



A Comprehensive and Brief Review on Appraisal of *Cucumber Mosaic Virus* Infecting Vegetable Crop *Capsicum spp.*

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ABSTRACT

Capsicum is an important vegetable crop in most part of the world. Viral diseases are the most important diseases that cause huge losses to Capsicum. Many viruses infect Capsicum and sometimes, plants are infected by a combination of viruses. Among them *Mosaic disease* is one of the most important viral disease caused by *Cucumber Mosaic virus* (CMV) of Capsicum worldwide. From the beginning of the discovery of CMV in Capsicum on 1960s, a number of reports published till date on the disease incidence, host range, diagnosis, pathogenicity, severity and losses due to infection, biological and molecular characterization, genome organization, several biological, cultural, biochemical and biotechnological approaches for disease management and even development of transgenic Capsicum resistant to this virus are mostly studied. Taking into consideration of the importance of this devastating virus causing great yield loss in Capsicum crop, there is an urgent need to thoroughly study about the trend of the infection and severity of CMV alone or in combination with other Chilli viruses in the host. In this brief review, most of the reports are basically focused on the disease incidence study in various geographical locations in the world along with detection techniques implied and possible management practices to control this disease in sustainable are also discussed.

Keywords: Chilli, *Cucumber Mosaic virus*, technique, symptom, reaction, host

Capsicum or chilli is an important vegetable worldwide for its green immature fruits used in various preparations and salads. It is important cash crop of temperate and tropical regions alike. It also grows well in cooler weather prevalent in different parts of temperate regions throughout the world (Kohli, 1996). Many bacterial, viral and fungal diseases cause economic losses to Capsicum cultivation. Among these, viral diseases are of great importance for farmers due to severe loss in quality and yield even though they have variable range of symptoms that differ greatly from one host to the other. This problem is predominant, because of lack of awareness, specific identification and screening strategies of virus infected plants in field condition and their proper management. Also, viruses are transmitted in different ways (mechanical, by aphids or thrips). So identification

of the causal agent and hygiene steps (as per most prevalent pathogens) is the key to reduce the spreading of viral infection in the field (Nederhoff, 1998).

Though a number of plant viruses are reported to infect the Capsicum vegetable but on the basis epidemiological review still studied on virus diseases of vegetables, Cucumber mosaic cucumovirus (CMV) is considered as one of the most primitive viruses (Tomlinson, 1987). This virus is well-known for its devastating nature of infection and even caused yield loss more than 60% in Capsicum (Pepper) (Joshi and Dubey, 1973; Florini and Zitter, 1987).

It is often difficult to distinguish CMV isolates from other cucumoviruses (i.e. *Tomato aspermy virus*, *Peanut stunt virus*). Its ubiquity may be attributed to its broad

natural host range, non-persistent transmission by a large number of aphid species, and transmission through seeds in some hosts (Brunt *et al.*, 1990; Douine *et al.*, 1979; Tomlinson *et al.*, 1970a; 1970b). These factors make prediction and management of CMV outbreaks difficult. As far as detection is concerned, another problem arises in case of CMV is that, many variants of this virus occur, making it difficult to identify the isolate from symptoms alone. Traditionally, diagnosis of plant viruses requires bioassays, an indicator plant, symptom observation, host range determination, and virus particle morphology, biochemistry and immunology has lead to the development of many new accurate, rapid and less labour-intensive methods of virus detection (Katoch *et al.*, 2003). Strategies need to be devised for detection of multiple virus infections and strategies for simultaneous detection of multiple plants (James *et al.*, 2006). So specific techniques are in great need to identify CMV in Capsicum to achieve the ultimate objective of viral management.

So considering the insufficiency of a compact review that depicts the overall incidence report as well as detection of CMV in Chilli in different time frames and concrete cum comprehensive study on the distribution, diagnosis and mostly adopted and newly encouraged advanced strategies for disease management would make a most promising way to combat against this vulnerable disease.

Review on studies carried out on CMV infecting Capsicum crop

At the beginning of the history of host range study of CMV, a number of vegetable crops including Capsicum were appeared as host for this virus. In 1960, it was reported that more than 25 vegetables act as host for CMV and they were cucumber, muskmelon, squash, tomato, spinach, celery, chilli, water cress, beet, sweet potato, turnip, chayote, gherkin, watermelon, pumpkin, citron, gourd, lima bean, broad bean, onion, ground-cherry, eggplant, potato, rhubarb, carrot, dill, fennel, parsnip and parsley (Chupp and Sherf, 1960).

