Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(5): 331-335

Received: 10-06-2020
Accepted: 12-08-2020

**Arvınd Mohanan**
Department of Plant Pathology, Punjab Agricultural University, Ludhiana, Punjab, India

**Abhıshek Sharma**
Department of Vegetable Science, Punjab Agricultural University, Ludhiana, Punjab, India

**Mamta Pathak**
Department of Vegetable Sciences, Punjab Agricultural University, Ludhiana, Punjab, India

**Corresponding Author:**
**Arvınd Mohanan**
Department of Plant Pathology, Punjab Agricultural University, Ludhiana, Punjab, India

# Germplasm screening and molecular characterization of begomovirus associated with bitter gourd yellow mosaic disease in Punjab

**Arvınd Mohanan, Abhıshek Sharma and Mamta Pathak**

**Abstract**
The screening study was conducted to identify the germplasm resistant to begomovirus associated with bitter gourd yellow mosaic disease (BGYMD). A total of sixteen bitter gourd germplasms, procured from Punjab Agricultural University (PAU) were grown in protrays for this purpose. The virulent whiteflies fed on previously characterized infected plant source were allowed to feed on these germplasms. The per cent disease ıncidence ranged from 40 to 100 per cent. Based on 0-5 disease scale, none of the entries were found to be immune to begomovirus. However, the germplasm PAU BG-61 was found to be moderately resistant and recorded lowest per cent disease incidence. The DNA extracted from the infected plants, cross checked with Polymerase Chain Reaction (PCR) confirmed the presence of begomovirus. The molecular characterization studies revealed that the associated begomovirus was close to Tomato leaf curl New Delhi virus (ToLCNDV).

**Keywords:** Begomovirus, BGYMD, PCR, per cent disease incidence, screening, ToLCNDV

## Introduction
Bitter gourd (*Momordica charantia* L.) (2n=22) is an important vegetable crop mainly cultivated in Asia and tropical countries. It belongs to the family *Cucurbitaceae* and has a unique bitter taste. Despite of bitter taste, it is susceptible to fungal, bacterial, virus and insects attack. Among viral diseases, BGYMD is most severe and widespread, encountered by farmers all over the world [1]. The disease leads to poor yield and reduction in fruit quality [2]. BGYMD is usually associated with viruses belonging to three distinct families *Bromoviridae*, *Potyviridae* and *Geminiviridae*. The family *Bromoviridae* and *Potyviridae* consist of positive sense single stranded RNA viruses and are transmitted by aphid (*Aphis* spp) whereas the begomoviruses belonging to *Geminiviridae* are transmitted by whitefly. A roving survey conducted in major bitter gourd growing areas of Punjab revealed that the per cent disease incidence ranged from 14-38 per cent and most of the symptoms were found to be associated with begomovirus. The symptoms ranged from mosaic or mosaic along with blistering and curling [3]. The most economical method of viral disease control includes development of resistant source. Therefore, it is necessary to identify the available resistant /moderately resistant germplasm in order to combat this disease.

## Materıals and Methods
The genotypes of bitter gourd were collected from vegetable science department of PAU, Ludhiana, Punjab for the evaluation of resistance to BGYMD (Table 2). The resistance to begomovirus was evaluated by whitefly transmission method. The naturally infected plants showing typical blistering, yellowing and mosaic were earlier confirmed for the presence of begomovirus with the help of Polymerase Chain Reaction (PCR) [4]. The inoculum was characterized and used as source for transmission studies. For this purpose, bitter gourd germplasms were grown in protrays and transferred to the whitefly screen house at vegetable farm, PAU at two true leaf stage. Infected whiteflies were allowed to feed on genotypes. The plants were observed daily for the development of symptoms. Days to first appearance of symptoms, percent plants infected and type of symptoms produced were recorded. Seedlings successfully infected by whitefly transmission were cross checked by PCR and sequenced.

**Maintenance of whitefly culture**

The culture of *Bemisia tabaci* was maintained on disease free cotton plants at vegetable farm, PAU. The cotton plants were grown in earthern pots and non-viruliferous whitefly were maintained all round the year by replacing the cotton plant.

