

Transmission of Cucumber Mosaic Virus (CMV) infecting banana by aphid and mechanical methods

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Abstract: Banana is the premier fruit of the Asia and Pacific region. It is the fourth most important food crop after rice, wheat and maize. In Tamil Nadu, banana is grown in 92,200 hectares with an annual production of 48.56 million tones. Among all biotic constraints, viruses cause huge economic loss to the crop. Some of the major diseases are caused by viruses which include Banana Bunchy Top Virus (BBTV), Cucumber Mosaic Virus (CMV), Banana Streak Virus (BSV), Banana Bract Mosaic Virus (BBMV) have been reported in our country. CMV virus concentration in host (Musa) tissue is very less. So it is necessary to transfer this virus to good propagation host such as *Nicotiana* sp for purification. The purpose of the study was the transmission of CMV infecting banana into propagation host either by aphid or mechanically. The transmission was confirmed through Direct Antigen Coating Enzyme Linked Immuno Sorbent Assay (DAC-ELISA).

Keywords: Cucumber Mosaic Virus, transmission, aphid method, mechanical method, Direct Antigen Coating ELISA.

نقل فيروس موزاييك الخيار سى ام في من نبات الموز المصاب بواسطة حشرة البقعة
وبالطرق الميكانيكية

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يعتبر الموز هو الفاكهة الرئيسية في آسيا وإقليم المحيط الهادي. وهو رابع أهم محصول بعد الأرز والقمح والذرة. في ولاية تاميل نادو ينمو الموز بمساحته 92,200 هكتار وإنتاج سنوي يقدر بـ 48.56 مليون طن. ولكن من بين كل القيود البيولوجية، تسبب الإصابة بالفيروسات خسائر طائلة للمحصول. من بين الأمراض الرئيسية سببها الفيروسات مثل فيروس الموز العنقودي بي بي تي في، وفيروس موزاييك الخيار سى ام في، وفيروس شريط الموز بي اس في، وفيروس موزاييك الموز الورقي بي بي تي في والذي أعلن عن وجوده في الهند. يعتبر تواجد فيروس سى ام في في أنسجة نبات الموز ضئيل. لذلك من الضروري نقل هذا الفيروس لمضيف آخر جيد النمو مثل نيكوتانيا اس بي وذلك من أجل التطهير. الهدف من هذه الدراسة هي نقل فيروس سى ام في من نبات الموز المصاب إلى النبات المضيف بواسطة حشرة البقعة أو بطريقة ميكانيكية. الانتقال كان مثبتاً من خلال تحليل طبقة الانزيم المضاد المرتبطة بالمادة المانعة (DAC-ELISA)

Introduction

Banana is the premier fruit of the Asia and Pacific region. It is the fourth most important food crop after rice, wheat and maize. World production of bananas is around 70 million tonnes, with plantation of 30 million tonnes. Nearly half the world production of banana comes from Asia. Worldwide, 4.5 million hectares are devoted to banana

cultivation. Approximately 98 per cent of world production is grown in developing countries. More than 73 per cent of total banana production is contributed by ten major banana-producing countries. India, Ecuador, Brazil and China alone produce half of total banana output.

India's share is around 24 per cent. Global banana trade is currently estimated at \$4.7 billion. India is the biggest producer of bananas with 17 million

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tones. The major banana growing states are Maharashtra, Tamil Nadu, Gujarat, Assam, Karnataka, Kerala, Bihar, West Bengal, Andhra Pradesh, Orissa and Madhya Pradesh. Banana and plantains are good source of dietary carbohydrates; vitamins C & B6 and minerals but is fat-free and low in proteins. It has several medicinal properties (Simmonds, 1966). Fruits are good for the treatment of gastric ulcer, diarrhoea, cancer and heart diseases. Banana is widely grown under tropical and subtropical conditions.

