into wide variety of granules, pastes, flours etc. or consumed after boiling. It is also used as an animal and poultry feed. Leaves of cassava are also fed to cattle and pet animals, especially pigs. Cassava starch is used as a binding agent, in the production of paper and textiles and as monosodium glutamate, an important flavoring agent for cooking. It is also used for alcohol production (IITA, 2001). Even though this crop was introduced into India as 'famine saver', presently it has attained
commercial status in Tamil Nadu and Andhra Pradesh for processing into starch and sago (Nair and Makeshkumar, 2000).

Among the diseases and pests of cassava, cassava mosaic disease (CMD) is a serious factor limiting the productivity of cassava, which can lead to yield reduction of $70-80 \%$ (Fauquet and Fargette, 1990) depending on the cultivars, disease severity and losses estimated at one pound sterling (Fargette et al., 1988). However in India, tuber yield loss due to CMD has been reported from 10 to $90 \%$, though a modest estimate could be about $30 \%$ on an average (Thankappan and Chacko, 1976; Edison, 1979). Cassava mosaic disease (CMD) is caused by viruses belonging to genus Begomovirus of the family Geminiviridae, which are characterized by small, geminate particles containing circular, single stranded DNA molecules (Briddon and Markham, 1995). Twenty different viruses have been reported from cassava (Thresh et al., 1994). Among these, in India, cassava mosaic disease is caused by two viruses - Indian Cassava Mosaic Virus (ICMV) and Sri Lankan Cassava Mosaic Virus (SLCMV) (Malathi et al., 1985; Dutt et al., 2005).

Even though, cassava is cultivated extensively in Kerala,
no systematic studies has been undertaken to generate information on CMD status in different parts. The present study reveals the CMD distribution pattern in the state and the virus species associated with it.

## Materials and Methods

## Survey

Surveys were conducted during 2005 and 2006 to record the incidence of cassava mosaic disease in cassava growing areas of all the 14 districts of Kerala. A total of 35 fields were visited. In each field, four plots of $10 \mathrm{~m}^{2}$ area were selected. Healthy and diseased plants were counted and percent incidence was calculated. Disease incidence refers to the number of visibly diseased plants, in relation to the total number assessed and so expressed as the proportion or percentage of plants in a stand with symptoms on a scale of $0-100 \%$ (Fargette, 1985). The symptom severity of each isolate were recorded using the standard $1-5$ scale described by Hahn et al. (1980) in which grade 1 represents cassava mosaic symptomless plants; grade 2 - Mild chlorosis, mild distortions at bases of most leaves, while the remaining parts of the leaves and leaflets appear green and healthy; 3 - Pronounced mosaic pattern on most leaves, narrowing and distortion of the lower one-third of the leaflets; 4 - Severe mosaic distortion of two thirds of most leaves and general reduction of leaf size and stunting of shoots and 5 - Very severe mosaic symptoms on all leaves, distortion, twisting, mis-shapen and severe leaf reduction of most leaves accompanied by severe stunting of plants. This method has been used in recent surveys and field experiments in Uganda (Sseruwagi et al., 2004) and elsewhere and is recommended for use more widely as it provides a true evaluation of disease severity in the stand assessed. In each plot, an assessment of adult white fly population was made by counting the adults on the top most fully expanded five apical leaves of the tallest shoot of 10 cassava plants per field, counts were added for each plants and the largest of these totals were recorded (Legg and Raya, 1998; Fargette, 1985). This is because the adults feed preferentially and oviposit on the youngest immature leaves (Khalifa and El-Khider, 1965; Avidov and Harpaz, 1969; Gameel, 1977; Ohnesorge et al., 1980; Fargette, 1985). Each leaf is held by the petiole and gently inverted so that the adults present on the lower surface can be counted (Seif, 1981; Fargette, 1985; Fargette et al., 1985; Fishpool et al., 1995).

Young cassava plants (3-6 months old) were selected to permit a clear distinction between cutting (planting material) and whitefly borne infection by observing the lower first formed leaves. Where all the leaves show symptoms, infection is considered to be cutting borne but where only upper leaves show symptoms, infection is considered to be as a result of current season whitefly borne infection. Farmers provided information on cassava variety, but were unable to provide names of 14 of the 70 isolates sampled. Altogether 70 isolates were collected from different areas and were serially numbered according to the order of collection made. Mean values were calculated for all factors measured both for each field as well as for each districts. Leaf samples of these isolates were put in sterile polythene bags for subsequent virus diagnosis in the laboratory.

## DNA isolation

DNA was extracted from the cassava leaf tissues using the protocol of Lodhi et al. (1994). DNA extracts were preserved at $-20^{\circ} \mathrm{C}$ for virus diagnosis and characterization using PCR techniques.

