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Screening of *Lagenaria siceraria* (Mol.) Standl. Genotypes against a *Tobamovirus* causing mosaic disease in Jammu

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Abstract
CGMMV, a member of *Tobamovirus*, family *Virgaviridae* is a predominant virus infecting bottle gourd and is known to cause serious losses worldwide. Many germplasm accessions showing resistance to CGMMV have been reported in *Cucumis* species like *C. sativus, C. melo, C. anguria* etc. However, at present, not much information is available regarding CGMMV resistance in bottle gourd. Therefore, in this study, sixty-six genotypes of bottle gourd were evaluated for resistance. Only one variety (Warad) was found resistant, while six were moderately resistant, twenty-nine were moderately susceptible, twenty-six susceptible and only four were highly susceptible. The presence of CGMMV in infected plants were confirmed using coat protein specific primers.

Keywords: Bottle gourd, screening, CGMMV, resistance, PCR

1. Introduction
Bottle gourd (*Lagenaria siceraria* (Mol.) Standl.) is a major pan tropical cucurbitaceous crop. It is a monoecious annual herb and the only species of *Lagenaria* which is cultivated. Bottle gourd is one of the major crops in India none the less in Jammu, J&K famous for its nutritional values. The bottle gourd is a source of carbohydrates, protein, fat and vitamin C and is used as vegetable also for pickling and in desserts (Gajera and Joshi, 2014) [4]. Bottle gourd is attacked by many biotic factors *viz.*, fungi (*Colletotrichum lagenarium, Erysiphe cichoracearum, Alternaria alternate, Fusarium oxysporum* f.sp. *lagenariae, Cercospora lagenariae* etc.), bacteria (*Pseudomonas syringae* pv. *lachrymansngae, Erwinia tracheiphila etc*.) and viruses. Around the world more than 59 viruses are known problematic to the bottle gourd production (Nagendran *et al.*, 2017a) [8]. The most important viruses reported from bottle gourd are *Cucumber Green Mottle Mosaic Virus* (CGMMV), *Cucumber Mosaic Virus* (CMV), *Watermelon Mosaic Virus* (WMV), *Papaya Ringspot Virus- W* (PRSV- W), *Pumpkin Yellow Vein Mosaic Virus* (PYVMV), *Zucchini Yellow Mosaic Virus* (ZYMV) (Nagendran *et al.*, 2017b) [9]. These are destructive and difficult to manage and have caused number of losses worldwide (Lecoq and Desbiez, 2012) [5].
CGMMV was first reported in Great Britain from *Cucumis sativus* and till date, is an important pathogen naturally infecting cucurbits like cucumber, pumpkin, melons, squash and gourds (Ainsworth, 1935) [1]. This virus has rapidly spread not only within the countries from where they have been reported but also from Europe to Asia, America and Australia (Dombrovsky *et al.*, 2017) [3]. CGMMV is a *Tobamovirus* (family: *Virgaviridae*), having a +ve sense ssRNA (6.4kb) and four open reading frames (ORFs). These ORFs encode one helicase, one RdRp enzyme, one movement protein (MP) and one coat protein (CP), wherein, MP & CP ORFs overlap each other. The replication related proteins are translated via genomic RNA while the MP& CP from two sub genomic RNAs (Mandal *et al.*, 2008; Liu *et al.*, 2020) [7, 6].
CGMMV symptoms varies from one cucurbit species to another as well as one cultivar to other cultivar within a species. The typical symptoms include mosaic mottling, leaf deformation as well as fruit distortion. CGMMV was identified in India by Vasudeva and Lal (1943) [16] as the virus causing mosaic of bottle gourd. It has an incidence as high as 100 per cent in North India and causes losses in various cucurbits i.e., up to 64 per cent alone in bottle gourd (Vasudev *et al.*, 1949; Raychaudhuri and Varma, 1978; Rao and Varma, 1984) [17, 13, 11]. Worldwide, CGMMV has caused many epidemics including in India (Rao *et al.*, 2016). CGMMV's rapid global increase over the past 15 years has left us to ponder upon its

importance, its effect on the crops and how to manage this virus? Given its spread and detrimental effects CGMMV has been placed under the category of quarantine pests.