Biotic factors caused by pests and diseases present constant threats to banana farmers. Biotic stresses like fungi, bacteria, nematodes, viruses and insect pests limit the production and productivity of banana. Among all biotic constraints, viruses cause huge economic loss to the crop. Bananas are affected by four known, relatively well-characterized viruses (Diekmann and Putter, 1996) these are Banana bunchy top virus (BBTV) genus Nanavirus, Banana streak virus (BSV) genus Badnavirus; Cucumber mosaic virus (CMV) genus Cucumovirus; and Banana bract mosaic virus (BBrMV) genus Potyvirus. Recently, a filamentous virus, Banana mild mosaic virus (BanMMV), (Thomas et al., 2000; Gambley and Thomas, 2001) has been shown to be widespread in banana and also been noted in bananas from Africa, the Americas, Southeast Asia, and Australia (Lockhart and Jones, 1995; Thomas et al., 2000) (Figures 1 and 2).

Banana bunchy top virus has wiped out the hill banana in Tamilnadu. A loss of about 80 million USD annually has been recorded due to BBTV in kerala (Metha et al., 1964). Banana bract mosaic and streak viral diseases of recent origin caused huge economic loss. The most of the commercial cultivars are known to be infected by BBMV. A hardy banana, Poovan grown throughout India has been a victim for BSV. CMV is still assuming importance in all the banana growing regions of India.

In India, the occurrence of CMV has been reported from many hosts such as Egyptian henbane (Samad et al., 2000), gladiolus (Raj et al., 2002), *Lycopersicum esculentum* (Sudhakar et al., 2006), *Geranium* (Verma et al., 2006), banana (Aglave et al., 2007), *Rauvolfia serpentina* and *Jatropha curcas* (Raj et al., 2007, 2008). However, only limited reports are available on biological and molecular characteristics of these isolates (Madhubala et al., 2005, Verma et al., 2004, 2006). CMV causes chlorosis, mosaic and heart rot in banana and has been found in most banana growing areas of the world.

Viral diagnosis of a virus infection in plants is the easy method of detection. Banana viruses known to exist in latent form without expressing any visual symptoms for long time. If mother plants used for mass multiplication are not indexed, the virus can easily pass through to the progenies. Hence it is a need to standardize and develop serodiagnostic techniques to identify the viruses, if they are in latent form.

The field identification of viral diseases caused by BBTV, BBMV, BSV and CMV based on the symptoms in banana and plantains is difficult at certain stages of disease development. Symptoms are of limited value in certain condition for the identification of the causative viral agent. Identification of the viruses by serological methods will be of more accurate, reliable, less time consuming and also cost effective. Banana is infected by several viral diseases of which Banana bunchy top virus; Banana bract mosaic virus, Banana streak virus and cucumber mosaic virus are the most serious viral diseases and have devastating effect on the cultivation of banana. Recently a new Banana mild mosaic virus has been reported by (Thomas et al., 1994).



Figure 1. Cucumber Mosaic Virus (CMV) infected Banana plant.



Figure 2. Banana Bunchy Top Virus (BBTV) infected plant.

CMV causes great losses in vegetables, ornamentals and fruits, and it is destructive due to its rapid spread by more than 60 aphid species in the field (Palukaitis and Garcia-Arenal, 2003). Transmission through planting material is also significant in some crop and weed hosts (Hsu et al., 2000).

Banana mosaic or infectious chlorosis is one of the important and widely distributed viral diseases of banana. Banana mosaic is cosmopolitan, and is found wherever bananas are grown. This virus is a member of the cucumovirus group and belongs to bromoviridae. CMV is easily transmitted by sap inoculation and by aphids in a non-persistent manner (Kaper et al., 1981). In some virus infections, the whole leaf may become chlorotic due to decreased chlorophyll production and the breakdown of chloroplasts.

CMV, which has alternate host and can be readily transferred by a member of insects vectors in a non – persistent manner (Bird and Wellman, 1962). Banana mosaic disease is transmitted successfully by *Aphis gossypii* and poorly by *Aphis craccivora* but not at all by *Pentalonia nigronervosa* in Puerto Rico. Shukla and Govinda (2000) made a detailed study to establish virus- vector relationship of aphid of CMV. He showed that a short preacquisition fasting (2hrs) and acquisition threshold (2min) given to *A. pisum* increased the efficiency of the aphid and that a single aphid can produce infection, although in low percentage and the percentage infection increases with an increase in the number of aphids.