In the year of 1971, CMV with other viruses viz. Potato virus Y (PVY), Nasturtium ringspot virus (NRSV) and Tobacco Mosaic virus (TMV) were found in Piemonte and Umbria crops in an area of Italy where CMV caused mild mosaic of leaves and ring spots on Capsicum fruits (Conti *et al.*, 1971). In Mexico, CMV and other two viruses Tobacco etch virus (TEV) and TMV were identified from their host range and serology from

infected samples of Capsicum which was used as a source of inoculums (Delgado-Sanchez, 1974). Bock *et al.* from Kenya isolated CMV from Capsicum and Notonia in the field in Kenya and identified on the basis of particle morphology, serology, host range and reaction (Bock *et al.*, 1975). It was reported in USA that a survey (during 1972-74) based observation indicated the infection of CMV alone or with PVY severely limited the yield of marketable Capsicum in several areas of Morocco (Lockhart *et al.*, 1976). In India, CMV infestation was first and frequently observed for 5 years (1971-76) on Capsicum at Kanpur on the basis of presented data on properties and host range tests (Singh and Singh, 1976). Conti and Masenga reported from Italy that CMV, AMV, PVY, TMV, Broad bean Mosaic virus (BBMV), and Tobacco rattle virus (TRV) were the viruses found to infect Capsicum in Piedmont (Conti and Masenga, 1977). In the same year from India, eleven virus isolates collected from Capsicum growing in Udaipur and Mathania and showing mosaic symptoms were identified as CMV, CMV + TMV complex and PVY (Shukla and Ram, 1977). At the same time in Mendoza of Argentina, the disease incidence studied on a group of viruses on *Capsicum annuum* and the result was found as: CMV 18%, PVY 78%, TMV 6%, Pepper severe Mosaic virus (PSMV) 4%, TSWV 4%, AMV 2% and Tobacco streak virus (TSV) 2% (Gracia and Feldman, 1977). It has been reported from Israel that, Enzyme-linked immunosorbent assay (ELISA) enabled a rapid and accurate detection of CMV, AMV, TMV and PVY in *C. annuum* (Marco and Cohen, 1979).

In 1980 from Venezuela a report stated that a CMV strain isolated from *C. annuum* fruits was identified from its host range study, non-persistent transmission by *Myzus persicae* and double diffusion in agar using CMV antiserum (Debrot, E.A., 1980). In the year of 1982 from Netherland, a report confirmed that two Hungarian isolates of CMV from Capsicum and melon were serologically closely related to each other and to other CMV isolates. According to symptoms it has been found that they belonged to different groups (Tobias *et al.*, 1982). In the same year from India, CMV was reported for the first time on *C. annuum* var. *grossum* in India (Nagaraju and Reddy, 1982). In the next year it has been reported by Bidari and Reddy that, Pepper vein banding virus (PVBV), PVMV, TMV, CMV, PVY, TEV, Tobacco ringspot virus (TRSV) and TSWV were the most prevalent viruses encountered in commercial Capsicum

fields at Karnataka (Bidari and Reddy, 1983). In Italy an observation based study on symptoms development on inoculated indicator plants and serological tests showed various degrees of infection by CMV, PVX and PVY, and TMV on tomato and *C. annuum* (Davino *et al.*, 1983).

It was reported from India that, CMV from melons in the Punjab was transmitted by seed, sap and aphids, but not by beetles, whitefly, *Sphaerotheca fuliginea* or contact and it systemically infected *C. annuum*, tobacco, *Nicotiana glutinosa*, *Nicotiana rustica*, and various cucurbits when inoculated mechanically (Sharma *et al.*, 1984). In Egypt, ELISA technique was used to detect CMV in *C. annuum* and several other hosts, including tomato, squash, cucumber, melon, celery and tobacco where CMV was found in all tested host samples (Khalil and Mikhail, 1987). Two virus-protecting strains (S51, S52) were obtained from China by local lesion selection after adding satellite RNA to the RNA genome of CMV. Both were found to protect *Capsicum* against virulent strains of CMV under greenhouse and field conditions (Tien *et al.*, 1987). Abdel-Salam *et al.* from Egypt developed a modified method of virus purification that produced unaggregated preparations of CMV with high virus yield from *Capsicum* plants (Abdel-Salam *et al.*, 1989). In China an experiment was carried out by Wu *et al.* where *Capsicum* and Tobacco plants were inoculated with a mild strain of CMV with or without satellite RNA, then challenged by a virulent CMV strain devoid of satellite RNA and protection effects detected by assessment of symptoms and protein sandwich ELISA for the challenge CMV strain (Wu *et al.*, 1989).

