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Properties, Diagnosis and Management of *Cucumber green mottle mosaic virus*

Seema Mandal[1] • Bikash Mandal[1*] • Qazi Mohd. Rizwanul Haq[2] • Anupam Varma[1]

[1] Plant Virology Unit, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi-110012, India
[2] Department of Biosciences, Jamia Millia Islamia, New Delhi-110025, India

Corresponding author: * **leafcurl@rediffmail.com**

ABSTRACT

Cucumber green mottle mosaic virus (CGMMV), a species under the genus *Tobamovirus*, is an important viral pathogen affecting cucurbit crops in Asia and Europe. The virion is rod shaped particle of 300 × 15 nm containing positive sense ssRNA of 6.4 kb as its genome. No insect vector is known to transmit the virus in a specific manner. The virus is highly stable, contagious and sap transmissible. Several isolates have been described from different countries in Eurasian regions infecting important cucurbits such as bottle gourd (*Lagenaria siceraria*), cucumber (*Cucumis sativus*), gherkin (*Cucumis anguria*), muskmelon (*Cucumis melo*) and watermelon (*Citrullus vulgaris*). Symptomatology of the virus is characterized by systemic green mottle mosaic on foliage. Some of the isolates induce serious fruit symptoms such as pulp deterioration ('blood flesh' disease) in watermelon. The complete genome of CGMMV is composed of 6424 nucleotides containing four open reading frames encoding replicase proteins, movement protein and coat protein. The CGMMV isolates form a distinct evolutionary clade separated from the other cucurbit-infecting tobamovirus species. Several sensitive methods are available for reliable diagnosis of CGMMV of which ELISA and RT-PCR are commonly used. Immunofilter paper assay and bioelectric recognition assay have been shown for rapid test of CGMMV. Control of CGMMV is difficult as commercial cultivars are susceptible. Limited source of resistance is known in muskmelon and wild *Cucumis* spp. The transgenic approach holds a greater potential in developing resistant varieties against CGMMV in any cucurbit.

Keywords: biological properties, CGMMV, diagnosis, management, molecular properties, tobamovirus
Abbreviations: CGMMV, *Cucumber green mottle mosaic virus*; **ELISA**, enzyme-linked immunosorbent assay; **NT**, nucleotide; **ORF**, open reading frame; **RT-PCR**, reverse transcription polymerase chain reaction; **ssRNA**, single stranded RNA

CONTENTS

INTRODUCTION

Cucumber green mottle mosaic virus (CGMMV), a member of *Tobamovirus*, was first reported in *Cucumis sativus* from Great Britain, by Ainsworth (1935). Subsequently, it was reported from several countries in Asia and Europe e.g., China (Chen *et al*. 2006), Israel (Antignus *et al*. 1990), Greece (Varveri *et al*. 2002), Japan (Komuro *et al*. 1971),

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Invited Review

Fig. 1 Distribution map of *Cucumber green mottle mosaic virus*. 1. Antarctica, 2.China, 3. Israel, 4. Greece, 5. Japan, 6. India, 7. Korea, 8. Netherlands, 9. Pakistan, 10. Poland, 11. Russia, 12. Saudi Arabia, 13. Spain, 14.Taiwan, 15. UK, 16. Ukraine.

India (Capoor and Varma 1948), Korea (Lee *et al*. 1990), Netherlands (van Koot and van Dorst 1959), Pakistan (Ali *et al*. 2004), Poland (Pospieszny *et al*. 1997), Russia (Slavokhotova *et al*. 2007), Saudi Arabia (Al-Shahwan 1992), Spain (Celix *et al*. 1996), Taiwan (Wang and Chen 1985) and Ukraine (Budzanivska *et al.* 2007). CGMMV is distributed in Eurasian region (**Fig. 1**), however, it is one of the few plant viruses that has been recorded in Antarctica (Polischuk *et al*. 2007). Several Cucurbitaceous vegetables such as bottle gourd (*Lagenaria siceraria*), cucumber (*Cucumis sativus*), gherkin (*Cucumis anguria*), muskmelon (*Cucumis melo*) and watermelon (*Citrullus vulgaris*) are affected by CGMMV (Capoor and Varma 1948; Vasudeva *et al.* 1949; Raychaudhuri and Varma 1978; Antignus *et al.* 1990; Lee *et al.* 1990; Celix *et al.* 1996; Varveri *et al.* 2002; Rashmi *et al*. 2005). The typical disease symptoms caused by CGMMV are systemic greenish mottle mosaic on foliage (**Figs. 2A, 2B**), however, symptomatology varies based on isolate/strain and the plant species affected. CGMMV causes serious distortion and decomposition of fruits. In grafted watermelon plants, CGMMV causes 'blood flesh' disease in Korea (Lee *et al.* 1990), 'Konnyaku' disease in Japan (Komuro *et al.* 1968) and similar disease in Greece (Varveri *et al*. 2002; Boubourakas *et al*. 2004). In cucumber, CGMMV produces systemic symptoms such as leaf mottling, mosaic, blistering and stunted growth. The acuba mosaic strain of CGMMV causes yellow mottling on leaf and yellow streak or fleck on fruit of cucumber (Ainsworth 1935). The incidence of CGMMV builds up gradually during cropping. High incidence of CGMMV has been recorded in the north Indian conditions, where by the end of the cropping season, about 100% incidence of CGMMV has been reported in bottle gourd, 80% in muskmelon and 75% in watermelon (Raychaudhuri and Varma 1978; Rao and Varma 1984). Serious outbreak of CGMMV in cucumber and watermelon is known as early as 1966-69 in Japan (Komuro 1971). Subsequently, CGMMV has emerged as a serious problem in 1995 in Greece (Bem and Vassilakos 2000), 2002 in Korea (Yoon *et al*. 2008) and 2005-06 in China (Chen *et al*. 2008). CGMMV being a potential threat to the production of cucurbits has been recognized as a quarantine pest by Chinese government in May 2007 (Chen *et al*. 2008).

Cucurbits are know to be affected by three additional tobamovirus species e.g., *Cucumber fruit mottle mosaic virus* (CFMMV) (Antignus *et al.* 2001), *Kyuri green mottle mosaic virus* (KGMMV) (Francki *et al*. 1986; Yoon *et al.* 2001) and *Zucchini green mottle mosaic virus* (ZGMMG)

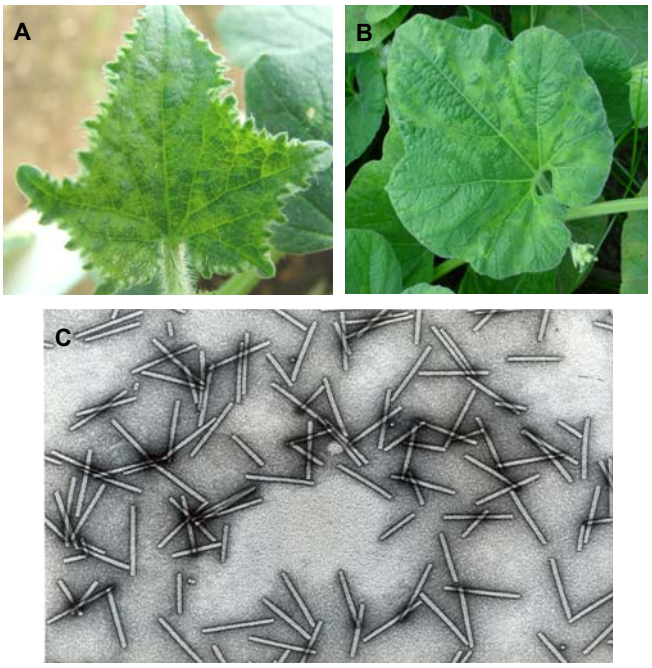

Fig. 2 Symptoms of *Cucumber green mottle mosaic virus* (CGMMV) in cucumber (A) and bottle gourd (B). Electron micrograph of purified virions of CGMMV from cucumber leaves (C). The virions were negatively stained with 2% aqueous solution of uranyl acetate.