**Raising of healthy bitter gourd germplasms**

Healthy seedlings of different bitter gourd germplasms at two true leaf stage were used for transmission studies. For this, ten seeds of sixteen available germplasm were sown in protrays filled with soil and compost mixture in 2:1 proportion and kept in insect proof cages. These protrays were transfered to whitefly screen house for screening studies.

**Collection of whiteflies and acquisition feeding**

A self made aspirator was used for the collection of whiteflies. The whiteflies were sucked into the aspirator from the cotton leaves and transferred into the collection bottle. The plastic collection bottle was 25 cm in length and 7.5 cm in diameter with a tapered end. The bottom portion was removed with a sharp knife and covered with white muslin cloth. The other end was closed with a cotton plug to prevent the whiteflies from escaping. A young twig of infected bitter gourd plant showing mosaic and curling symptoms was inserted and held upright into the bottle containing the whitefly and closed with the cotton plug immediately.Whiteflies were moved to this bottle carefully with the help of aspirator. An acquisition feeding period of 24 hours was given and after this period, whitefly were again collected with the aspirator and transfered to bitter gourd germplasms for further studies.

**Inoculation feeding**

Plastic bottles 6.0 cm long with 4.0 cm diameter were taken and the top and the bottom portions were removed with a sharp knife .White muslin cloth was pasted to one end to prevent whitefly from escaping and to avoid accumulation of excess moisture. The viruliferous white flies after feeding period of 24 hrs were released into the bottle with the help of aspirator at the rate of 5-10 whiteflies per plant. After 24 hours of inoculation feeding, the plastic bottles were sprayed with triazophos @ 0.15 per cent to kill the whitefly in order to prevent it from infecting other plants and plants were left undisturbed in insect proof cage to observe development of symptoms.

**Screening process**

Ten plants of each germplasm was screened at two true leaf stage along with susceptible check PAU BG-14. The number of diseased and healthy plants were recorded after 15 days. The per cent disease incidence was calculated using following formula:

$$\text{Per cent Disease Incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

The resistance against BGYMD was evaluated using self made disease scale as shown below:

**Table 1:** Disease rating scale

| Scale | Percent infection | Infection category | Reaction group |
|---|---|---|---|
| 0 | All plant free of disease symptoms | Highly resistant | HR |
| 1 | 1-20 | Resistant | R |
| 2 | 21-40 | Moderately resistant | MR |
| 3 | 41-60 | Moderately susceptible | MS |
| 4 | 61-80 | Susceptible | S |
| 5 | 81-100 | Highly susceptible | HS |

**Virus confirmation**

The total DNA was extracted from the plants showing symptoms using CTAB method [5] with suitable modifications and subjected to PCR analysis using PALIc1960/ PARIv722 [6] for the confirmation of begomovirus. PCR was performed as described by [7].

**Genome sequencing and analysis**

One of the infected plant sample which produced required band of 1280 bp with PALIc1960/ PARIv722 [6] was purified using FavorPrep GEL/ PCR Purification Kit (Favorgen, Germany) as per its protocol, concentration was checked and sent for sequencing (Eurofin, Karnataka). The genome sequence was retrieved and used for further analysis.

The retrieved forward and reverse sequences were edited using Editseq and Seqman software and saved as a single contig file. The contig file was used as a query sequence and screened against all available online GenBank nucleotide sequence database using BLAST (Basic Local Alignment Search Tool) program. The top ten hits of blastn analsyis were downloaded in the FASTA format and used for phylogenetic analysis. The multiple sequence alignment (MSA) and phylogenetic analysis was performed using ClustalW [8] of Molecular Evolutionary Genetics Analysis (MEGA X) [9], respectively. The evolutionary history was studied by using the Maximum Likelihood method and Tamura-Nei model [10]. The bootstrap consensus tree inferred from 500 replicates was taken to represent the evolutionary history of the taxa analyzed [11]. Pairwise distances (p-Hamming distances with pairwise deletion of gaps) were calculated using SDT V1.2 [12].