During 1979-80, a survey based study confirmed that 53% of commercial *Capsicum* growing areas were infected by *Chilli Mosaic virus* in Karnataka, India. Isolates of *Chilli Mosaic virus* were divided into 9 basic reacting types (Bidari and Reddy, 1990). Based on host range and sap tests, the virus isolated from infected *C. annuum* plants in Yugoslavia was identified as CMV. Double radial immuno-diffusion showed its affinity with unstable CMV isolates (Subasic *et al.*, 1990). During 2-year survey (1984 and 1985), a total of 1195 *Capsicum* samples were collected from Ventura, Tulare and Imperial counties in California, USA. An indirect ELISA was used to test all samples for the presence of one or more of 7 *Capsicum* viruses and CMV was found as major virus with disease incidence of 39% and 97.5% for Ventura and Tulare countries respectively (Abdalla *et al.*, 1991). During 1991 in Australia, *Capsicum* plants became infected

with an aggressive viral disease, the symptoms of which resembled with Tobacco etch virus (TEV) and CMV. Presence of these viruses was confirmed by DAS-ELISA (Perry *et al.*, 1993). In a study of the viruses in plants, TMV and CMV were found in *Capsicum* and cucumber in greenhouses and plastic tunnels in Adana, Icel and Hatay provinces of Turkey (Depestre *et al.*, 1993). It was reported from the same country that CMV-CP gene was introduced into *Capsicum* by co-culturing cotyledon explants of 3 cultivars with *Agrobacterium tumefaciens*. Transformants were selected and regenerated. ELISA, southern and northern blotting indicated that the CMV-CP gene was incorporated, transcribed and translated in transgenic plants and their offspring which showed that they were resistant to CMV (Gullu and Cali, 1994). In a report from China, a total of 33 isolates of CMV from *Capsicum* were differentiated using differential hosts and identified as CMV-U (20 isolates) and CMV-N (13 isolates) strains. Non leguminous isolates (CMV-UnL) were found as predominant isolates in the CMV-U group (Zong-Jiang *et al.*, 1994). A total of 24 CMV field isolates from *Capsicum* crops in California, USA, were characterized and compared by nucleic acid hybridization sub-grouping, virion electrophoresis and biological effects in several hosts (Hristova and Kim, 1994). In USA, CMV, TMV, CGMMV and ZYMV were detected from individual fruits and seeds of *Capsicum* and cucumber by reverse transcription-polymerase chain reaction (RT-PCR) (Rodriguez-Alvarado *et al.*, 1995).

On the basis of host range study, transmission, serology and physicochemical properties, commercial tomato, chilli and brinjal crops were tested for isolation of viruses in Chittoor district, Andhra Pradesh where cucumber mosaic cucumovirus (CMV) were identified as strains. The virus was further confirmed in different diagnostic host plants, vector transmission in non-persistent manner by *Myzus persicae* and Direct Antibody Coating- ELISA (DAC-ELISA) (Kiranmai *et al.*, 1997). A biologically active cDNA clone of CMV was generated from Fny- and Sny-strains of CMV and that was capable of infecting tobacco but not squash plants in systemic way. The virus after even mutation in specific codons of 3a amino acid shown systemic infection in various solanaceous hosts (Tobacco, tomato, and pepper) but did not some cucurbit hosts (cucumber, melon, or squash) (Kaplan and Palukaitis, 1997). In Korea Republic, about 29 isolates of CMV obtained from *Capsicum* and 7 other plant species collected were characterized by host range, RT-PCR,

restriction digestion and sequencing of CP gene (Choi *et al.*, 1998).