(Ryu *et al.* 2000; Yoon *et al.* 2002). Of all the cucurbit-infecting tobamoviruses, CGMMV is the most widely occurring and best studied virus. Since its discovery by Ainsworth in 1935, extensive studies have been made on various aspects of CGMMV. Brief description of CGMMV is available (Hollings *et al*. 1975; Francki 1988). Okada (1986) reviewed the work on virus structure, composition, assembly and partial genome sequence characteristics. The present review provides up-to-date information of biological and molecular properties, diagnosis and management of CGMMV.

PROPERTIES OF VIRION

The virion of CGMMV is rod shaped measuring 300 × 15 nm. In addition to the normal size particle, shorter rods of about 50 nm are also present in the cucumber plants infected by CGMMV (Fukuda *et al.* 1981). The genomic RNA

and two major sub-genomic RNAs are encapsidated in normal length of particle. Whereas, the shorter particle has been shown to encapsidate mRNA, that encodes coat protein (Whitefield and Higgins 1976; Fukuda *et al*. 1981; Kim *et al*. 2003a). Sedimentation co-efficient of CGMMV varies from 185 *S* to 195 *S*. The isoelectric point of the virus is about pH 4.98 (Hollings *et al*. 1975).

CGMMV can be easily purified from cucumber leaves by precipitation with polyethylene glycol followed by sucrose density gradient ultra centrifugation (**Fig. 2C**). The purified preparation is infectious. The molecular weight of CGMMV is 17 K, which is similar to *Tobacco mosaic virus* (TMV). Interestingly, CGMMV migrates faster than TMV in SDS-polyacrylamide gel electrophoresis (Ohno *et al*. 1977; Sano *et al*. 1978). However, if the gel electrophoresis is carried out in the presence of 8M urea, both CGMMV and TMV migrate at the same rate. The anomalous mobility of the CGMMV protein is attributed to the higher intrinsic negative charge density, smaller hydrodynamic volume and slightly higher α-helix content compared to TMV (Sano *et al*. 1978).

The particle feature and biophysical characters of CGMMV are similar to TMV (Knight 1952). The RNA location in CGMMV protein disk has been studied by negative staining, which shows a dense narrow ring around the central hole (Nonomura and Ohno 1974). The RNA occupies in a position corresponding to that of the ring like area. Like TMV, CGMMV particle can be reconstituted *in vitro* with its RNA and coat protein (Kurisu *et al*. 1976). The RNA of CGMMV can form infectious virus particle with the coat protein of TMV and *vice versa* (Kurisu *et al*. 1976; Ohno *et al*. 1977).

The stability of CGMMV in leaf extract has been studied based on thermal inactivation point (TIP), dilution end point (DEP) and longevity *in vitro* (LIV). The TIP of different strains ranges between 85-100°C indicating high thermo-stability. LIV of CGMMV is for several months depending on the storage temperature, for example the LIV of CGMMV-M described from India is 60 days at 30-32°C and 90 days at 10°C. In desiccated leaves, CGMMV-M has been shown to be infective for more than four years (Raychaudhuri and Varma 1978). CGMMV has high DEP, which varies from 10^{-5} to 10^{-7}.

BIOLOGICAL PROPERTIES

Host range

The host range of CGMMV is narrow and mainly restricted to Cucurbitaceae and Chenopodiaceae. Some isolates however, infects a few species of Solanaceae. The natural host range of CGMMV includes *Cucumis sativus*, *Cucumis anguria*, *Cucumis melo*, *Citrullus lanatus* and *Lagenaria siceraria*. In Antarctica, CGMMV has been detected in unusual hosts *viz*., mosses (*Barbilophozia* and *Polytrchum*) and Antarctic hairgrass (*Deschampsia antarctica*) (Parnikoza *et al*. 2007; Polischuk *et al*. 2007). In India, so far, no natural infection of CGMMV is known in cucumber; only bottle gourd, gherkin, muskmelon and watermelon are known to be affected by CGMMV (Varma and Giri 1998; Rashmi *et al*. 2005).

The experimental host range of CGMMV includes the following plant species: *Chenopodium amaranticolor*, *Citrullus lanatus*, *Cucumis sativus*, *C. melo*, *C. melo* var. *utilissima*, *Cucurbita pepo*, *C. moschata*, *Datura stramonium*, *Lagenaria siceraria*, *Luffa acutangula*, *Nicotiana benthamiana*, *N. debneyii* and *Trichosanthes anguina. Momordica charantia* and *N. tabacum* are symptomless hosts. The insusceptible plant species are *Capsicum annuum*, *Carica papaya*, *Lycopersicon esculetum*, *Phaseolus vulgaris*, *N. glutinosa* and *Vigna unguiculata*. The majority of the host species produce systemic symptoms and a few (*C. amaranticolor* and *Datura stramonium*) produce local lesion symptoms. *C. sativus* and *C. amaranticolor* are good maintenance and assay hosts, respectively.

Biological variants

Several biological variants of CGMMV have been reported from Asia and Europe (**Table 1**). The local lesion symptoms in *C. amaranticolor* and the specific symptoms in the original host from where the virus is isolated are generally used to distinguish the biological variants (**Table 1**). The biological variants of CGMMV were first isolated from UK and referred as cucumber virus-3 (CV3) and cucumber virus-4 (CV4) (Ainsworth 1935). CV3 is the type strain of CGMMV, which induces systemic mosaic and blistering symptoms on leaves and does not induce fruit symptoms in cucumber. CV3 induces local lesions in *C. amaranticolor*, but not in *Datura stramonium* or *Petunia hybrida* (Ainsworth 1935). CV4 induces bright yellow mottling on leaves and yellow streaks on fruit in cucumber and induces local lesions in *C. amaranticolor* but not in *D. stramonium*. CV3 and CV4, however, are serologically very similar (Nozu *et al*. 1971). CGMMV described from Spain (Sp) induces mottling mosaic and blistering on greenhouse grown cucumber (Celix *et al.* 1996). Sp does not induce local lesions in *C. amaranticolor* and its host range is restricted within Cucurbitaceae only.