Banana bunchy top is another serious virus found to reduce the banana production in India. Dale (1987) reported that the virus is present round the year in hill area but less significant in plains. BBTV spreads via undetected infection in suckers and the secondary spread is through the banana aphid, *Pentalonia nigronervosa* in a semi- persistent manner (Magee, 1927, 1940). Attempts on

mechanical transmission of BBTV have failed (Magnaye and Valmayor, 1995). CMV is transmitted in the non-persistent manner by several aphid species (Mali and Rajgore, 1980; Palukaitis and Garcia-Arenal, 2003).

Even though CMV infecting other than banana can be transferred to many hosts for the purpose of propagation and purification, it is always difficult to transmit the CMV banana isolate to propagation hosts either mechanically through sap or by aphid vectors. Considering the importance of banana and its viral pathogens, the present investigations were undertaken with a main objective of diagnosing viruses through serodiagnostic techniques such as Enzyme Linked Immuno Sorbent Assay (ELISA), Dot Immuno Binding Assay (DIBA) and Electro Blot Immuno Assay (EBIA). To accomplish the above objective, the studies on collection, maintenance of virus culture; transmission, host range and detection of CMV infecting banana were performed.

Materials and Methods

The present investigations were undertaken to identify the causal virus based on transmission and serological testing. The research work has been carried out at Molecular Virology Laboratory, National Research Center for Banana (NRCB), Indian Council of Agricultural Research, Tiruchirappalli, Tamil Nadu, India.

Maintenance of virus

The Banana Mosaic Virus, symptomatic samples collected were maintained at 28°C in the insect proof glass house at NRCB, farm. Healthy plants were also maintained in the same glass house.

Maintenance of other hosts

For mechanical sap inoculation and transmission through aphid species were done on *Commelina* sp, *Nicotiana glutinosa*, *Vigna radiata*, *Vigna mungo* and

Chenopodium spp in addition to banana. The experimental hosts were raised through seed and maintained in 10-inch pots with standard pot mixture.

Maintenance of aphids

Three aphid species such as *Aphis craccivora* maintained in cowpea, *Aphis gossypii* maintained in cotton seedlings and *Pentalonia nigronervosa*, the banana black aphid was multiplied in small healthy banana plants in separate room.

Methods of transmission

Mechanical transmission

For transmission and host range studies, mechanical inoculations were carried out by extracting banana tissues infected with CMV in 0.1 M phosphate buffer, pH 7.0 containing 1.0% sodium sulphite (1:2W/V). The infectious sap was applied to healthy *Commelina* sp, *Nicotiana glutinosa*, *Vigna radiata*, *Vigna mungo* and *Chenopodium* spp in addition to banana. Leaves of the inoculated plants were previously dusted with 400 mesh carborandum. For control treatment carborandum dusted leaves were inoculated with phosphate buffer alone. Inoculated plants were maintained in the greenhouse at 25-30°C for 30 days and the plants were inspected daily for symptom development. The inoculated plants were serologically tested using CMV antiserum.

Aphid transmission

Individuals of the aphids *Aphis gossypii* collected from cotton plants and *A. craccivora* collected from cowpea. Individuals of insect were reared onto healthy cotton and cowpea seedlings. New generation of aphids were transferred to other healthy plants of cotton and cowpea growing in insect proof cages and the aphids were left for reproduction. Several virus free adults of each of the above mentioned aphids were starved for two hours.

They were allowed to feed for 20 min (acquisition period). After that the aphids were transferred to healthy *Commelina* sp, *Nicotiana glutinosa*, *Vigna radiata*, *Vigna mungo* and *Chenopodium* spp in addition to banana allowed to feed for 10-15 min. (inoculation period), and then the insects were sprayed by insecticide (Malathion) The plants were maintained in insect proof cage at 25-30°C for 30 days and the plants were inspected daily for symptoms development.