Two different accession of *Capsicum frutescens* viz. 'BG2814-6' and 'Perennial' were validated for resistance to several isolates of CMV. ELISA based detection confirmed a varietal difference in the reading values of inoculated leaves that established a mechanistic difference in resistance between the two genotypes (Grube *et al.*, 2000). During the period between 1997-1999, 162 *Capsicum* samples with typical virus symptoms in Chongqing, China were identified by the diagnostic hosts, stability tests and indirect ELISA where CMV and TMV were appeared as major viruses with disease incidence of 79.0% and 54.2%, respectively (Boari *et al.*, 2000). The CMV infecting *Capsicum* in Guangdong, China was confirmed as a member of subgroup I based on CP and 2b gene sequencing. It shared 90.9-93.8 and 76.1-76.9% CP and 2b DNA sequence similarity with strains of subgroup I (Dong-Lin *et al.*, 2001). In Korea Republic, transgenic *Capsicum* plants co-expressing coat proteins (CPs) of CMV (CMV-Kor) and ToMV were produced by *Agrobacterium*-mediated transformation (Shin *et al.*, 2002). Chilli mosaic disease caused by CMV in *C. annuum* and several other eight plant species has been reported in severe basis as host plants of CMV in eastern U.P, India Chilli mosaic disease caused by CMV in *C. annuum* and several other eight plant species has been reported to serve as host plants of CMV in eastern U.P., India (Tewari and Tripathi, 2003). On the basis of *Agrobacterium tumefaciens*-mediated transformation method, the coat protein (CP) genes of CMV and TMV have been transferred to *Capsicum* (*Capsicum annuum* var. Longunt) cultivar 8212. The putative transgenic lines confirmed by PCR analysis, Southern blot, RT-PCR and western blot analyses. The newly developed transgenic plants exhibited deferred symptom development wild milder disease severity in comparison with wild chili pepper plants under field conditions (Wen *et al.*, 2003). During the survey on diseases in chilli, symptoms like green to yellow mosaic, mottling of leaves with or without deformation, stunting and extremely deformed shoestring leaves in advanced stages of diseases were found in *Capsicum* cultivar in Kalimpong, Darjeeling of West Bengal and Sikkim, India. The presence of CMV and PVY was confirmed by EM decoration test and DAC-ELISA (Biswas *et al.*, 2005). A survey was conducted during 2001-2003 on *Capsicum* in various locations of Coquimbo, Chile. Using DAS-ELISA, mixed

infection of CMV (23.3%) with Tomato spotted wilt virus (TSWV) (20.8%), Alfalfa Mosaic virus (AMV) (14.8%), PVY (14.5%), Impatiens Necrotic Spot (INSV) (3.1%), Tomato Mosaic virus (ToMV) (2.2%) and TMV (4.9%) were detected as major viruses (Sepulveda *et al.*, 2005). The DAS-ELISA test was used for virus detection of viruses in *Capsicum* plants grown in some parts of China. The relative importance of the viruses encountered was as follows: CMV (23.3%), TSWV (20.8%), AMV (14.8%), PVY (14.5%), INSV (3.1%), ToMV (2.2%) and TMV (4.9%) (Xu *et al.*, 2006).

Seed transmission technique was standardized in young pepper seedlings by mechanical inoculation with CMV-Fny (Fast New York) isolate. The experimental most of the inoculated plants with rigorous mosaic symptoms were extended up-to seed harvesting stage. Thus RT-PCR analysis was carried out from all seeds derived RNA samples displayed an infection ranged from 95 to 100%. Similar test was repeated by using RNA from sprouted seeds with an infection ranged between 53 and 83% claimed as the first report of CMV seed transmission in chilli (Ali and Michelle, 2010). To determine the disease incidence of CMV in chilli, a pack of surveys were done during 2006-07 in 73 fields to cover the major vegetables growing areas of Pakistan. DAC-ELISA was tested in 706 samples confirmed the overall severity of this disease over Pakistan along with 44.7% relative incidence where highest and lowest disease incidence found in Sindh (51.8%) and KPK (7.8%) respectively (Iqbal *et al.*, 2012). A comparative study on the resistance mechanisms was carried by using five different antiviral constructs with all possible combinations in two diverse vector transformation systems and two model host plants. It was also identified that the most efficient construct could be capable of transforming even in *Capsicum* to develop transgenic plant against CMV (Xinqiu *et al.*, 2012). Biological and molecular characterization of major chilli viruses was carried using DAS-ELISA, mechanical transmission, DNA-RNA hybridization and RT-PCR in western Himalayan regions of India. The mostly identified viruses were CMV, TSWV, poty virus and gemini viruses where CMV was found to be most abundant. The CMV-CP gene was characterized by RT-PCR along with cloning and sequencing. The resulting gene sequence confirmed the CMV isolate belong to subgroup II class (Biswas *et al.*, 2013). To evaluate the reaction of 2 local and 7 exotic cultivars of chilli-pepper against the viruses under natural condition, a biological