Five variants of CGMMV have been reported from India, CV2B and CV2C isolated from bottle gourd (Capoor and Varma 1948; Vasudeva *et al.* 1949; Vasudeva and Nariani 1952), Gh from gherkin (Rashmi *et al*. 2005), M from muskmelon (Raychaudhuri and Varma 1978) and IW from irrigation water (Vani and Varma 1993). CV2B and CV2C induce similar systemic mosaic symptoms on bottle gourd and their response on *C. amaranticolor* is not known. However, they differ in host reaction on watermelon and pumpkin (*C. moschata*). In watermelon, CV2B induces mottle mosaic and CV2C causes symptomless infection. In pumpkin, CV2C induces yellow spots along the veins, whereas, CV2B causes no infection (Vasudeva *et al.* 1949). The M isolate induces well defined mosaic and green vein banding symptoms on muskmelon leaves, and no symptoms on *C. amaranticolor*. The host range of M isolate is restricted only to family, Cucurbitaceae. It differs from CV2B and CV2C by not infecting watermelon (Raychaudhuri and Varma 1978). The IW isolate induces systemic mottle mosaic symptoms in bottle gourd, cucumber, muskmelon and watermelon, and induces pinpointed local lesions on *C. amaranticolor*. The IW differs from the other three Indian isolates by inducing mottle mosaic symptoms on *Luffa acutangula* as other isolates cause either symptomless infection or no infection in *L. acutangula.* The Gh isolate, which is reported from southern India, induces yellowing, blistering, mosaic mottling, chlorotic spots, necrotic lesions and leaf cupping on gherkin (Rashmi *et al*. 2005).

In Japan, two important isolates, W and SH have been studied. The W isolate causes severe disease symptoms in watermelon and induces local lesions in *C. amaranticolor* but not in *D. stramonium* (Komuro *et al*. 1968). The SH isolate obtained from greenhouse muskmelon exhibits leaf mosaic and necrotic lesions on the fruit and causes local lesions on *C. amaranticolor* (Ugaki *et al.* 1991). Two additional Japanese isolates, C (Inouye *et al*. 1967) and Y (Kitani *et al*. 1970) originally considered as CGMMV variants, proved to be different from CGMMV based on serological reaction and genome sequence information (Francki *et al*. 1986). Based on the complete genome sequence data, the C isolate was classified as a new species under *Tobamovirus*, *Kyuri green mottle mosaic virus* (KGMMV) (Yoon *et al*. 2001) and the Y isolate as KGMMV-Y (Tan *et al*. 2000).

In Korea, KOM and KW isolates have been characterized at biological and molecular levels. KOM and KW have been isolated from oriental melon and watermelon, respectively. Biologically they are very similar in inducing systemic symptoms in several cucurbits and they differ only by their responses on indicator plant, *C. amaranticolor,* where only KW induces local lesions (Kim *et al*. 2003a). Biological diversities have been studied in another three isolates, NW-1, MW-2 and KC1-2 from Korea (Yoon *et al*. 2008).

Table 1 Biological variants of *Cucumber green mottle mosaic virus* described from Asia and Europe.

Name of isolates/strains	Original host/source	Country reported from	Symptoms on		Reference
			Original host	*C. amaranticolor*	
CV3	Cucumber	UK	Leaf mottling and blistering, no fruit symptoms	Local lesion	Ainsworth 1935
CV4	Cucumber	UK	Yellow leaf mottling, yellow streak on fruit	Local lesion	Ainsworth 1935
CV2B	Bottle gourd	India	Mottle mosaic on leaves and fruit discoloration	-	Capoor and Varma 1948
CV2C	Bottle gourd	India	Mottle mosaic and green blisters on leaves	Local lesion	Vasudeva *et al*. 1949
Gh	Gherkin	India	Yellowing, blistering and mottle mosaic on leaf	-	Rashmi *et al*. 2005
IW	Irrigation water	India	-	Local lesion	Vani and Varma 1993
M	Muskmelon	India	Mosaic blistering	No local lesion	Raychaudhuri and Varma 1978
W	Watermelon	Japan	Severe leaf mosaic and stunting of plant	Local lesion	Komuro *et al*. 1971
SH	Muskmelon	Japan	Necrotic spot on fruit	Local lesion	Ugaki *et al*. 1991
Is	Cucumber	Israel	Mottle mosaic on leaf	Local lesion	Antignus *et al*. 1990
GR	Watermelon	Greece	Fruit pulp deterioration	Local lesion	Varveri *et al*. 2002
KW	Watermelon	Korea	Mottle mosaic on leaf	Local lesion	Kim *et al*. 2003a
KoM	Oriental melon	Korea	Mottle mosaic on leaf	No local lesion	Kim *et al*. 2003a
NW-1	Watermelon	Korea	Severe mosaic	Local lesion	Yoon *et al*. 2008
NW-2	Watermelon	Korea	Severe mosaic	Local lesion	Yoon *et al*. 2008
KC1-2	Cucumber	Korea	Severe mosaic	Local lesion	Yoon *et al*. 2008
Sp	Cucumber	Spain	Mottle mosaic and blistering on leaf	No local lesion	Celix *et al.* 1996
Ti	Bottle gourd	Taiwan	Mosaic on leaf	Local lesion	Wang and Chen 1985

-: not tested

All these isolates produce severe mosaic symptoms on leaves of watermelon and cucumber, and local lesions on *C. amaranticolor.* The NW-1 isolate differ from other two by not infecting cucumber and KC1-2 by not infecting *N. debneyii*. A new strain of CGMMV (Ti) has been reported infecting bottle gourd in Taiwan (Wang and Chen 1985). The Ti strain induces small chlorotic local lesions on *C. amaranticolor* and symptomless infection on inoculated leaves of *D. stramonium* and *P. hybrida*.

In planta movement and distribution

Systemic movement in infected host plants is one of the key processes of viral pathogenesis. Movement of virus in plant takes place through two major steps- short distance movement from cell to cell through plasmodesmata and long distance movement through vascular tissues (Carrington *et al*. 1996). Number of indirect evidences suggests that viruses systemically move through phloem in the form of intact virus particle (Esau and Cronshaw 1967; Dolja *et al.* 1994; Ding *et al.* 1996). Simon-Buela and Garcia-Arenal (1999) analysed CGMMV in the phloem sap of cucumber plant and showed that the CGMMV particles in the phloem exudes were indistinguishable from the purified virions. No evidence of free RNA or other CGMMV related structure was found in phloem exudates of infected plants. These observations provide evidence that CGMMV systemically moves through the phloem of infected cucumber plant in the form of virus particle and not as free RNA.

Moreno *et al.* (2004) described the systemic colonization of CGMMV in cucumber plants, which was studied by detecting coat protein in different plant organs at different days post inoculation (dpi) by immunochemical and immunocytochemical methods. Systemic infection is first detected at 12 dpi in the young expanding leaves. In minor veins of inoculated cotyledons, bundle sheath and vascular parenchyma cells are infected at 8 dpi. CGMMV accumulates in xylem-associated vascular parenchyma cells at 8 to 20 dpi.

