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

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ABSTRACT

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 Myzus persicae 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.

Keywords
Cucumber mosaic virus, Serology, Symptoms, Virus Indexing, Vector, Serology

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 is grouped under family Bromoviridae, 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 (Galli et al., 2005) and Ilarivirus (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 tomato 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 suckers are 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 IB of CMV is limited to Asia, and the other two subgroups (i.e., IA 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., 2005). 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, cucurbaceous vegetables as intercrops in banana. In Kerala, infection of CMV was noticed in banana varieties such as Karpooravally, Nendran, Palayankodan, Peykunnan, Kosthabontha, Mottapooovan, Bhimkel, 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 as 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 of 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 (Kirnma 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 orchards (Sheikh et al., 2013). Southern hybridisation test 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 from Kerala (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- CI/ 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. colistarin 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) (Komorewoska 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 Immuno-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; El 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 Ceccharelli, 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 ultracentrifugation, 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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