study was performed in the coastal savanna zone of Ghana. On the basis of symptoms severity and diseases incidence, the inoculated plants were assessed where incidence increased from 0.5% to 47.2% at 14 week after transplanting. ELISA was tested against PVMV, TMV, CMV and PMMV for each cultivar where mixed types of infections of multiple viruses were reported in all the experimental varieties (Appiah *et al.*, 2014). For the evaluation of genetics of resistance of chilli pepper to CMV in Pakistan, a total of 40 genotypes were tested by mechanical transmission where the level of resistance was assessed by visual observations and DAS-ELISA. As per 0-5 disease rating scale and ELISA result, 9 genotypes were found to be totally infection free and considered as highly resistant where others exhibited as variable disease symptoms (Ashfaq *et al.*, 2014). To determine the presence of viral particle in naturally virus infected plants, sampling was done from symptomatic *Capsicum chinenses* var. Scotch Bonnet in a field in Florida. Viral inclusion assays and lateral flow immunoassays specific to CMV, PVY, PMMoV, TMV, ZYMV and Papaya ringspot virus (PRSV) were performed where samples only displayed positive response to CMV. Mechanical sap inoculation confirmed the infectivity by developing symptoms like mild mosaic and mottling of post inoculation in healthy plants. Appearance of Mosaic virus was further validated through CMV Immunostrips, RT-PCR and sequence analysis (Babu *et al.*, 2014). A survey based detection of viruses in 500 leaf samples of Pepper (*Capsicum annum* L.) was carried out from 23 fields of Dezful on the basis of typical symptoms. The collected were further diagnosed by DAS-ELISA using several polyclonal antibodies. The diseased samples displayed positive titer for PVMV, Pepper mild mottle virus (PMMV), Pepper mottle virus (PMV), PVY, AMV, CMV and TSWV in single and mix infection manner where highest incidence noticed in case of PVMV in Dezful, Iran (Soleimani *et al.*, 2014). To trace the disease severity on chilli by viruses, surveys were conducted in nine locations in Bangladesh based on visual disease symptoms. To further confirm the presence of different viruses in separate manner, DAC-ELISA based test was carried out using the antisera of CMV, PVY and CVMV where positive titer value was only noticed for CMV antisera (Myti *et al.*, 2014). In order to assess the incidence, diversity and distribution of viruses in chilli (*Capsicum spp.*), surveys in six different states were conducted in South-west Nigeria during 2010-2011. On the basis of result obtained from Antigen coated plate-

ELISA (ACP-ELISA), the viruses detected in leaf samples were PVY, Potato virus X (PVX), PVMV, PMMV, TMV, CMV, Tobacco etch virus (TEV) and ToMV. It was further affirmed that PVY served as the most severe virus with highest incidence (79%) where ToMV (23%) shown lowest level of incidence (Olawale *et al.*, 2015). Another study was carried out to screen out fifty *Capsicum* genotypes on resistance against CMV and CVMV by using mechanical sap inoculation and graded according to their reaction. Crossing among ten parental genotypes in possible combinations was performed and selected 45 F1 hybrids were evaluated for resistance against both the viruses in separate manner. The inheritance studies with advanced populations validated that the CMV resistance is a polygenic where CVMV resistance is monogenic characteristics (Naresh *et al.*, 2016). A yeast two hybrid system was generated from *C. annum* 'Bukang' cDNA library and that was screened to get 76 potential clones involved in CMV-P1 infection in chilli by interacting with host P1 helicase gene. On the basis of Beta-galactosidase filter lift assay, PCR screening, and sequencing analysis results, it was further assured that 10 candidate genes involved in virus infection. The host genes silencing in *Nicotiana benthamiana* plants inoculated with CMV-P1 tagged with green fluorescent protein (GFP). It was finally demonstrated that formate dehydrogenase and calreticulin-3 precursor plays a vital role for CMV-P1 infection in host plant (Choi *et al.*, 2016).

Beside these, a number of disease diagnosis techniques and possible biotechnological approaches to control of this devastating virus have been appeared in to the lime light of scientific success for disease management in a sustainable way.

CONCLUSION

A high assortment of advanced strategies have been utilized to distinguish, recognize and measure to traces of virus particle and even to find out dynamic plant genes showing highly resistance response to virion molecule by creating a permanent barrier. Moreover, these methods have a limit in detection and recognition of biological substances however how they are reacting at various expression levels with pathogenic molecules during infection would not be very much experienced. But, current understanding of specio-temporal behavior of some valuable plant genes may be attributed to perfectly correlate the pathogenesis with disease development by thoroughly studies on symptomatology of several host

plants. In this way, a compact and complete study of disease severity and host range evaluation would make a helping hand in development of several diagnostic aids to detect virus at nano level. Moreover the day is not so far when we could see a dramatic advancement in biotechnology that would be able to develop such antiviral particles with features of complete protection barrier in plant system when integrated and sustains the same property in plant from generation after generation.

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CONFLICT OF INTEREST

Authors have no conflict of interest.

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