The long distance movement of CGMMV is from photoassimilate source to sink, which supports phloem transport mechanism. In systemically infected sink leaves, CGMMV is simultaneously detected in the xylem and phloem. CGMMV accumulates in high levels in the differentiating tracheids of young leaves. These observations indicate involvement of xylem in systemic movement of CGMMV. The more convincing evidence of xylem transport of CGMMV has been experimentally demonstrated by inducing cell death by steam treatment of a portion of stem (Moreno *et al*. 2004). However, xylem transport of CGMMV appears to be less efficient than phloem transport with reference to the time requires for systemic infection and the percentage of infected plants.

TRANSMISSION

CGMMV being highly stable and contagious, natural spread is largely through contact of infected plant materials. No insect vector is known to transmit the virus in a specific manner. CGMMV is efficiently transmitted through mechanical sap inoculation.

CGMMV is one of the important seed-transmitted viruses. Commercially important watermelon varieties are generally cultivated by grafting on the rootstock of bottle gourd or other curcurbits in China, Greece, Japan and Korea, where seed- and graft-transmission have been attributed to introduction and emergence of CGMMV. In Japan and the Netherlands, up to 8% seed transmission of CGMMV has been reported in bottle gourd, cucumber and watermelon (van Koot and van Dorst 1959; Komuro *et al.* 1971; Nagai *et al*. 1974). Seed transmission gradually declines in storage for example, in cucumber, 8% seed transmission was detected one month after harvesting the seeds, which reduced to 4% after four months (van Koot and van Dorst 1959). In India, CGMM has been shown not to be seed transmitted in bottle gourd or vegetable marrow (*C. pepo*) (Rao and Varma 1984). The differences in virus isolates and environmental conditions between the countries may influence variability in seed transmission.

CGMMV is transmitted through soil and irrigation water contaminated with infected plant debris (Inouye *et al*. 1967; Nagai *et al*. 1974; van Dorst 1988; Vani and Varma 1993). Under experimental conditions, about 18% transmission of CGMMV has been demonstrated in bottle gourd when soil was mixed with freshly dried infected plant debris (Rao and Varma 1984). CGMMV remains infective in the contaminated soil for at least 10 months (Varveri *et al*. 2002). The debris of diseased plants possibly contributes as primary source of infection to a limited number of plants and successively the virus spreads through plant-to-plant contact (Rao and Varma 1984).

Insect transmission of CGMMV has been investigated by many workers and no specific virus-vector relationships with any insect could be established (Inouye *et al.* 1967; Nagai *et al.* 1974; Rao and Varma 1984). In India, a low-level (10%) transmission of CGMMV by cucumber leaf beetle, *Raphidopalpa faeveicollis* has been demonstrated (Rao and Varma 1984). In Japan, the effort to transmit CGMMV by another cucumber leaf beetle, *Aulocophora feumoralis* was unsuccessful (Nagai *et al*. 1974). The mouthparts of *R. faeveicollis* are contaminated with the virus, which remain infective even after passage through the ele-

Table 2 Transmission of *Cucumber green mottle mosaic virus* from bottle gourd to bottle gourd through razor blade.

Source[1] plant	Number of plants given serial cuts[2]							
	1	2	3	4	5	6	7	8
1	+	-	-	-	-	-	-	-
2	+	+	+	-	-	-	-	-
3	+	+	+	+	-	-	-	-
4	+	+	-	-	-	-	-	-
5	+	+	-	-	-	-	-	-

[1] Symptomatic plant at 14 days post inoculation was used as source of infection
[2] The stem of an infected plant was cut with a razor blade and then the same blade was used for serial cutting of the upper stem of three weeks old test plants
+ : Symptomatic plant; – : Asymptomatic plant

mentary canal. The feeding habit of *R. faeveicollis* seems to influence transmission of CGMMV. The beetle starts feeding, nibbling at the leaf tissues and they sometimes change the site of their feeding immediately after a short nibble. If this happens soon after feeding on disease plants, there is a fair chance of transmission. Often, after eating tissues, beetles clean up contamination from their mouthparts resulting in reduced chance of virus transmission especially to non-lethally injured cells due to nibbling.

The cutting knives used by growers for harvesting fruits from diseased plants can potentially contribute additional means of field-spread of CGMMV during fruiting stage of crop. Under the experimental conditions, the CGMMV could be transmitted up to four healthy bottle gourd plants through serial cuttings by contaminated razor blade (**Table 2**).

MOLECULAR PROPERTIES

Genome organization

In 1983, Meshi and coworkers for the first time generated sequence information from the 3′ end of the genomic RNA of CGMMV-W from Japan. In the same year, Nozu and Tsugita (1986) independently generated amino acid sequence information of coat protein of the same isolate by conventional protein sequencing method. In 1988, Saito and coworkers sequenced the region covering the 30K protein gene and compared it with that of other tobamoviruses. The 5′ terminus of CGMMV is blocked with a cap like structure, as is the case in TMV RNA (Kurisu *et al*. 1976). The Poly-A tail at 3′ end of CGMMV genome is absent. The 3′ untranslated region (UTR) forms secondary structure having tRNA-like activity (Meshi *et al*. 1983). The assembly origin of CGMMV-W RNA is located at about 320 nucleotides away from the 3′ end as determined by electron microscopic serology (Fukuda *et al*. 1981). The sequence in assembly region forms highly base-paired hairpin loop structure similar to those found in the other tobamoviruses (Meshi *et al*. 1981; Takamatsu *et al*. 1983). The sequence at the loop, GAGGUUG is identical among all the isolates of CGMMV, but it differs from the other cucurbit-infecting tobamoviruses (CFMMV, KGMMV and ZGMMV) by only the third base composition (**Fig. 3**), where G has been substituted by A in CGMMV (Okada 1986; Ryu *et al.* 2000).

The first complete nucleotide sequence of CGMMV genome was achieved for the CGMMV-SH isolated from Japan (Ugaki *et al*. 1991). The genome of SH is 6424 nucleotides long containing four open reading frames (ORFs) (**Fig. 4**). The ORF-I begins at 61 nucleotide encoding a 129K short-replication associated protein (S-Rep) composed of 1142 amino acids. The ORF-I terminates at an amber codon, UAG, located at 3493 to 3495 nucleotides.

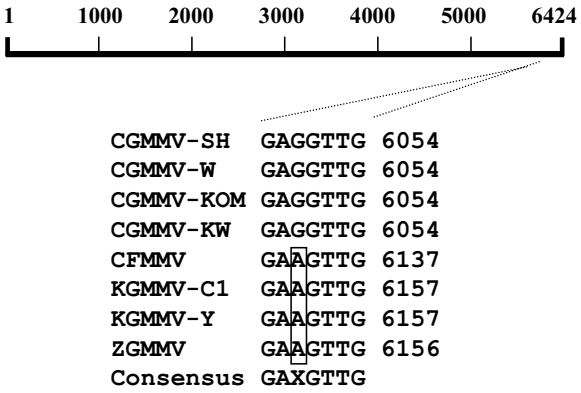

Fig. 3 The conserved nucleotide sequence in the loop of putative assembly origin of cucurbit-infecting tobamoviruses. The values indicate nucleotides number in the genome. The difference in the sequence between CGMMV and other cucurbit-infecting tobamoviruses are indicated by a open box. CGMMV-SH: *Cucumber green mottle mosaic virus*-Japanese SH isolate; CGMMV-W: Japanese watermelon isolate; CGMMV-KOM: Korean oriental melon isolate; CGMMV-KW: Korean watermelon isolate; CFMMV: *Cucumber fruit mottle mosaic virus*; KGMMV: *Kyuri green mottle mosaic virus*; ZGMMV: *Zucchini green mottle mosaic virus*.