## PCR amplification

PCR reaction was performed using primer pairs specific for Indian Cassava Mosaic Virus (ICMV) and Sri Lankan Cassava Mosaic Virus (SLCMV) ie., multiplex PCR primers SLCMV A-F, ICMV A-F and I/SLCMV (Patil et al., 2005 and Dutt et al., 2005) and primers specific for African Cassava Mosaic Virus (ACMV) and East African Cassava Mosaic Virus (EACMV) (Zhou et al., 1997) were used to amplify fragments of DNA-A of cassava mosaic Geminivirus (CMGs) in reaction mixture per tube containing $5 \mu \mathrm{l}$ of 10 x Taq polymerase buffer (containing 100 mM Tris $\mathrm{HCl}, 500 \mathrm{mM} \mathrm{KCl}$ and $15 \mathrm{mM} \mathrm{MgCl}{ }_{2}$, $1.0 \mu \mathrm{l}$ of dNTPs 10 mM$), 1.0 \mu \mathrm{l}$ each of forward and reverse primer ( 5.0 pM ), $0.5 \mu \mathrm{l}$ ( 2.5 units) of Taq DNA polymerase and $5 \mu \mathrm{l}$ of template DNA ( 50 ng ) and 36.5 $\mu \mathrm{l}$ of sterile distilled water to make a final volume of 50 $\mu \mathrm{l} /$ tube. The negative controls were the buffer used for DNA extraction and DNA from meristem derived healthy cassava leaves. The thermal cycling profile was 3 minutes of initial denaturation at $94^{\circ} \mathrm{C}$ followed by 30 cycles of: 1 minute at $95^{\circ} \mathrm{C}, 2$ minutes at $55^{\circ} \mathrm{C}, 3$ minutes at $72^{\circ} \mathrm{C}$ and 5 minutes of final extension at $72^{\circ} \mathrm{C}$. Reaction was carried out in DNA engine model PTC 200 (MJ Research, USA). The PCR products were separated by electrophoresis in $1 \%$ agarose gel having
ethidium bromide as stain $\left(10 \mathrm{mg} \mathrm{ml}^{-1}\right)$ at 60 volts for about 1 hour in 1X- Tris-Acetate-EDTA (TAE) buffer of pH 8.0 (Sambrook et al., 1989).

## Details of primers used in this study are:

| Primer | Sequence |
| :--- | :--- |
| SLCMV-A-F | 5'-TGT AAT TCT CAA AAG TTA CAG TCN-3' |
| ICMV-A-F | 5'-GCT GAT TCT GGC ATT TGT AN-3' |
| I/SLCMV-A-R | 5'-ATA TGG ACC ACA TCG TGT CN-3' |
| ACMV AL1/F | 5'-GCG GAA TCC CTA ACA TTA TC-3' |
| ACMV ARO/R | 5'-GCT CGT ATG TAT CCT CTA AGG CCT G-3' |
| EACMV LCP | 5'-TCT TTA TTA ATT TGT CAC TGC AT-3' |
| EACMV T | 5'-CAC TGG TAT GGT CCG ATG TG-3' |

## Results

A survey was undertaken to assess the incidence of cassava mosaic disease and also to collect diseased samples from different cassava growing areas of Kerala to assess the virus diversity. The symptoms on infected cassava plants were recorded while collecting the diseased leaf samples. During the course of surveys, the percentage of cassava mosaic incidence, symptom severity and adult whitefly populations were recorded and the data is presented in Table 1. The maximum incidence of CMD (100\%) was recorded at Neyattinkara in Thiruvananthapuram district and Anchal and Kottarakkara in Kollam district. Disease incidence through cutting borne infection was also found maximum in Kollam (69.25\%), followed by Thiruvananthapuram (66\%) and minimum in Kottayam (27\%) and Kozhikode (28\%) districts. Disease incidence through whitefly infection was found maximum (37.5\%) in Kozhikode district and minimum (9\%) in Wayanad district.

The symptom of CMD, in general, on cassava leaves under field conditions were


Fig. 1. Typical mosaic symptom of cassava mosaic disease characterized by the presence of chlorotic spots on the leaf lamina, which may be yellow or pale green depending on the cultivar and season. The chlorotic areas intermixed with normal green tissues gave a typical mosaic pattern (Fig. 1). The
mosaic pattern was sometimes restricted only to basal portion of lamina or seen throughout the lamina. In some severely diseased plants, leaves were reduced in size due to distortion of lamina and twisted with yellow areas separated normally by green areas (Fig. 2). Some showed drying of leaf margin and vein clearing (Fig. 3). Further, when compared to healthy cassava plants the diseased


Fig. 2. Leaf twisting symptom of cassava mosaic disease


Fig. 3. Leaf distortion and drying symptom of cassava mosaic disease
plants appeared stunted very much. Masking of symptoms was also observed in some cassava plants.