Methods to manage any viral disease includes insecticidal spray schedules for insect- vectors, herbicides for the weeds (alternate hosts for the viruses) and resistance. The most economical method for managing the viral disease is to utilize the genetic resistance. Although bottle gourd accessions have been screened against viruses like ZYMV, PRSV- W, CMV in many countries, however, resistant sources in bottle gourd germplasm has not been screened extensively against CGMMV. Therefore, in our experiment an effort was made to find sources of resistance towards CGMMV in bottle gourd accessions as well as varieties.

2. Material and Methods

2.1 Plant material & raising healthy seedlings

Twenty indigenous collections of bottle gourd were procured from NBPGR, New Delhi. Also, forty-six varieties/ hybrids were also collected from various places (Table 1). The seedlings were raised in 6-inch plastic pots containing potting mixture (sterilized soil and Vermicompost @ 2:1) grown in insect- proof conditions in a green house.

Table 1: list of the bottle gourd genotypes (germplasm / varieties) screened for resistance against CGMMV

S. no	Genotypes	S. no	Genotypes
1	IC- 371695	34	Gaurav
2	IC- 342081	35	lattu Manvik
3	IC- 40890	36	Urvashi F1
4	IC- 371675	37	Pooja
5	IC- 276524	38	NE lauki
6	IC- 284965	39	NE lattu
7	IC- 322274	40	VC- 038
8	IC- 331101	41	Komal Kiran
9	IC- 337077	42	Kanchan
10	IC- 382192	43	Imperial G2
11	IC- 339215	44	Imperial GTK
12	IC- 382240	45	ES Gola
13	IC- 385816	46	Uttam
14	IC- 392192	47	Lata
15	IC- 398534	48	Makhmal
16	IC- 531896	49	Rani
17	IC- 536536	50	Pooja-9
18	IC- 546147	51	Ratan
19	IC- 550725	52	Megha Star
20	IC- 342077	53	M-11
21	MGH-1	54	Kundal BSS- 687
22	Vardan	55	Research-10
23	Sharada	56	Naveen F1
24	Bhushan	57	Pusa Komal
25	MHBG-8	58	Sudha
26	MGH-4	59	Akash
27	JS-651	60	Earth Co Lauki
28	GC-S27	61	Neo lauki
29	GC-S28	62	HP local-1
30	NO.17	63	HP local-2
31	HY- 401	64	JK local-1
32	PSPL- 101	65	JK local-2
33	Shiva- 305- F1	66	Bihar local

2.2 Maintenance of Virus Culture & Method of inoculation: The viral isolate was collected from the farms of SKUAST-J, Chatha, Jammu. This isolate was multiplied and maintained on a susceptible bottle gourd variety *viz*., 'MAHY-1' for future use. The inoculum was prepared by macerating 100 mg of infected 'MAHY-1' leaves showing typical dark green mottled mosaic symptoms in 100 ml ice cold 0.01 M phosphate buffer (Sodium Phosphate, 7.0 pH). The sap was then filtered through cheese cloth into a sterile Petri dish. The healthy seedlings to be inoculated were dusted with an abrasive i.e., carborundum powder (600 mesh) to create wounds for facilitating virus entry in to the host plants. Standard "leaf rub method" was used for inoculating the germplasm.

2.3 Screening of germplasm

The healthy seedlings of the germplasm/ cultivar were inoculated at 2 to 4 leaf stage. The symptom development was observed at 21, 36 and 55 days post inoculation (dpi). The germplasm was graded into different categories using the disease rating scale by Rajamony *et al.,* (1990) [10] (Table 2).

Table 2: Scale for scoring the germplasm

Scale	Symptoms	Reaction
0	Immune (I)	No symptoms
1	Resistance (R)	Slight vein clearing, very light mottling of light & dark green colour in younger leaves
2	Moderately Resistance (MR)	Mottling of leaves with light and dark green colour
3	Moderately Susceptible (MS)	Blisters & raised surfaces on the leaves
4	Susceptible (S)	Distortion of leaves
5	Highly Susceptible (HS)	Stunting of the plants with negligible or no flowering and fruiting

The germplasm appearing to be resistant was back inoculated on to the susceptible cultivar 'MAHY-1' in order to detect any symptomless carrier.