The ORF-II (61-5007 nucleotides) is in a read through frame of ORF-I encoding a 186K long-replication associated protein (L-Rep) composed of 1646 amino acids. The ORF-III (4994-5788 nucleotides) begins overlapping 14 terminal nucleotides of the ORF-II encoding a 29K movement protein (MP) composed of 264 amino acids. The ORF-IV (5763-6248 nucleotides) initiates overlapping 25 terminal nucleotides of ORF-III encoding 17.3 K coat protein (CP) composed of 161 amino acids.

The complete genome sequences of four more isolates have been obtained, one from Japan (CGMMV-W) (Tan *et al*. 2000), two from Korea (CGMMV-KW and CGMMV-KOM) (Kim *et al*. 2003a) and one from India (bottle gourd isolate; Accession No. DQ767631-35 &AY309021). The genome organisation of all these four isolates is identical. The genome size in all these isolates is same (6424 nucleotides), except W strains, which is short by only one nucleotide (6423 nucleotides). The nucleotide sequence composition is slightly different among all these isolates. The CGMMV-SH and CGMMV-W are different by 73 nucleotides in the ORF-I, 45 in ORF-II, 10 in ORF-III and 7 in ORF-IV. Of these total 135 different nucleotides, nine causes amino acid substitutions (6 in the ORF-I and 3 in ORF-III). No amino acid sequence difference was found either in ORF-II or in ORF-IV between SH and W. The Korea isolates (KOM and KW) differ from that of Japanese by 56 nucleotides resulting in 4-15 amino acid substitutions. The genome organization of the other cucurbit-infecting tobamovirus species (CFMMV, KGMMV and ZGMMV) is slightly different from CGMMV as the ORF-III and -IV are not arranged in overlapping manner, they are situated one after another often separated by 1-5 nucleotides (Ugaki *et al*. 1991; Antignus *et al*. 2001; Yoon *et al*. 2001; Kim *et al*. 2003a).

Phylogenetic relationships

CGMMV isolates those have been completely sequenced from Japan and Korea have limited genetic diversity as they share 97.7-99.2% identity. The differences in the sequence

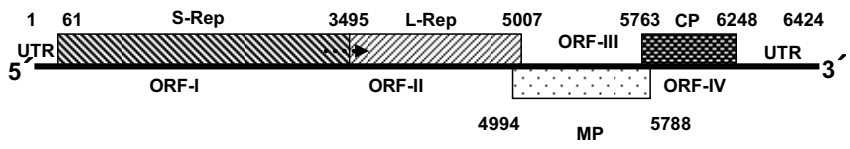

Fig. 4 Schematic presentation of the genome organisation of *Cucumber green mottle mosaic virus*. The genome encodes putative short-replicase protein (S-Rep), large-replicase protein (L-Rep), movement protein (MP) and coat protein (CP). The numbers indicate the start and end of each coding and untranslated region (UTR).ORF: open reading frame.

Table 3 Percent identity matrix of *Cucumber green mottle mosaic virus* (CGMMV) and members of *Tobamovirus* based on complete nucleotide seque genomes.

Virus*	-KOM	-KW	-SH	-W	CFMMV	KGMMV	ZGMMV	ObPV	ORSV	PMMoV	RMV	TMGMV	TMV	ToMV	TVCV	YoMV
-KOM	ID															
-KW	99.1	ID														
-SH	99.1	99.2	ID													
-W	97.6	97.6	97.7	ID												
CFMMV	59.5	59.5	59.7	59.5	ID											
KGMMV	60.3	60.3	60.3	60.4	71.8	ID										
ZGMMV	59.1	59.1	59.1	59.0	70.7	83.6	ID									
ObPV	47.3	47.3	47.3	47.2	46.4	45.5	45.5	ID								
ORSV	46.2	46.2	46.1	46.0	45.0	44.4	43.9	56.8	ID							
PMMoV	48.5	48.4	48.5	48.2	46.3	46.8	46.1	61.9	58.3	ID						
RMV	48.6	48.6	48.5	48.5	45.5	46.3	45.6	46.8	59.5	57.9	ID					
TMGMV	49.3	49.2	49.2	49.2	46.3	46.2	46.1	60.9	58.6	63.3	59.1	ID				
TMV	47.8	47.9	47.8	47.9	46.4	46.5	46.2	62.1	57.6	66.9	58.0	63.2	ID			
ToMV	48.7	48.6	48.6	48.4	46.0	45.9	45.8	62.7	58.1	68.4	57.9	64.2	78.2	ID		
TVCV	48.4	48.4	48.4	48.3	46.2	46.1	45.5	56.5	59.6	57.2	82.1	59.6	57.6	57.7	ID	
YoMV	48.6	48.5	48.6	48.5	45.7	46.3	45.6	56.9	59.5	57.9	95.9	59.0	58.0	58.0	82.3	ID

*Acronyms used in this table: CGMMV-KOM: Korean oriental melon isolate (AF417242); CGMMV-KW: Korean watermelon isolate (AF417243); CGMMV-SH: Japanese SH isolate (D12505); CGMMV-W: Japanese watermelon isolate (AB015146); CFMMV: *Cucumber fruit mottle mosaic virus* (NC_002633); KGMMV: *Kyuri green mottle mosaic virus* (AB015145); ZGMMV: *Zucchini green mottle mosaic virus* (NC_003878); ObPV: *Obuda pepper virus* (D13438); ORSV: *Odontoglossum ring spot virus* (NC_001728); PMMoV: *Pepper mild mottle virus* (M81413); RMV: *Ribgrass mosaic virus* (NC_002792); TMGMV: *Tobacco mild green mosaic virus* (AB078435); TMV: *Tobacco mosaic virus* (D63809); ToMV: *Tomato mosaic virus* (NC_002692); TVCV: *Turnip vein-clearing virus* (U03387); YoMV: *Youcai mosaic virus* (U30944). ID: identical.

Similarities among the CGMMV isolates are indicated with dark shade; similarities of CGMMV isolates with the other cucurbit-infecting tobamoviruses are indicated with light shade; similarities with other tobamovirus species are indicated with a box.

Table 4 Percent sequence similarity of *Cucumber green mottle mosaic virus* isolates with the cucurbit-infecting tobamoviruses and other tobamoviruses in the coding and non-coding regions of the their genome.

