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The Virus Causing Infectious Chlorosis in Banana (*Musa* sp.): A Review

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A B S T R A C T

Banana fruit crops are high in potassium, contain a high level of protein and dietary fiber which makes it world's most popular. The crop gives a maximum return to the farmers when good cultivation practices are followed, which include using disease-free planting material. The crop is infected by several viruses viz., Banana bunchy top virus, Cucumber mosaic virus, Banana streak virus, Banana bract mosaic virus and Banana mild mosaic virus (Tripathi et al., 2016). Among these viruses, CMV causes a devastating effect on tissue culture banana plants. Various symptoms of CMV were reported under natural conditions like diamond-shaped discontinuous lesions, severe mosaic with extreme distortion and reduction of leaf lamina. KAU (2016) opined that infection due to CMV was observed in widely cultivated banana varieties in Kerala, such as Nendran, Palyankodan, Karpooravally and Poovan (Rasthali). Hence, virus indexing is a must to select healthy planting materials. Cucumber mosaic virus is transmitted through more than 60 species of aphids, including *Aphis gossypii* and *Myzuspersicae*etc. The serological methods are widely used in the detection of Cucumber mosaic virus from the field.This review paper is focused on various aspects of novel detection methods of CMV infecting banana.

Introduction

Banana (*Musa* spp.) belongs to the genus Musa and family Musaceae of the order Zingiberales. The plant plays an amicable role in religious and cultural occasions, hence the crop has the name Kalpatharu (Plant of virtues). When banana is infected with virus it effects the production directly by reducing plant growth and yield. It causes yield losses of about 40-100 percent (Gambley and Thomas, 2001). Economically important

viruses infecting banana are *Banan bunchy top virus, Banana streak virus, Banana bract mosaic virus and Cucumber mosaic virus (CMV).* Banana viruses also have important indirect effects by restricting germplasm movement and predisposing plants to damage from other biotic and abiotic stress factors.

Cucumber mosaic virus was first reported simultaneously by Doolittle (1916) and Jagger(1916) in cucumber. This is an emerging viral disease in Kerala, India which causes leaf distortion, stunting of plant and yield reduction. *Cucumber mosaic virus* taxonomically grouped under family *Bromovirdae*, which contain five genera i.e., *Alfamovirus, Ilavirus, Cucumovirus, Oleavirus* and *Bromovirus*. It is important to understand the characteristics of each virus for effective control of viral diseases and the development of reliable virus detection methods. Biological, serological and molecular methods like Direct antigen coating enzyme-linked immunosorbent assay (DAC-ELISA) and Reverse transcriptase-polymerase chain reaction (RT- PCR) are used to detect CMV from field. Antiserum production is an essential prerequisite for serological detection. Coat protein (CP) region of CMV in a banana is sufficient enough to provide a reliable method for the detection of virus. This review article comprises the chronicle of biological, genomic, post-genomic and diagnostic studies of *Cucumber mosaic virus.*

Genome organisation of *Cucumber mosaic virus*

Members of the *Bromoviridae* family show significant diversity in their coat protein architectures. *Alfamovirus* having genome encapsidated in 19 nm wide bacilliform capsids (*Hull et al.,* 1969). Bacilliform or quasi-spherical particles of varying sizes virus structure is observed in Oleavirus (Martelli and Grieco, 1997). *Anulavirus* (Gallitelli *et al.,* 2005) and Ilarvirus (isometric labile ringspot virus) particles are quasi-spherical (Lister *et al.,* 1972) and have sizes which depend on the type and length of packaged RNA. According to ICTV (2012), Cucumber mosaic virus causing infectious chlorosis in banana belongs to the family *Bromoviridae.* The entire genome consists of approximately 8 kb in length. Genomes comprise of three linear, positive sense ssRNAs with 5'-terminal cap. The 3'termini are not polyadenylated but generally are highly conserved within an isolate or species. They are either tRNA-like and can be aminoacylated (genera *Bromovirus* and *Cucumovirus*). Major viral proteins associated with *Bromoviridae* family are enlisted in Table 1.

Cucumber mosaic virus is the type species of the genus *Cucumovirus* in the family *Bromoviridae*. It encloses three spherical particles, each approximately 28 nm in diameter. Subgenomic RNA (sgRNA) expresses a third nonstructural protein P2b. This helps in movement from cell-to-cell. Nonstructural movement protein (i.e., P3a, cell-to-cell MP) post-transcriptional gene silencing and RNA 3 represents the structural capsid protein or coat protein (P3b, CP) that is expressed via subgenomic RNA (*i.e*., RNA4) (Hull, 2009; Zitter and Murphy, 2009).