Disease intensity was also found maximum in Thiruvananthapuram (4.25) and Kollam (4.02) and minimum in Wayanad (2.3). The maximum white fly population (88 flies per topmost leaves of ten plants/field) was in Thiruvananthapuram district of Kerala followed by Kollam, Idukki and Alappuzha
Table 1. Cassava mosaic disease incidence, symptom severity and whitefly population in different cassava growing districts of Kerala

| Districts | Number of regions surveyed | Varieties grown | Cassava mosaic disease incidence |  |  | CMD symptom severity (1-5 scale) | Number of whiteflies per plant |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | Total (\%) | Cutting borne (\%) | Whitefly borne (\%) |  |  |
| Thiruvananthapuram | 4 | Kalikalan, Etha kappa | 96.75 | 66.00 | 30.75 | 4.25 | 88 |
| Kollam | 4 | Arumasa kappa, Singapore vella, Nanjuvella | 95.00 | 69.25 | 25.25 | 4.02 | 83 |
| Pathanamthitta | 2 | Malayan | 77.70 | 49.20 | 28.50 | 3.80 | 69 |
| Alappuzha | 2 | Pathinettu, Arumasa kappa | 90.00 | 58.00 | 32.50 | 3.85 | 80 |
| Kottayam | 2 | Manja kappa, Malayan | 60.00 | 27.00 | 33.00 | 2.55 | 79 |
| Ernakulam | 2 | Malayan | 74.50 | 44.50 | 18.00 | 3.50 | 57 |
| Idukki | 2 | Manja kappa, Malayan | 48.00 | 32.00 | 16.00 | 2.70 | 80 |
| Thrissur | 2 | Karutha kalikalan, Vella kappa | 70.00 | 54.00 | 16.00 | 2.95 | 69 |
| Palakkad | 3 | Nilagiri kappa, Arumasa kappa | 67.60 | 41.30 | 26.30 | 3.20 | 45 |
| Malappuram | 4 | Pindi kappa, Arumasa kappa, Chuvandha kappa | 88.75 | 59.20 | 29.50 | 2.70 | 58 |
| Kozhikode | 2 | Arumasa kappa | 65.50 | 28.00 | 37.50 | 3.10 | 66 |
| Kannur | 3 | Arumasa kappa | 66.60 | 36.30 | 30.30 | 3.50 | 43 |
| Wyanad | 1 | Vella kappa | 44.50 | 35.50 | 9.00 | 2.30 | 42 |
| Kasargod | 2 | Arumasa kappa, Vella kappa | 62.00 | 44.50 | 18.00 | 2.90 | 54 |

districts respectively and minimum of 42 was found in Wayanad district.

The isolates collected in the study were from different varieties grown in the sampling locations which included 17 isolates from Arumasa kappa, 11 of Kalikalan, six of Malayan, five of Etha kappa, four each of Nanjuvella and Karutha kalikalan, two each of Singapore vella, Vella kappa, Chuvandha kappa and Pathinettu, one each of Nilagiri kappa and Pindi kappa. Among the varieties collected, Kalikalan showed maximum disease incidence (100\%) followed by Karutha kalikalan (96\%) and least incidence was shown by Arumasa


Fig. 4. Detection of ICMV and SLCMV infection using multiplex PCR of samples

Lanes: 1 - 500bp DNA ladder; 2 - ICMV infected sample from Ernakulam district showing band at 900bp; $3 \& 4$ - SLCMV infected sample from Thrissur \& Palakkad districts showing band at 600bp; 5 - Sample from Thiruvananthapuram district having mixed infection of ICMV and SLCMV

Table 2. Cassava mosaic virus detected in the CMD samples collected from different cassava growing districts of Kerala

| District | SLCMV (\%) | ICMV (\%) | Mixed infection of both ICMV and SLCMV (\%) | ACMV (\%) | EACMV (\%) |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Thiruvananthapuram | 25.0 | 62.5 | 12.5 | - | - |
| Kollam | 87.5 | 12.5 | - | - | - |
| Pathanamthitta | 66.7 | - | - | 33.3 | - |
| Alappuzha | 100.0 | - | - | - | - |
| Kottayam | 100.0 | - | - | - | - |
| Ernakulam | 75.0 | 25.0 | - | - | - |
| Idukki | 75.0 | - | - | - | - |
| Thrissur | 100.0 | - | - | - | - |
| Palakkad | 100.0 | - | - | - | - |
| Malappuram | 50.0 | 33.3 | 16.7 | - | - |
| Kozhikode | 75.0 | - | - | - | - |
| Kannur | 100.0 | - | - | - | - |
| Wyanad | 100.0 | - | - | - | - |
| Kasargod | 100.0 | - | - | - | - |