Serodiagnosis of CMV

DAC-ELISA

DAC- ELISA test was performed as described by Clark and Joseph (1984) to test the presence of CMV in collected plants using CMV antiserum.

One-gram midrib portion of leaf sample collected and ground in liquid nitrogen. One ml of 0.2M potassium phosphate buffer with 0.5% sodium sulphite and 1% polyvinyl pyrrolidone (PVP) was added. The sample squeezed gently with absorbent cotton and equal volume of chloroform was added and vortexed thoroughly. The homogenate was centrifuged at 5000rpm for 5min and the supernatant was used for coating the ELISA plates.

Source of antiserum

Cucumber Mosaic Virus antiserum provided by Dr. Ishwar Bhat, Senior Scientist, IISR, Calicut, Kerala, India was used for serodiagnosis study.

Plates were coated with 200µl of partially purified viral antigen sample and incubated at 37°C for 1 hr or over night at 4°C. After incubation, the well's contents were discarded and washed with PBS-T buffer thrice (flooding with PBS-T for 3min every time). The blocking solution was added (200 µl / well) and incubated for 1 hr at 37°C. After washing the plate (thrice with PBS-T), 200µl of diluted rabbit antiserum (1: 500) was added in the wells and incubated at 37°C for 1½ hrs.

The plate was washed and 200 µl of enzyme conjugate (anti - rabbit alkaline phosphate) was added and incubated at 37°C for 1½ hrs. After incubation, the plates were washed with PBS-T. After final washing, 200µl of substrate (p-Nitro phenyl phosphate- 0.6 mg/ml) was added and allowed for color development at room temperature by avoiding exposure to light. Then the reaction was stopped by adding 3M NaOH and the color intensity was read in (Asys Hi-tech ELISA) reader at 405nm.

Results and Discussion

Sap Transmission of CMV

Fifty plants of *Tobacco*, *Chenopodium* and *V. mungo* were sap inoculated by using carborundum powder with CMV. The results revealed that Tobacco plants expressed the CMV symptom after three months of inoculation. Transmission of CMV causes severe mosaic and leaf deformations in inoculated plants, which was confirmed through DAC-ELISA.

Aphid Transmission of CMV

Vector transmission studies using two aphid species *Aphis craccivora* and *A. gossypii*, both species are efficiently transmitted the virus in a non-persistent manner within 20 min of acquisition and inoculation of 10 to 15 min. Thirty plants of Robusta and Tobacco were inoculated with infective *Aphis gossypii* and thirty plants of Tobacco, Cowpea and Commulina inoculated with infected *Aphis craccivora*. Some of the Tobacco plants showed the symptoms of CMV after three months of inoculation (Figures 3 and 4). These results revealed that the CMV is aphid transmissible and agreed with the data recorded by Rao (1980) who reported that the successful transmission of CMV to test plants by *A. gossypii*. Mali and Rajegore (1980) who indicated that CMV isolated from banana was transmitted by *A. craccivora* to healthy plants.

Serological diagnosis for confirmation of transmission

This method is very efficient even in detecting symptom less plant. The results revealed that positive control recorded a mean absorbance of 0.358. The negative control recorded a mean absorbance of 0.148. Among the 18 samples collected from the aphid inoculated plants, only two samples of Tobacco recorded positive result with values similar to that of positive control and the other samples recorded values lower absorbance than that of the positive control (Table 2).

On inoculating two months old healthy plant seedling with virus extract, symptoms appeared on tobacco leaves after 90 days. Out of nine sap inoculated plants only three samples of Tobacco recorded an absorbance similar to the positive control. Except Tobacco the other plants were recorded lower absorbance value, which is similar to that of the negative control at 405nm, which revealed that CMV was not detected in the following plant spp. *Chenopodium* and *V. mungo* (Table 1 and Figure 5).