Coding and non-coding regions	% Similarity	
	Cucurbit-infecting tobamoviruses[1]	Other tobamoviruses[2]
5′ Untranslated region	30.4-40.5	45.3-63.7
3′ Untranslated region	44.2-52.7	27.3-54.6
Replicase protein	63.3-64.6	42.4-45.7
Movement protein	57.9-61.7	22.8-34.3
Coat protein	44.7-46.5	34.2-43.9

[1]CFMMV, KGMMV and ZGMMV

[2]ObPV, ORSV, PMMoV, RMV, TMGMV, TMV, ToMV, TVCV and YoMV. The virus acronyms and sequence data are as used in **Table 3**.

among the isolates are located in UTRs, Rep, and MP; whereas, the CP is identical. The closest relatives of CGMMV are other cucurbit-infecting tobamoviruses, KGMMV, CFMMV and ZGMMV. In the complete genome sequence, CGMMV shares 59.1-60.4% similarity with the other cucurbit-infecting tobamoviruses and only 46.0-49.3% with rest of the tobamovirus species, *Obuda pepper virus*, *Odontoglossum ring spot virus*, *Pepper mild mottle virus*, *Ribgrass mosaic virus*, *Tomato mosaic virus*, *Tobacco mosaic virus*, *Tobacco mild green mosaic virus*, *Turnip vein-clearing virus* and *Youcai mosaic virus* (**Table 3**). The coding and non-coding regions of CGMMV are significantly different from the members in the genus, *Tobamovirus* with the maximum sequence similarity only up to 64.6% (**Table 4**).

Phylogenetic analysis with CGMMV isolates and 13 other tobamovirus species based on complete genome sequence reveals a distinct evolutionary clustering of the cucurbit-infecting tobamoviruses (**Fig. 5**). The CGMMV isolates constitute a different clade from the other cucurbit-infecting tobamoviruses (CFMMV, KGMMV and ZGMMV). Similar evolutionary distinction of CGMMV within *Tobamovirus* is consistent when each coding and non-coding regions are compared separately.

DIAGNOSIS

Bioassay

Biological assay based on specific host response following sap inoculation of CGMMV has been used to identify and differentiate CGMMV and their variants. The important diagnostic and differential hosts of CGMMV are *C. amaranticolor*, *C. sativus*, *D. stramonium* and *N. glutinosa*. Bioassay alone is not a conclusive diagnostic method for CGMMV, as several viruses like *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus* (WMV) and *Papaya ring spot virus* (PRSV) produce similar symptoms as CGMMV in cucurbits (Varma and Giri 1998). However, considering high thermal inactivation property of CGMMV, pretreatment of the inoculum at 85-100°C for 10 min can potentially enhance the specificity of bioassay by eliminating non-tobamoviruses. Bioassay has been employed to judge efficacy of disinfection of bottle gourd seed contaminated by CGMMV (Kim and Lee 2000).

Electron microscopy

The CGMMV particles are easily observed in the transmission electron microscope as high concentration of virus particles occurs in infected leaf tissues. The particle morphology of CGMMV however is indistinguishable from the other tobamoviruses. Therefore, mere presence of full-length rod-shaped virus particles of about 300 nm cannot lead to conclusive diagnosis of CGMMV. Positive decoration of virus particle with CGMMV specific antibody in immunosorbent electron microscopy is however, a valuable diagnostic method (Milne and Lesemann 1984).

Serological assay

Various serological techniques such as tube precipitation test (TPT), dot immunobinding assay (DIBA), Western blotting (WB), enzyme-linked immunosorbent assay (ELISA) and immunofilter paper assay (IPA) have been utilized for detection of CGMMV. Polyclonal antibodies raised against CGMMV-M isolate were used in TPT, which showed different degrees of serological relationship among CGMMV-M, CGMMV-CV2 and TMV-coconut isolate (Raychaudhuri and Varma 1978). The DIBA was employed in field diagno-

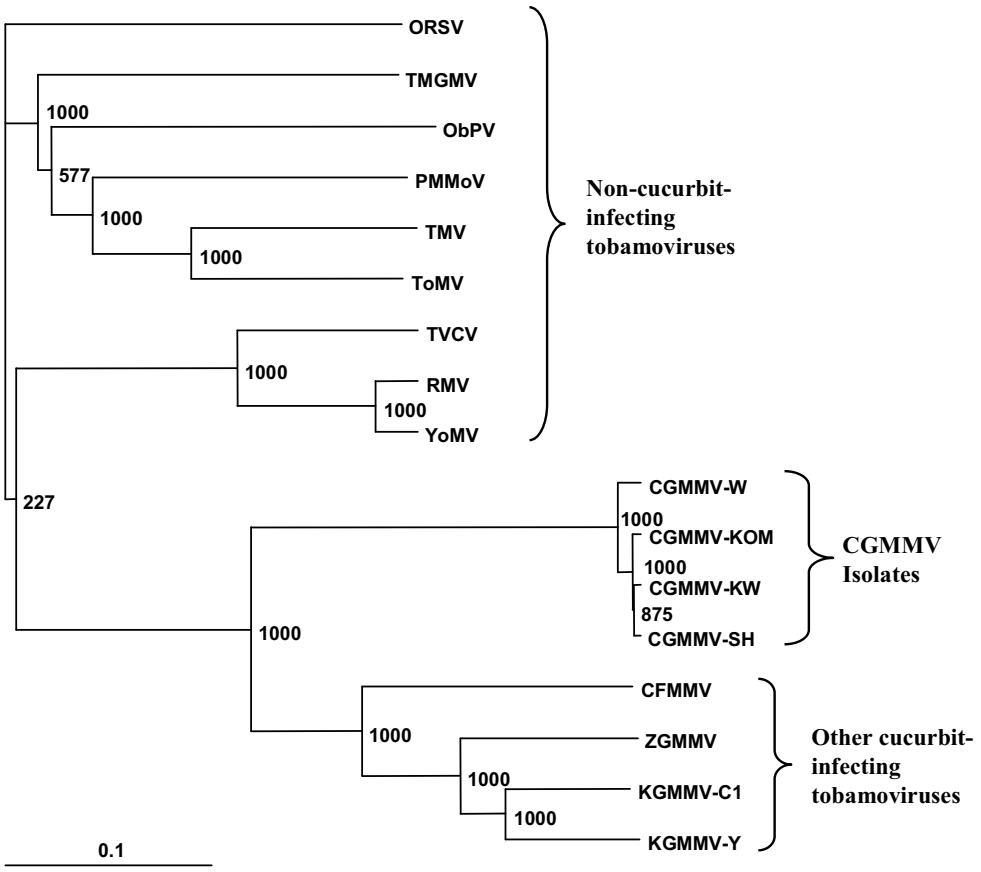

Fig. 5 Phylogenetic relationships of *Cucumber green mottle mosaic virus* with the members of tobamoviruses based on complete genome sequences. Phylogenetic tree was constructed using the neighbour-joining method with bootstrapping (1000 replicates) in TreeView (Win32 version) on sequences aligned using CLUSTALW 1.7 version. Vertical distances are arbitrary. Horizontal distances are proportional to genetic distances. The numbers at nodes refer to number of times in which the branching was supported. The virus acronyms and sequence data are as used in **Table 3**.

sis of CGMMV in Pakistan (Ali *et al.* 2004). The DIBA assay was useful to test a large number of samples for assessing the wide spread occurrence of CGMMV compared to potyviruses in cucurbits in Pakistan (Ali *et al*. 2004). Lactose has been shown as an effective blocking agent compared to egg albumin and skim milk for minimizing non-specific reaction in ELISA. The WB technique is not suitable for diagnosis of a large sample; however, it is used as a confirmatory serological test. Antignus *et al*. (2001) used WB for serological differentiation of CGMMV-Is, -CV4 and CFMMV. ELISA is a widely used serological technique for the detection of CGMMV in crude extract. The polyclonal antibodies raised against CGMMV are able to distinguish CGMMV from other cucurbit-infecting tobamovirus species by ELISA. Antignus *et al*. (2001) used double antibody sandwich (DAS) ELISA to differentiate CGMMV-Is and CFMMV.