kappa (31\%), Malayan (30\%) and Nanjuvella (28\%). Of the 70 samples analyzed, 68 gave positive results (Table 2 ), of which 55 samples were
 identified as SLCMV, nine samples were ICMV and two samples showed mixed infection of both ICMV and SLCMV (Fig. 4) and two samples, were identified as ACMV (Fig. 5). EACMV was not detected in any of the samples tested.

## Discussion

The survey conducted to study the incidence of CMD in cassava growing areas of Kerala revealed that the

Fig. 5. Detection of ACMV infection using ACMV specific primers in samples from Pathanamthitta district
Lanes 1-1kb DNA ladder; 2 \& 3 - ACMV infected sample from Pathanamthitta district showing band at 1 kb
percentage of incidence and whitefly populations varied with localities and cultivars grown. The disease was found in all the cassava growing areas of Kerala with varying intensity. In Thiruvananthapuram and Kollam districts of Kerala, the disease incidence was more when compared to the other 12 districts of Kerala. The maximum disease incidence of 100 percent was recorded at Neyattinkara in Thiruvananthpuram district of Kerala. In Kottayam district the incidence of disease was only $60 \%$ but the whitefly population was more. In Thiruvananthpuram, Kollam, Alappuzha, Ernakulam and Kottayam districts, the whitefly population on cassava was high. Higher incidence of disease in Thiruvananthpuram, Kollam, Alappuzha and Malappuram districts could be attributed to large areas under the crop, indiscriminate use of diseased cuttings for planting and high whitefly populations. Infected planting material is the primary source of infection and vector (Bemisia tabaci) association for the secondary spread. Less disease incidence in Idukki and Wayanad districts may be due to the cool climate, where whitefly population was also low. The whitefly is an important vector of the virus causing Indian Cassava Mosaic Disease
(ICMD) as it can account for 4-30\% secondary spread of the disease depending upon the variety, under field conditions (Palaniswami et al., 1996). The secondary spread of disease was significantly high (52.5\%) in highly susceptible variety. Among the cultivars, highest secondary spread (69.09\%) was seen only in one cultivar, viz., Kalikalan, followed by $16.35 \%$ in $\mathrm{H}-226$ and the other varieties (H-165, H-1687, H-2304 and M4) together had only $15.56 \%$ spread. This indicated that varietal susceptibility was the major factor influencing secondary spread. Pillai and Daniel (1979) studied the variations in the population build up of whitefly in relation to climatic factors, suggesting that temperature is the only important parameter influencing the population build up of whitefly and high population build up was recorded during February-March and lower during August-September. Edison (1979) made a survey of whitefly population on cassava at ten different centres in Kerala and found that Ambalavayal (comparatively low temperature area) and Nileswar centres harboured the lowest population of whiteflies. The adult whiteflies were found more on young leaves as well as on old yellow leaves. Cultivars, H-226 and Kalikalan were more susceptible to field dissemination of the disease, which harboured more number of whiteflies (Shanta et al., 1984). The present study also showed that among the different varieties observed, Kalikalan was the most susceptible one with very high disease incidence. Malathi and Sreenivasan (1983) and Palaniswami and Nair (1995) in India reported unsuccessful transmissions of ICMV from cassava to cassava by Bemisia tabaci.

Cassava mosaic virus infected plants exhibited chlorotic areas on the leaves giving a mosaic pattern, severe leaf distortion and reduction in the size of the leaf lamina, stunting of plant growth and twisted leaf. Early infected plants were severely stunted. These symptoms were similar to those reported earlier (Alagianagalingam and Ramakrishnan, 1966; Sam Raj, 1966; Menon and Raychaudhuri, 1970; Mathew et al., 1989; Jos et al., 1984; Malathi et al., 1985). However, in the present studies, drying of leaf margin and vein clearing were also observed in cassava mosaic infected leaves. Malathi et al. (1985) reported that intensity of symptom expression varied with season and varieties, similar to the findings of the present study.

Survey results indicate that SLCMV is wide spread in all the districts of Kerala, except Thiruvananthapuram, where ICMV is predominant. In Pathanamthitta district, SLCMV and ACMV were found. In Malappuram district, ICMV, SLCMV and mixed infection of both ICMV and SLCMV were found.

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