Symptoms developed by the virus

Downward bending of the petiole and leaf surface along with leaf reduction and severe epinasty, are the common symptoms of the virus in cucurbits. Plants infected at the early stage are severely stunted, leaves are malformed, and fruits are unmarketable because of obvious rugosity (Agrios, 2005; Zitikaite *et al.,* 2011). Shoestring of upper leaf blades in tomatoas a distinctive symptom in the crop was reported by Sudhakar *et al.,* (2006) and Aglave *et al.,* (2007). Srivastava

et al., (1992), Madhubala *et al.,* (2005) and Bhadramurthy *et al.,* (2009) recorded the symptoms caused by CMV in chrysanthemum, vanilla (*Vanilla planifolia*) and paprika (*Capsicum annum* L.) respectively.

The first signs of *Cucumber mosaic virus* infection in banana were noticed in Australia by Magee (1930). According to the external symptoms observed the disease was named infectious chlorosis, heart rot, virus sheath rot, cucumber mosaic and banana mosaic (Stover, 1972). Mosaic patterns or discontinuous linear streaking bands, extending from leaf margin to midrib are the characteristic symptoms of infectious chlorosis. Curling of leaves, rosette appearance of leaf arrangement and dead or dying suckersare noticed in advanced cases (Niblett *et al.,* 1994; Rodoni *et al.,* 1997; Sivaprasad *et al.,* 2016; Tripathy, 2016). Banana mosaic is categorised as cosmopolitan and is found wherever bananas are grown. Even at a low titer of the virus, the whole leaf may become chlorotic due to decreased chlorophyll production and breakdown of chloroplasts (Dheepa and Paranjothi, 2010). The symptoms occurred sporadically and the majority of the leaves appeared healthy. The expression of symptoms can be influenced by virus strain and temperature (Hitchborn, 1956). Among all the strains of *Cucumber mosaic virus* infecting banana, heart rot strain causes significant losses, due to the rotting of inner leaves leading to the death of the plant (Lockhart, 2000).

In Kerala, the disease is considered as an emerging one and the symptoms were documented. The infected plants become dwarf and lag in growth. The infected plants mask the symptoms and act as a virus reservoir. But the plants become dwarf and lag in growth. Infected leaves produce parallel chlorotic streaks on younger leaves; later leaves become distorted, irregular wavy leaf margin along with necrotic tissues (KAU, 2016; Mujtaba, 2017). So far, no strains of the virus, causing heart rot are reported from Kerala, India (Antony, 2019).

Distribution of the virus

Cucumber mosaic virus is geographically widespread and having broad host range, including some annual crops in temperate zones, tropical regions and Mediterranean countries (Tomlinson, 1987). *Cucumber mosaic virus* (CMV) was first reported in detail on cucumber and other cucurbits but is now known to occur worldwide in most of the crops (Roosinck *et al.,* 1999; Zitter and Murphy, 2009; Sokhandan-Bashir *et al.,* 2012). *Cucumber mosaic virus* isolates were phylogenetically analysed and the subgroup I have subdivided into IA and IB. Among the subgroups, subgroup 1B of CMV is limited to Asia, and the other two subgroups (*i.e.,* 1A and 2) are distributed worldwide (Sivaprasad *et al.,* 2016).

In India, CMV occurrence has been reported in commercially grown flowers and spices such as chrysanthemum (Srivastava *et al.,* 1992); carnation (Raj *et al.,* 1993); black pepper (Sharma *et al.,* 2001); and periwinkle (*Catharanthus roseus*) (Samad *et al.,* 2008). Bhadramurthy (2008) reported that CMV causes mosaic symptoms in vanilla. The virus has been reported in *Oxalis corymbosa* in Aligarh, India (Sheikh *et al.,* 2013).

According to Estelitta *et al.,* (1996) and Mujtaba (2017), CMV is an emerging threat in banana farmers in Kerala, especially in the fields where, cucurbitaceous vegetables as intercrops in banana. In Kerala, infection of CMV was noticed in banana varieties such as *Karpooravally*, *Nendran*, *Palayankodan*, *Peykunnan*, *Kosthabontha*, *Mottapoovan*, *Bhimkhel*, *Dhakhinsagar*, *Madhuraga*, *Rasthali* and *Musa ornate* (KAU, 2016).

Detection of the virus

So far, there is no protocol for the treatment of plant viral diseases, hence its detection is very crucial.As far there is no treatment protocol for plant viral diseases, detection of the virus causing infection is very crucial. The techniques applied for the diagnosis of plant viral diseases include biological, serological and molecular means (Lopez *et al.*, 2003). When the sample size to be tested is large, double antibody sandwich-enzyme linked immunosorbent assay is widely used. Virus detection methods have upgraded greatly in recent years with the development diagnostic techniques that can be applied directly in the field.

