**PhD thesis title**

# "Molecular characterization of *Cucumber green mottle mosaic virus* occurring in India and development of transgenic plant using coat protein gene"

**by**

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## Summary

*Cucumber green mottle mosaic virus* (CGMMV), a member of the genus *Tobamovirus* is known to affect bottle gourd and muskmelon in India. No molecular information about CGMMV occurring in India was known and no transgenic resistance to CGMMV in bottle gourd has been studied. Therefore, the present study was undertaken with the objective to (1) characterize CGMMV isolates commonly occurring in India, (2) determine genome organization of CGMMV, (3) determine the phylogenetic relationships, (4) develop protocol for transformation of bottle gourd using CP gene. The complete genome (6.4 Kb) sequence of CGMMV infecting bottle gourd was achieved and a phylogenetic relationship with the members of the genus *Tobamovirus* was established. The coat protein gene of the virus was successfully used to develop transgenic bottle gourd plant.

Two isolates of CGMMV, one from Delhi (CGMMV-D) and the other from Sri Ganganagar, Rajasthan (CGMMV-Raj) were initially identified based on electron microscopy and amplification of CP gene by RT-PCR. The isolates were easily transmitted by sap inoculation to bottle gourd plants that developed typical green mottle mosaic disease of CGMMV. CGMMV could be transmitted through contaminated razorblade from bottle gourd to bottle gourd. Host range for CGMMV-D has been identified. In Cucurbitaceae, bottle gourd (*Lageneria siceraria*), cucumber (*Cucumis sativas*), long melon (*C. melo* var. Utilissimus), muskmelon (*C. melo*) and watermelon (*Citrullus lanatus*) produced greenish mosaic symptom. Pumpkin, ridge gourd and sponge gourd were identified to be symptomless carrier of CGMMV. Only one plant species under family, Solanaceae, *Nicotiana benthamiana* produced green mosaic symptoms. The host-range reaction for both the isolates were similar in all the inoculated plant species except in *Chenopodium amaranticolor*, which produced chlorotic lesions following inoculation with CGMMV-Raj isolate, but not with CGMMV-D.

Purification of CGMMV was achieved by precipitation with polyethylene glycol followed by ultracentrifugation. The length of the majority of the intact virus particles was measured as 300 nm. The molecular weight of the capsid protein was estimated about 14.0 kDa in SDS-PAGE. Molecular cloning and sequencing of CP gene

of both the isolates showed 98.7% similarities. As biological and molecular properties of both the isolates were similar only CGMMV-D isolate was studied further.

The complete genome of CGMMV-D was cloned as seven overlapping fragments and sequenced. The nucleotide sequencing of all these clones showed that CGMMV-D genome is 6424 nucleotide (nt) long containing four open reading frames (ORF) and terminal untranslated regions (UTR) of 60 nt at 5' end and 176 nt at 3' end. The ORF-1 (61-3493 nt) and ORF-2 (61-5007 nt) encoded a short (129 kDa) and long (186 kDa) putative replicase proteins, respectively. The ORF-3 (4994-5785 nt) encoded a 29 kDa protein, which is a putative cell-to-cell movement protein. The ORF-4 (5763-6281 nt) encoded a 17.4 kDa protein, a putative coat protein. The complete genome of CGMMV-D is highly conserved having 97.7-98.7% identity with that of the other isolates of CGMMV (KOM, KW, SH and W) reported from Japan and Korea. Whereas, CGMMV-D shared only 44.4-60.5% identities with the other *Tobamovirus* species. The ORF-3 (MP) and ORF-4 (CP) was the most conserved region of the genome of CGMMV-D. The phylogenetic trees constructed based on the coding and non-coding sequences of all the known tobamoviruses revealed that the CGMMV-D clustered very closely with the other isolates of CGMMV. The molecular characterization based on complete genome sequence was reported for four isolates of CGMMV, SH (muskmelon isolate), W (watermelon isolate), KW (Korean watermelon isolate), KOM (Korean oriental melon isolate) from Japan & Korea. In the present study, for the first time a bottle gourd isolate of CGMMV occurring in India was completely sequenced and characterized at molecular level.

A transgenic construct was developed from the CP gene (486 nt) of CGMMV-D and a commercial cultivar of bottle gourd; Pusa Naveen was successfully transformed through *Agrobacterium* mediated gene delivery. The cotyledonary explants of bottle gourd were surface sterilised by a single treatment of 0.1% mercuric chloride for 5 min. The cotyledonary basal explant from 5-6 days old seedlings was found most suitable for organogenesis following co-cultivation with the *Agrobacterium* containing CP gene. Co-cultivation in induction medium (MS medium containing 3-6 mg/l BA and agar 0.7%) resulted in high level of callus induction in 80.0-94.5% of the explants by 4-6 days. The shooting medium was composed of MS with 2 mg/l BA and 1 mg/l IAA and 400 mg/l cefotaxim sodium. The rooting medium composed of only MS or with 0.1 mg/l NAA. The present method of transformation resulted in about 7.5% rooted plants by 8 weeks. The transgenic plants were analysed by PCR with the specific primers followed by Southern hybridization with the homologous DNA probe. The results showed transformation frequency of 3.61%. The present study demonstrates, for the first time, transformation of a commercial Indian cultivar of bottle gourd with the viral gene construct. The transformation protocol developed in the present study will be useful in development of transgenic bottle gourd resistant to CGMMV.