The IPA has been demonstrated for rapid and simultaneous detection of CGMMV, KGMMV and ZGMMV (Choi *et al*. 2001). The virus from crude leaf extract is trapped on a glass filter paper strip pretreated with white latex coated with viral antibody. The positive result is visualized by the presence of coloured band when the virus bound filter paper is treated with dyed latex coated with antibody (Tasuda *et al.* 1992). The sensitivity of IPA in detecting CGMMV is lower than DAS-ELISA. The minimum detection limit of IPA has been shown as 5 ng/ml of the purified virus and 2^{-12} dilution of sap (Choi *et al.* 2001).

Nucleic acid hybridization

Nucleic acid hybridization (NH) was employed to distinguish CGMMV isolates/strains reported from UK and Japan (Francki *et al*. 1986). The NH analysis showed that the CV3, CV4 and W isolates were very closely related, whereas C isolate from Japan was quite different. Based on this study, Francki *et al*. (1986) suggested C isolate, as a new virus, KGMMV. Antignus *et al.* (2001) developed riboprobe from the CP gene of CGMMV-Is, which reacted strongly with the total RNA extracted from plants infected with CGMMV-Is.

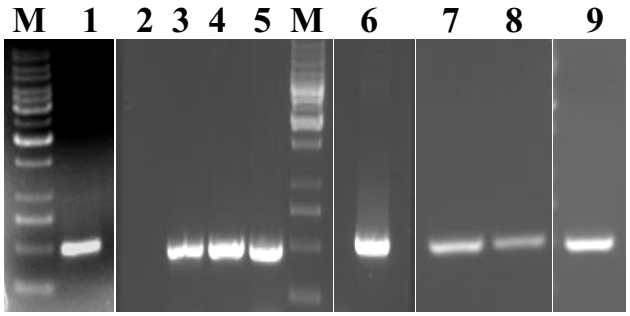

Fig. 6 Detection of *Cucumber green mottle mosaic virus* by RT-PCR in experimentally inoculated plant species. The amplified DNA bands with forward primer: 5′ATGGCTTACAATCCGATCAC3′ and reverse primer: 5′ACCGTCGAAACTAAGCT TTCG3′ are of coat protein gene of 500 bp. M: Marker; lane 1: Positive control; lane 2: Negative control; lane 3: bottle gourd; lane 4: cucumber; lane 5: watermelon; lane 6: muskmelon; lane 7: sponge gourd (*Luffa cylindrica*); lane 8: ridge gourd (*L. acutangula*); lane 9: long melon (*Cucumis melo* var. *utilissima*).

The probe distinguished CGMMV from CFMMV, another cucurbit infecting *Tobamovirus* occurring in Israel.

RT-PCR and real-time RT-PCR

Genome sequences among CGMMV isolates/strains are highly conserved. The primers from the terminal sequences of CP gene serve as valuable tool for reliable diagnosis of CGMMV in RT-PCR (**Fig. 6**). The RT-PCR is accomplished by cDNA synthesis on the RNA template followed by PCR. Both the reactions (RT and PCR) can be conducted either as single tube reaction or as two-steps reactions. CGMMV can be detected efficiently in crude RNA isolated from infected plant tissues by RT-PCR. Kim *et al.* (2003b) used CP based primers in RT-PCR for assessing heat inactivation of CGMMV in contaminated seeds, which showed that CGMMV contained heat sensitive and resistant regions on its genome. The specific primers corresponding to 2 to 2.5

kb and/or 4 to 4.8 kb regions of the genome could be employed for rapid detection of CGMMV inactivated by heat treatment. Immunocapture-RT-PCR (IC-RT-PCR) was used to detect CGMMV affecting watermelon in Greece (Varveri *et al.* 2002). IC-PT-PCR has been showed to be 10^5 times and 10^2 times more sensitive in detecting CGMMV than DAS-ELISA and F(ab')$_2$-ELISA, respectively.

Real-time RT-PCR has been developed for the detection and quantification of CGMMV (Park *et al*. 2006; Chen *et al*. 2008). The technique is highly sensitive, specific and rapid, and thereby ideal for quarantine measure. The one step assay technique has been designed using a pair of primers and a TaqMan probe targeting the conserved sequence in 3′ UTR of CGMMV. The sensitivity of the technique is 0.13 pg of total RNA or 50 copies of RNA (Chen *et al*. 2008).

Bioelectric recognition assay

Bioelectric recognition assay (BERA) is based on the principle of a biosensor constructed with electro-conductive probe containing immobilized cells in agarose gel matrix and microelectrode or data acquisition system. When a positive sample is added to the probe, a change in electrical potential is measured in a characteristic signature upon contact between the virus and the gel matrix. Kintzios *et al.* (2001) demonstrated the use of BERA for detection of CGMMV in a remarkably specific, rapid (within 1-2 min), reproducible and cost-effective manner. In a time course analysis of cucumber plants infected with CGMMV, the virus could be detected in leaf extracts even on the second day after inoculation by BERA biosensor. Whereas, in the same experiment CGMMV was detected by F(ab′)$_2$-ELISA only 16 days post inoculation (Kintzios *et al.* 2001) The BERA is highly sensitive with detection limit of ~0.01 ng/µl and thus can complement immunological or molecular methods of virus detection.

Microarray

Molecular techniques *viz*., PCR, RT-PCR, multiplex PCR, real-time PCR and nucleic acid hybridization assays (dot blot and Southern blot hybridization) were extensively used for the detection of plant viruses. All these traditional molecular techniques have limitation in detecting one or a few target viruses at a time. Microarray system is shown as a powerful high throughput technique for the simultaneous detection of a large number of human viruses (Wang *et al*. 2002). Microarray is a new emerging method in plant virology currently being developed by various laboratories. Lee *et al.* (2003) reported plant virus detection technique based on cDNA chip technology. The cDNA chip was designed for detection and differentiation of the four species of cucurbit-infecting tobamoviruses, CGMMV, CFMMV, KGMMV and ZGMMV. The cDNA chip, which was named as 'cucurbitvirus chip', successfully detected specific target virus. The fluorescent probe made from ZGMMV infected samples reacted strongly with its homologous cDNA and moderately with KGMMV and CFMMV, while it did not react with CGMMV on the same chip. The CGMMV probe reacted strongly with its homologous cDNA spot and weakly with ZGMMV, KGMMV and CFMMV.