Nucleic acid probes methods , RT-PCR and ELISA were used for the differentiation of CMV isolates in chrysanthemum (Srivastava *et al.,* 1992), carnation (Raj *et al.,* 1993), banana (Kiranmai *et al.,* 1996), geranium (Verma *et al.,* 2004), gladiolus, pepper and vanilla (Madhubala *et al.,* 2005), and anthurium (Miura *et al.,* 2013).

Molecular diagnosis

Molecular techniques are powerful, sensitive and popular methods used for detection of plant viruses and viroids (Hu *et al.,* 1995).It is widely used by researchers in scientific field such as molecular cloning, gene manipulation, gene expression analysis, sequencing, and mutagenesis (Lundberg *et al.,* 1991; Makkouk and Kumari, 2006; Verkuil *et al.,* 2008). RT-PCR is used to detect RNA viruses like CMV, which includes reverse transcription of RNA followed by normal PCR (Choi *et al.,* 1999; Ghangal *et al.,* 2009; Jeong *et al.,* 2014).

This method was well-known to detect seed-borne infection and seed transmission frequency of CMV in pepper seed (Ali and Kobayashi, 2010). The RT-PCR in turn is used for genomic and post genomic studies of RNA viruses. Amplification of CP (using gene specific primers) of CMV infecting banana and other crops at ~700bphas reported (Zein and Miyatake, 2009; Ali *et al.,* 2012; Khan *et al.,* 2012; El- Borollosy and Hassan, 2014; Shetti *et al.,* 2014; Antony, 2019). Sudhakar *et al.,* (2006), detected virus infecting tomato by RT-PCR and restriction fragment length polymorphism analysis (RFLP).Molecular detection has standardised for CMV infecting *Oxalis corymbosa*, a common weed of banana orchads(Sheikh *et al.,* 2013). Southern hybridisation test is is also used for sensitive detection of CMV from gladiolus leaf and corms (Pandey, 2015).

Molecular detection of virus variability

Deoxy ribonucleic acid sequencing is the process of determining the molecular sequence of particular gene, which determines the order of the four nitrogen bases *viz*., adenine, guanine, cytosine, and thymine.The sequence of the CP gene of the CMV from paprika (*Capsicum annuum* L.) contained a single open reading frame of 657 nucleotides potentially coding for 218 amino acids (Bhadramurthy *et al.,* 2009).

Indian isolates of CMV, infecting various crops were sequenced and found out its homology with subgroup II of CMV (Kumar *et al.,* 2005; Kumari *et al.,* 2013). High sequence identities and evolutionary tie in coat protein gene has been observed with CMV isolate fromKerala (Mujtaba, 2017). Serological detection of BSV has been problematic due to serological and genomic heterogeneity of the virus isolates (Selvarajan *et al.,* 2016).

Table.1 Details of viral protein encoded in *Bromoviridae*

Protein	Size (kDa)	mRNA	Function
1a	102.7–125.8	RNA 1	Helicase, Methyltransferase
2a	78.9–96.7	RNA 2	Replicase
3a	30.5–36.5	RNA 3	Cell to cell movement
Coat protein	19.8–26.2	Sub-genomic RNA- 4	Encapsidation, intercellular movement

Table.2 Major plant virus coat protein expressed through *in vivo* protein expression system

Expressed gene	Expression system used	Reference
***Cucumber mosaic virus* (Cucumber isolate) coat / capsid protein (CP)**	pET21a/*E. coli* strain Rosetta	Rostami *et al.*, (2014)
***Tobacco streak mosaic virus* CP**	pRSET- C/ *E. coli* (DE3) BL21	Gulati *et al.*, (2016)
***Alfalfa mosaic virus* CP**	*E. coli*/ pTrcHisB	Yusibov *et al.*, (1996)
***Pepper vein banding virus* encoded protein**	pRSETC/*E.coli*(DE3)BL21	Sabharwal (2017)
***Cardamom mosaic virus* CP**	pProEXHTb/ *E. coli*	Jacob and Usha (2002)
***Banana bract mosaic virus* CP**	pMAL-c2/*E.coli* (DE3)BL21	Wanitchakorn *et al.*, (1997)
***Grapevine leafroll associated closterovirus*-3 CP**	pRSET-C/*E. coli* (DE3)BL21	Ling *et al.*, (2000)
***Sugarcane streak mosaic virus* CP**	pRSET-A/E. coli (DE3)BL21	Hema *et al.*, 2003
***Prune dwarf virus* CP**	pRSET/*Epicurian coli* BL 21-Gold	Jawdah *et al.*, (2004)
***Nipah virus* matrix protein**	*Spodopterafrugiperda*- 9 (sf- 9) cell line using baculovirus expression system	Dezfooli *et al.*, (2016)
Viral associated protein of *Banana streak virus*	*E. coli* based expression system	Selvarajan *et al.*, (2016)
***Papaya ringspot virus* CP**	pRSET-B/*E.coli* DH5α	Valekunja *et al.*, 2016
***Banana bunchytop virus* CP**	pET28a (+)/*E. coli* BL21	Arumugam *et al.*, (2017)
***Grapevine fanleaf virus* CP**	pET28a/ E. coli (DE3)BL21	Shibaei *et al.*, 2018
***Cucumber mosaic virus* CP**	pQE30/*E. coli* M 15	Khan *et al.*, (2012)
	pRSET-B/*E. coli* DH5α	Pandey (2015)
	pET21- d(+)/E. coli (DE3)BL21	Kim *et al.*, (2016)
	pET21a/*E. coli*starin Rosetta	Koolivand *et al.*, (2017)
	pRSET-C/*E. coli* (DE3) BL21	Antony (2019)