2.4 Confirmation of Virus

The presence or absence of the virus was confirmed using RT-PCR based on coat protein specific primers of CGMMV. The total RNA from the resistant and infected plants was isolated using "Plant Total RNA kit, Sigma Aldrich" according to the manufacturer's protocol. The RNA was isolated 15 dpi. The RNA was checked on 1 per cent agarose gel electrophoresed at 90 volts for 1 hour in 0.5 X TAE buffer. Complementary DNA (cDNA) was synthesized using Verso cDNA synthesis kit (Thermo scientific) according to the manufacturer's instructions and amplification was performed as described by Nagendran *et al.,* 2017b [9]. The presence or absence of amplicon was detected through agarose gel electrophoresis (1.2%, in 0.5 X TAE buffer).

3. Results

The germplasm/ varieties inoculated with CGMMV isolate, expressed differentiated symptoms like vein clearing at younger leaf stages, mosaic mottling, blistering on leaves, leaf deformation etc. and were scored accordingly (Table 3). The time taken for symptom expression by individual plants varied according to the genotype and ranged between 15 days to 20 days on an average. All the replicated plants of a genotype showed similar symptoms. Among all the 66 genotypes inoculated, only one i.e., 'Warad'/ 'MGH-4' was resistant to the test isolate, while zero genotype had immunity. Six genotypes *viz.*, IC-40890, IC- 337077, IC-550725, Gaurav, Ratan, Pusa Komal showed moderate resistance towards the CGMMV isolate. About twenty-nine

were moderately susceptible. Four germplasm/ varieties were highly susceptible, whereas remaining twenty- six were rated as susceptible (Table 4).

The resistant cultivar 'MGH-4' showed vein clearing symptoms in early 2 leaf stage and later recovered and produced no symptoms after 55 dpi therefore was categorized as resistant. The plants categorized under moderately resistant either had a delayed onset of symptom expression or the rate of incidence was low (or both the factors). A large fraction of the genotypes accessed were labelled as moderately susceptible and had symptoms like blisters on leaves. The infection even though being systemic had negligible effect on the overall growth and fruit development when compared to the plants kept as control. In case of susceptible plants, they had an early expression of symptoms in comparison to the moderately susceptible plants. They exhibited symptoms like mosaic mottling, leaf deformation and substantial effect on fruit formation (deformed). We found only a few handfuls of cultivars/ germplasm as highly susceptible. Such lines had high incidence of virus and mostly exhibited stunted growth or with dark mottled mosaic, distorted leaves. Such vines if produced fruits were deformed and unconsumable (Fig. 1).

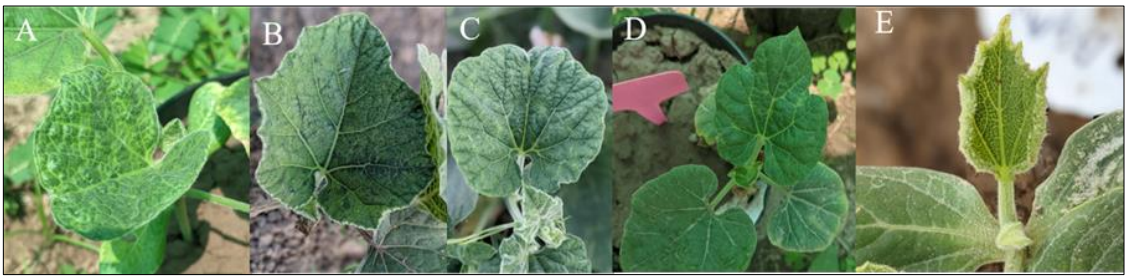

Fig 1: Various symptoms exhibited by the genotypes at 15- 20 dpi. A- mottling with slight blisters, B- mosaic mottling, C- leaf deformation, D- vein clearing in younger leaves

The most common symptom observed at 21 dpi was green mosaic mottling on leaves which later progressed in many genotypes into blisters on leaves or deformation of leaves as well as stunting in one case. The symptoms remained same for 36 dpi and 55 dpi. The experiment was repeated twice (May-July 2021 and Aug- Oct 2021) again under controlled conditions, and the results obtained remained identical. The RT-PCR using CGMMV CP primers, revealed an amplicon of 604 bp in case of the infected plants therefore confirming the presence of the CGMMV in them (Fig. 2).