MANAGEMENT

Cultural management

In general, management of viral diseases is more difficult compared to the diseases caused by other phytopathogens. This is because viral disease has complex disease cycle, efficient vector for natural spread, wide alternate sources of infection and no effective viricide. No single approach offers solution to the virus disease problems and as a result, multiple approaches are adopted to avoid or minimize the impact of viruses.

The traditional method of management of CGMMV is through cultural practices, which is principally based on the mode of transmission of the virus. Soil and irrigation water contaminated with infected plant debris are shown as primary sources of infection of CGMMV. Therefore, maintenance of sanitation in the field is important to prevent the initial spread of CGMMV. After the cropping season, plant debris should be removed and burnt to avoid the presence of CGMMV in the field from the previous crop. The other way to eliminate CGMMV is by composting infected plant materials, which is subsequently useful for growing plants as the virus is inactivated due to high temperature in compost pile (Avgelis and Manios 1992). Contaminated irrigation water also should be avoided, which will ensure avoidance of introduction of CGMMV from a distant place (Vani and Varma 1993). As CGMMV is known to be seed borne (van Koot and van Dorst 1959; Komuro *et al.* 1971), virus free seeds from healthy plants should be used. Heat treatment at 75°C for 3 days has been shown to inactivate CGMMV from bottle gourd seeds (Kim and Lee 2000). Heat treatment can potentially reduce seed germination and therefore, older seeds are not suitable for heat treatment (Macias 2000). As CGMMV spreads through contact, increase in plant-to-plant distance may help to avoid contact between the plants especially at early stage of crop growth (Rao and Varma 1984). In Japan, multiple tactics were adopted to control CGMMV, seed disinfection by soaking in 10% sodium phosphate for 20 min followed by washing in water, soil fumigation using 80 g methylbromide/m^2 and early removal of diseased plants (Komuro 1971).

Breeding for resistance

The most practical approach to control the virus is to develop virus resistant varieties that prevent movement, replication and symptom development. However, resistant varieties to CGMMV are hardly available. The source of resistance to CGMMV has been reported only in melon and wild *Cucumis* spp. Rajamony *et al*. (1987, 1990a, 1990b) have screened a large collection of dessert and non-desert types of muskmelon, and wild species of *Cucumis* against CGMMV under natural field as well as greenhouse conditions. All the desert type genotypes are susceptible, while the wild species *C. africanus*, *C. figarei*, *C. ficifolius*, *C. jeffrey*, *C. meeusii* and *C. zeyheri*; two non-desert types 'Phoot' or snapmelon (*C. melo* var. *momordica*) and 'Kachri' that are mostly grown in north India, and Cornell breeding lines, FM1 and FM2 are resistant to CGMMV (Rajamony *et al*. 1987, 1990b). Snapmelon has been utilized to develop highly resistant lines, DMR-1, DMR-2, VRM 29-1, VRM 42-4 and VRM 43-6 (More *et al*. 1993). *C. figarei* is immune to CGMMV, while all the other resistant types, snapmelon, Kachri, and other *Cucumis* spp. are symptomless carrier (Pan and More 1996). CGMMV resistance in muskmelon is polygenic and recessive in nature (Rajamony *et al*. 1990a). In Japan, new source of resistance has been identified in oriental melon accession, 'Chang Bougi' (Sugiyama *et al*. 2006). The resistance in Chang Bougi is virus isolate specific as it is resistant to CGMMV-SH isolate but not to CGMMV-W.

Transgenic resistance

Development of resistant varieties through conventional breeding has limitation when no suitable source of resistance is available. Advancement of recombinant technology, plant transformation methodology and molecular biology of plant viruses led to develop genetic engineering approach for developing virus resistant varieties (Powell-Abel *et al*. 1986). A plant with a new trait developed by transferring specific gene from any source through the method of genetic engineering is referred as 'transgenic plants'. The transgenic plant conferring resistance against pathogen is known as transgenic resistance. For developing virus resistant transgenic plants, the gene conferring resistance (transgene) can be derived from virus, plant and microbes (Varma *et al*.

2002). The nucleotide sequences from plant viral genome such as coat protein gene, replicase gene, movement protein gene, antisense RNA, defective interfering RNA and sat-RNA, untranslated sequences have been found useful in developing transgenic resistance against viruses (Beachy 1993; Lomonossoff 1995; Pappu *et al*. 1995; Tabler *et al*. 1998). The CP mediated resistance has been most promising against many different plant viruses and it has been successfully utilised to develop transgenic cucumber, cantaloupe, squash (*C. pepo*), and watermelon resistant to single or multiple infections caused by CMV, WMV-2, PRSV-W and *Zucchini yellow mosaic virus* (Fuchs *et al.* 1997). Although, transgenic resistance is highly promising, not many efforts are known having directed for the management of CGMMV. In Korea, Park *et al.* (2005) developed transgenic watermelon rootstock resistant to CGMMV. The popular watermelon rootstock, 'Gongdae', which is used for grafting watermelon, cucumber and muskmelon in Korea and Japan, has been transformed with the CP gene of CGMMV. The transgenic rootstock, which showed resistance against CGMMV, is expected to reduce initial infection of CGMMV through contaminated soil. Recently, high level transgenic resistance to CGMMV has been demonstrated by using CGMMV CP specific short interfering RNA in experimental plant, *N. benthamiana* (Kamachi *et al*. 2007).

CONCLUDING REMARKS

CGMMV is an economically important tobamovirus affecting several cucurbit crops in Asia and Europe. CGMMV is known since 1935 and until 2000; this was the only cucurbit infecting tobamovirus. During 2001-2002, three more cucurbit-infecting *Tobamovirus* species have been identified. The biological and molecular properties of CGMMV are unique and thereby it is recognised as a species under the genus *Tobamovirus*. Symptomatology of at least 18 isolates has been described from eight countries in Asia and Europe and complete genome sequence data are available for five isolates two each from Japan and Korea and one from India. The sequence data of CP is an important taxonomical criterion for differentiation of strains of the species under *Tobamovirus*. The CP sequences of the CGMMV isolates studied since last 15 years from different countries and different host plants are remarkably conserved. Functional genomics of CGMMV has not been investigated well. Of all the CGMMV-encoded proteins, so far, only CP has been identified *in vivo* (Okada 1986). CGMMV having positive sense monopartite genome is an attractive choice for generating edible vaccine in cucurbits (Ooi *et al*. 2006). The virus being highly stable and contagious is difficult to control. Resistant cultivar is the best option for the management of the virus. However, development of resistant cultivar in different cucurbits to CGMMV through classical breeding has limitation as limited source of host resistance is known only in melon and wild *Cucumis* spp. The transgenic approach holds good potential as resistance can be engineered in any cucurbits. However, so far limited efforts have been made in utilising transgenic approach for the management of CGMMV.

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