The present investigation results has clearly revealed the occurrence and association of cucumber mosaic virus with banana chlorosis was identified based on mechanical transmission, insect vector transmission, host range, serology DAC-ELISA detection. The virus was able to transmit mechanically on the plants used in the study very efficiently and produced the systemic and local symptoms characteristic of CMV. In our present study both the aphid species (*A. gossypii* and *A. craccivora*) transmitted the virus in non persistent manner. Patil (1982) reported that CMV from banana could be transmitted by rhizome inoculation. The ability of CMV to be transmitted declines after about 2 minutes and is usually lost with in 2 hours (Francki et al., 1979). Some investigators (Magee, 1930; Calinisan, 1938; Silberschmidt and Nobrega, 1941) have reported the successful transmission of banana mosaic

virus by the banana aphid *P.nigronevosa*, where as others have reported negative transmission (Bird and Wellman, 1962; Mali and Rajegore, 1980). CMV has the broadest host range among the plant viruses (Roosinck, 2002) and has been reported infecting banana and causes banana chlorosis disease. CMV has been identified in other vegetative propagated crops such as Geranium (Verma et al., 2004), Carnation (Raj et al., 1993), Chrysanthemum (Srivastava et al., 1992), based on biological characterization, serological relationship and electron microscopy.

Serology is the most reliable and quick method of detection of CMV infection in banana (Thomas et al., 1991). Presence of CMV in banana can easily be detected by RT-PCR technique (Zora singh

et al., 1995). The virus can also be detected by cDNA probe to RNA genome of CMV (Kiranmani et al., 1997). Mali and Rajegore (1980) found that the banana mosaic virus precipitated against the antiserum of CMV using tube ring inter- phase and agar gel diffusion tests. The DAC- ELISA was adopted to detect viruses in plants. It has been reported infecting banana and causes banana chlorosis disease from various parts of the world including Maharashtra (Mali and Deshpande, 1976).

In our study the transmission was confirmed through DAC-ELISA technique. It has been concluded that the transmission of CMV was more successful to *N. glutinosa* by mechanically and through aphids.

Table 1. Detection of CMV from asymptomatic host plants through DAC-ELISA.

	1	2	3	4	5	6	7	8	9	10	11	12
A	N	+	++	+	B	+	++	+	B	+	+	+++
B	B	+	+	+	B	+	++	+	B	+	+	+++
C	N	-	-	-	B	-	-	P	B	-	-	B
D	B	-	-	-	B	-	-	N	B	-	-	N
E	-	-	-	-	B	-	-	-	-	-	N	-
F	-	-	-	-	N	-	-	-	-	-	B	-
G	N	P	-	P	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
H	B	B	-	B	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

N-Negative control, P-Positive control, B- Buffer control

A₂-A₄ & B₂-B₄ - Mechanical Transmission of CMV into *Nicotiana glutinosa*

A₆, A₇, A₁₁, B₆, B₇, B₁₁ - Transmission of CMV by *Aphis craccivora* into *Nicotiana glutinosa*

A₈, A₁₀, A₁₂, B₈, B₁₀, B₁₂ - Transmission of CMV by *Aphis gossypii* into *Nicotiana glutinosa*

C₂-C₄, C₆, C₇, D₂-D₄, D₆, D₇ - Transmission of CMV by *Aphis gossypii* into *Musa*

C₁₀, C₁₁, D₁₀, D₁₁-Mechanical transmission of CMV into *Chenopodium*

E₁-E₄, E₆&F₁-F₄, F₆ - Transmission of CMV into *Vigna unguate* by *Aphis craccivora*

E₇-E₁₀ & F₇-F₁₀- Mechanical transmission of CMV into *Vigna Mungo*

E₁₂, G₃ & F₁₂, H₃-Transmission of CMV by *Aphis craccivora* in *Commelina*

Table 2. OD values recorded in ELISA. The colour developed was read at 405 nm in the ELISA reader.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.043	0.269	0.358	0.248	-0.002	0.178	0.268	0.125	0.001	0.131	0.171	0.409
B	0.036	0.218	0.294	0.201	-0.003	0.158	0.269	0.118	-0.013	0.093	0.101	0.496
C	0.038	0.08	0.085	0.053	-0.01	0.033	0.02	0.307	-0.011	0.018	0.023	-0.015
D	0.006	0.086	-0.016	0.05	-0.009	0.04	0.036	0.008	-0.007	0.022	0.065	0.015
E	0.012	0.05	0.016	0.039	-0.001	0.017	0.025	0.016	0.019	0.002	0.001	0.079
F	-0.016	0.07	0.021	0.03	0.066	0.017	0.018	0.012	0.03	0.014	-0.006	0.051
G	0.03	0.329	0.006	0.353	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
H	0.019	0.025	0.012	-0.003	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil