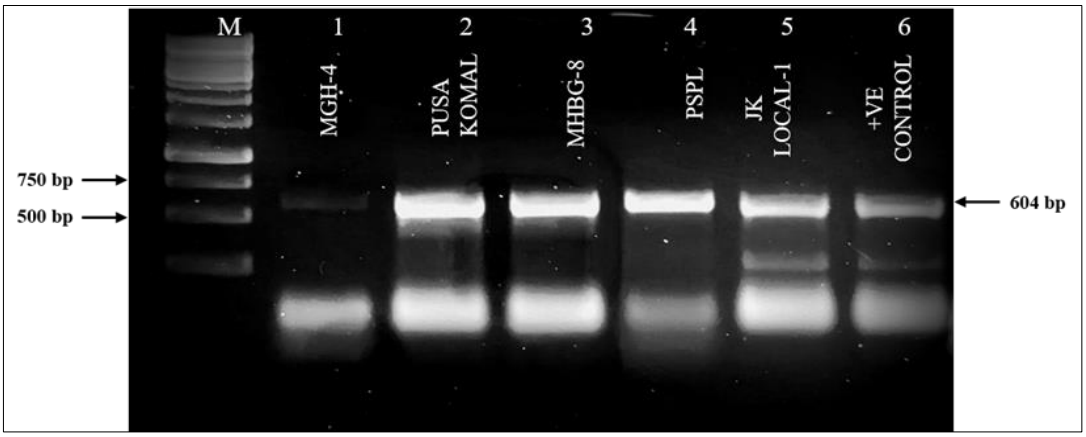

Fig 2: Detection OF CGMMV based on RT-PCR using CP specific primers in the bottle gourd germplasm: M- DNA ladder 1kb, 1- Resistant, 2- Moderately Resistant, 3- Moderately Susceptible, 4- Susceptible, 5- Highly Susceptible, 6- Positive control.

4. Discussion

CGMMV has serious impact on various cucurbits like watermelon, cucumber, and bottle gourd. CGMMV has been known to cause frequent epidemics across the globe. In field the CGMMV spread through seeds, contact, soil, plant debris as well as irrigation water. Therefore, identification and utilizing of host resistance is said to be the best method of managing this virus and the yield loss it causes.

Table 3: Screening of bottle gourd germplasm for the exploration of resistant sources for mosaic disease under artificial conditions

S. No	Genotypes/varieties	Score			Reaction
		21 dpi	36 dpi	55 dpi	
1	IC- 371695	2	3	3	MS
2	IC- 342081	2	3	3	MS
3	IC- 40890	2	2	2	MR
4	IC- 371675	2	3	3	MS
5	IC- 276524	2	4	4	S
6	IC- 284965	3	3	3	MS
7	IC- 322274	1	3	3	MS
8	IC- 331101	2	4	4	S