Serological diagnosis

Detection of plant viruses based on symptoms are of limited value in certain condition. So, identification of the viruses by serological methods will be of more accurate, reliable, less time consuming (Dheepa and Paranjothi, 2010).Enzyme Linked Immuno-Sorbent Assay has been successfully used for the large scale detection of plant viruses including *Banana bunchy top virus*, *Banana bract mosaic virus* and *Cucumber mosaic virus*(Clark and Adams, 1977; Clark, 1981; Espino *et al.,* 1989; Geering and Thomas 1996; Kiranmai *et al.,* 1996; Ling *et al.,* 2000; Shetti *et al.,* 2014). Several other serological methods are available for the detection of plant viruses like lateral flow test and Immunocapture-Reverse Transcriptase-Polymerase Chain reaction (IC- RT- PCR) (Komorowska and Malinowski, 2009; Zein and Miyatake, 2009).

Different serological assays used for detection of CMV are immunodiffusion (Scott, 1968), tube and ring precipitin tests (Mink *et al.,* 1975), western blotting (Towbin *et al.,* 1979), SDS immunodiffusion in agarose gel (Purcifull *et al.,* 1981). *Cucumber mosaic virus* isolates are detected using Triple Antibody Sandwich Enzyme Linked Immune-Sorbent Assay (TAS-ELISA) and IC-RT-PCR (Yu *et al.,* 2005). Wu and Su (1990) developed plate-trapped antigen (PTA) - ELISA using monoclonal antibodies, to detect BBTV. Agindotan *et al.,* (2003) reported the higher sensitivity of immune- electron microscopy (IEM) for detecting *Banana streak virus* (BSV). Hosseinzadeh *et al.,* (2012) detected CMV by DAS- ELISA in 10 crops *viz*., tomato, pea, watermelon, tobacco, broad bean, soybean, squash, eggplant, cucumber and lettuce. Among these, the highest and the lowest CMV infection was associated with watermelon (62.44 per cent) and lettuce (Zero per cent), respectively. Detection of CMV has been done using antisera developed against recombinant coat protein (rCP) of the virus (Khan *et al.,* 2012; EI- Borollosy and Hassan, 2014).

Protein expression and purification

High quality viral antibody with less contamination of host proteins is an essential pre requisite for virus indexing. Hence, Hochuli *et al.,* (1987); Chow (2006); Hartley (2006) standardized the protocols for cloning of virus coat protein gene in expression vectors and purification of recombinant protein. Through this method, virus coat protein with less contamination of host proteins can be prepared and thus, the same can be used for antiserumproduction.

Expression of plant viral coat protein is highly preferred by cell based (*in vivo*) expression system, which include suitable expression host and vector (Nettleship *et al.,* 2010). *E. coli* BL21 (DE3) pLysS is the most commonly used expression host, which is a derivative of *E. coli* BL21(DE3). DE3 is an arrangement of T7 RNA Polymerase gene, under the control of LacUV 5 promotor on a phage genome and pLysS is a plasmid that encodes T7 lysozyme gene. The T7 RNA lysozyme bind to T7 RNA polymerase gene, and block the induction until the addition of IPTG. After the addition of IPTG, number of T7 RNA polymerase gene increases and overcomes the inhibition of LysS (Rosano and Ceccarelli, 2014). Major plant viral coat proteins expressed through in vivo system of protein expression are enlisted in Table 2.

In conclusion, infectious chlorosis caused by CMV has attained a serious status in most of the banana growing states of India. Realizing the potential threats of cucumber mosaic disease of banana, it is feared that in future Indian banana growing areas might be highly affected by this disease (Khan *et al.,* 2011).

Aim of this paper illustrates immune-detection and cloning of CP gene of field isolates to correctly diagnose the disease and to assess similarity or variability among isolates of CMV infecting banana. This paper compares the conventional partial purification of virus over, recombinant coat protein production. During high speed ultra-centrifugation, plant proteins are also get contaminated with virus coat protein, in turn the same will contaminate the antiserum, which often led to false result during immune detection of the virus. But recombinant coat protein mediated antiserum, as it doesn't contain plant protein, detection the virus with maximum efficiency.

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