Figure 3. Transmission of CMV by *Aphis gossypii* into *N. glutinosa*.



Figure 4. Transmission of CMV into *V. unguate* by *Aphis craccivora*.

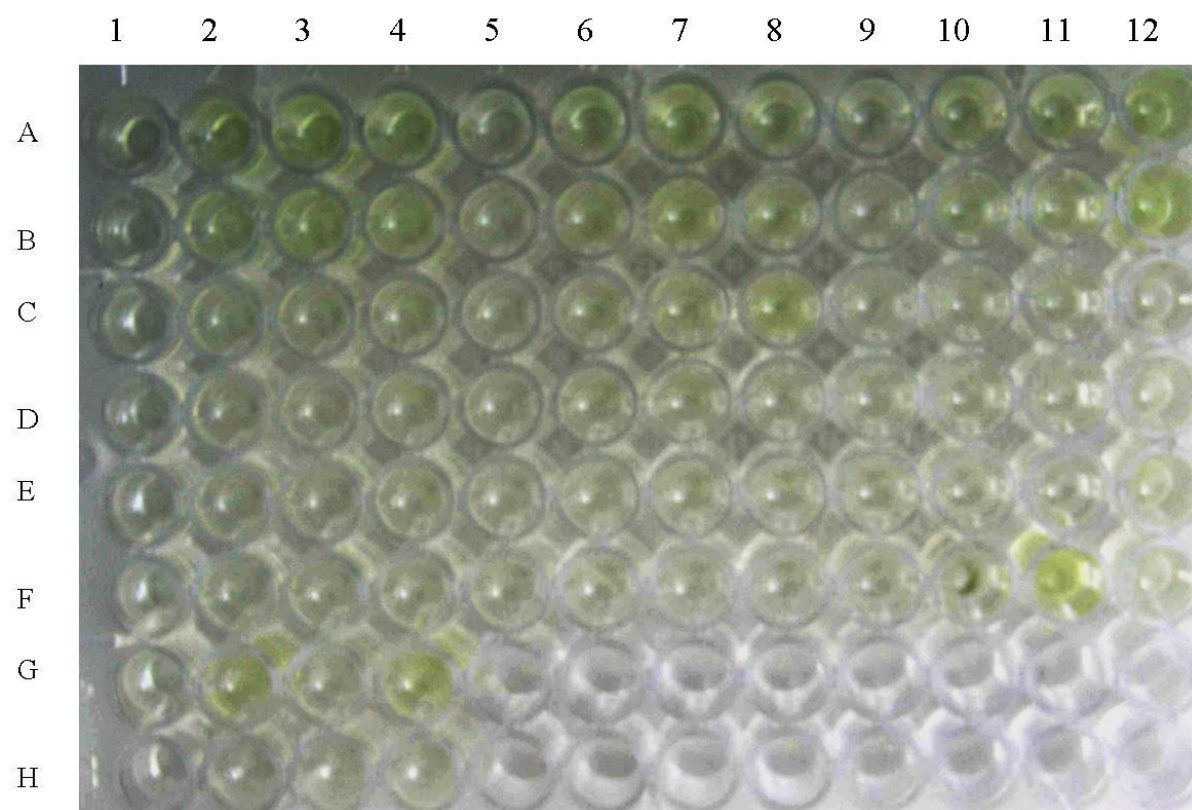


Figure 5. Detection of CMV from asymptomatic host plants through DAC-ELISA. Yellow colour - positive; No colour development - Negative.

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