9	IC- 337077	0	2	2	MR
10	IC- 382192	3	4	4	S
11	IC- 339215	3	5	5	MS
12	IC- 382240	3	4	4	S
13	IC- 385816	2	3	3	MS
14	IC- 392192	2	3	3	MS
15	IC- 398534	3	4	4	S
16	IC- 531896	3	4	4	S
17	IC- 536536	3	3	3	MS
18	IC- 546147	2	3	3	MS
19	IC- 550725	1	2	2	MR
20	IC- 342077	1	3	3	MS
21	MAHY-1	3	4	4	S
22	Vardan	3	4	4	S
23	Sharada	3	4	4	S
24	Bhushan	1	3	3	MS
25	MHBG-8	2	3	3	MS
26	MGH-4	1	1	0	R
27	JS-651	2	3	3	MS
28	GC-S27	3	4	4	S
29	GC-S28	3	4	4	S
30	NO.17	2	3	3	MS
31	HY- 401	2	4	4	S
32	PSPL- 101	3	4	4	S
33	Shiva- 305- F1	2	3	3	MS
34	Gaurav	2	2	2	MR
35	lattu (Manvik)	3	3	3	MS
36	Urvashi F1	2	3	3	MS
37	Pooja	2	3	3	MS
38	NE lauki	3	5	5	HS
39	NE lattu	2	3	3	MS
40	VC- 038	2	3	3	MS
41	Komal Kiran	3	3	3	MS
42	Kanchan	2	4	4	S
43	Imperial G2	2	3	3	MS
44	Imperial GTK	2	4	4	S
45	ES Gola	3	4	4	S
46	Uttam	3	4	4	S
47	Lata	3	3	3	MS
48	Makhmal	3	3	3	MS
49	Rani	3	4	4	S
50	Pooja-9	3	4	4	S
51	Ratan	1	2	2	MR
52	Megha Star	1	3	3	MS
53	M-11	2	3	3	MS
54	Kundal BSS- 687	3	4	4	S
55	Research-10	3	4	4	S
56	Naveen F1	3	4	4	S
57	Pusa Komal	1	2	2	MR
58	Sudha	3	4	4	S
59	Akash	3	4	4	S
60	Earth Co Lauki	3	3	3	MS
61	Neo lauki	3	4	4	S
62	HP local-1	3	5	5	HS
63	HP local-2	3	3	3	MS
64	JK local-1	3	5	5	HS
65	JK local-2	3	4	4	S
66	Bihar local	3	4	4	HS

Table 4: Disease reaction of bottle gourd varieties/ genotypes against mosaic disease

Disease Reaction	Germplasm	No. of entries
Immune	-	-
Resistant	MGH-4	1
Moderately Resistant	IC-40890, IC- 337077, IC-550725, Gaurav, Ratan, Pusa Komal	6
Moderately	IC- 371675, IC-371695, IC-342081, IC- 284965, IC- 322274, IC-385816, IC-392192, IC- 536536, IC- 546147,	29

Susceptible	IC-342077, IC-339215, Bhushan, MHBG8, JS-651, No. 17, Shiva- 305- F1, Lattu Manvik, Pooja, NE lattu, Komal kiran, Earth Co lauki, Urvashi F1, VC- 038, Imperial G2, Lata, Makhmal, Megha star, M-11, HP local- 2	
Susceptible	IC- 276524,IC-331101, IC- 382192, IC- 382240, IC-398534, IC- 531896, MGH-1, Vardan, Sharada, GC S27, GC S28, HY- 401, Neo lauki, PSPL- 101, Kanchan, Imperial GTK, ES Gola, Uttam, Rani, Pooja-9, Research – 10, Kundal BSS- 687, Naveen F1, Sudha, Akash, JK Local-2	26
Highly Susceptible	NE lauki, HP Local-1, JK Local-1, Bihar local	4

Therefore, we tried to explore genotypes in search of resistance against CGMMV. In the present study out the 66 germplasm/ varieties evaluated under controlled conditions during 2021 only one was found to be resistant towards this cucurbitaceous *Tobamovirus* i.e., CGMMV. Six showed moderately resistant reaction while, 29 were moderately susceptible, 26 were susceptible whereas, 4 were highly susceptible. Similar studies have been done by various workers across the globe, but in cucurbits like *C. sativus* and *C. melo*. Little to no literature of such work in bottle gourd is available. In India Rajamony *et al.*, 1990 [10] screened melon germplasm and found non dessert types *viz.*, 'Phoot', 'Kachri', 'FM1' and 'FM5' resistant. Crespo *et al*., (2018) [2] evaluated germplasm of *C. sativus*, *C. anguria* as well as *C. metuliferus* using two strains of CGMMV and found only *C. anguria* resistant to both. Only two *C. sativus* accessions showed mild symptoms while rest had severe infections. Similar study was conducted by Sugiyama *et al*. (2006) [15] and screened 152 melon accessions for resistance to CGMMV and found only Chang Bougi (*C. melo* var. *makuwa* Makino) was resistance to the test isolate CGMMV-SH. Ruiz (2021) screened forty-seven accessions of *C. melo* CGMMV and scored based on symptoms and used qRT-PCR for determining the viral load.

5. Conclusion

In our effort of searching the source of resistance towards CGMMV in bottle gourd we found only one variety, resistant towards CGMMV while 29 were moderately resistant. These germplasm/ varieties can be utilized in programs for exploring resistant genes and in breeding programme to develop a CGMMV resistant cultivar.

6. Future Prospect

CGMMV is predominant in the bottle gourd plants grown in the Northern India. The use of resistant sources can be effective method for withstanding this virus. Therefore, the search for more resistant sources against bottle gourd mosaic disease must be continued. More bottle gourd germplasm should be collected and evaluated to identify more sources of resistance against CGMMV. Study on inheritance in indigenous as well as exotic collections of germplasm should be conducted. R gene linked markers can be identified and used in such investigations making the search for resistance more accurate and authentic.

7. References

1. Ainsworth GC. Mosaic diseases of the cucumber. Annals of Applied Biology. 1935;22(1):55-67.
2. Crespo O, Janssen D, Robles C, Ruiz L. Resistance to Cucumber green mottle mosaic virus in Cucumis sativus. Euphytica. 2018;214(11):1-1.
3. Dombrovsky A, Tran-Nguyen LT, Jones RA. Cucumber green mottle mosaic virus rapidly increasing global distribution, etiology, epidemiology, and management. Annual review of phytopathology. 2017;55:231-56.
4. Gajera RR, Joshi DC. Processing and storage stability of bottle gourd (*L. siceraria*). Agricultural Engineering International: CIGR Journal. 2014;16(2):103-7.
5. Lecoq H, Desbiez C. Cucurbit viruses in the Mediterranean region: an ever- changing picture. Advances in Virus Research. 2012;84:67-126.
6. Liu M, Liang Z, Aranda MA, Hong N, Liu L, Kang B, *et al*. A cucumber green mottle mosaic virus vector for virus-induced gene silencing in cucurbit plants. Plant Methods. 2020;1:1-3.
7. Mandal S, Mandal B, Haq QM, Varma A. Properties, diagnosis and management of Cucumber green mottle mosaic virus. Plant viruses. 2008;2(1):25-34.
8. Nagendran K, Mohankumar S, Aravintharaj R, Balaji CG, Manoranjitham SK, Singh AK, *et al*. The occurrence and distribution of major viruses infecting cucurbits in Tamil Nadu state, India. Crop Protection. 2017a;99:10-6.
9. Nagendran K, Pandey KK, Rai AB, Singh B. Viruses of Vegetable Crops: Symptomatology, Diagnostics and Management. IIVR Technical Bulletin No. 75, IIVR, Varanasi, 2017b, 48.
10. Rajamony L, More TA, Seshadri VS, Varma A. Reaction of muskmelon collections to cucumber green mottle mosaic virus. Journal of Phytopathology. 1990;129(3):237-44
11. Rao DC, Varma A. A strain of cucumber green mottle mosaic virus (CGMMV) from bottlegourd in Saudi Arabia. Phytopathol Z. 1984;109:325.
12. Rao LX, Guo Y, Zhang LL, Zhou XP, Hong J, Wu JX. Genetic variation and population structure of Cucumber green mottle mosaic virus. Archives of virology. 2017;162(5):1159-68.
13. Raychaudhuri M, Varma A. Mosaic disease of muskmelon, caused by a minor variant of cucumber green mottle mosaic virus. Phytopathologische Zeitschrift. 1978;93(2):120-5.
14. Ruiz L, López C, Picó B, Janssen D. Resistance to cucumber green mottle mosaic virus in Cucumis melo. Plants. 2021 May 27;10(6):1077.
15. Sugiyama M, Ohara T, Sakata Y. A New Source of Resistance to Cucumber Green Mottle Mosaic Virus in Melon. Journal of the Japanese Society for Horticultural Science. 2006;75(6):469-475.
16. Vasudev RS, Lal TB. A mosaic disease of bottle gourd. Indian Journal of Agricultural Sciences. 1943;13:182-191.
17. Vasudeva RS, Raychaudhuri SP, Singh J. A new strain of Cucumis virus 2. Indian Phytopathology, 1949, 2(2).