**Results and Discussion**

The per cent disease incidence varied from 40-100 per cent. None of the varieties showed immunity to the whitefly transmitted virus though the variety PAU BG-61 showed moderately resistant reaction and least incidence (40 per cent) among all the varieties used (Fig. 1). The variety PAU BG-70 and PAU BG-77 developed symptoms earlier (18 days) than the susceptible check PAU BG-14 (20 days) (Table 2). The symptoms on PAU BG-70 and PAU BG-77 were severe compared to other varieties used in screening (Fig. 1). Giri and Mishra in the year 1986 [2] conducted similar experiment in case of BDMV and found that the symptoms developed in 8-20 days varying from one host to other. Arunachalam in the year 2002 [13] conducted a similar experiment to find the resistant source against BDMV where eighty six genotypes of bitter gourd were screened. Out of eighty six, nine genotypes

were found to be resistant viz., IC 68296, IC 68335, IC 68263B, IC 68275, IC 68250A, IC 85620, IC 68285, IC 68312 and IC 68272.

The PCR analysis proved that the symptoms produced on different varieties used in screening was due to begomovirus infection. The purified PCR product was directly sequenced and submitted to GenBank (Accession No. MT875454). The sequence of source used for transmission studies was submitted to GenBank earlier (MN527535) [4]. The Blastn results with top ten hits of Blastn suite of NCBI revealed that the sequence of inoculums shared highest similarity (99.80 and 98.66 per cent) with ToLCNDV B4 coat protein (AV1) gene, partial cds (MN527535.1) [4] and ToLCNDV partial AV1 gene coat protein, clone 4-PCR A1 (FN645904.1) [14]. Phylogenetic analysis using Mega X revealed that the query sequence (MT875454) was closely related to ToLCNDV isolate (MN527535.1) forming a clade and distantly related to other ToLCNDV isolates used for analysis (Fig. 2). Pairwise distance analysis done using SDT V1.2 also indicated the same result and confirmed the association of *Tomato leaf curl New Delhi virus* (Fig. 3). The association of *Tomato leaf curl New Delhi virus* with BGYMD has been already reported from *M. charantia* [4, 15, 16, 17, 18].

## Conclusion

The yellow mosaic disease of bittergourd is caused by viruses belonging to three genera viz., *Potyvirus*, *Cucumovirus* and *Begomovirus*, out of which begomovirus has been found be most problematic in major bitter gourd growing areas. The crop is highly susceptible to begomovirus associated with BGYMD due to warm tropical climate and intensive crop cultivation, which supports the survival of whitefly population all round the year. Host plant resistance is one of the most economical and environmental friendly strategy for reducing yield loss. In the present study, none of the cultivated accessions showed immune reaction to begomovirus associated with BGYMD. However, the variety PAU BG-61 was found to be moderately resistant to this disease. The begomovirus associated with this disease was identified to be close to ToLCNDV. The present study is important to know the resistance status of available variety which may help us to develop completely resistant varieties in the future. The attempts should be made to incorporate broad spectrum resistance through gene pyramiding and other biotechnological tools. Further to confirm the resitant reaction of PAU BG-61, the germplasm need to be screened in the hot spot areas of the world.

## Conflict of Interest

The authors declare that there is no conflict of interest for this study.

**Table 2:** Screening of bitter gourd germplasm against whitefly transmitted virus

| Germplasm | Symptoms observed | Days to first appearance of symptoms | Per cent Disease İncidence | Disease Scale | Reaction Group |
|---|---|---|---|---|---|
| PAU BG-14 | Downward curling and blistering | 20 | 100 | 5 | HS |
| PAU BG-27 | Downward curling and mosaic | 22 | 100 | 5 | HS |
| PAU BG-30 | Downward curling and mosaic | 20 | 100 | 5 | HS |
| PAU BG-33 | Downward curling | 21 | 80 | 4 | S |
| PAU BG-44 | Blistering, mosaic and curling | 20 | 100 | 5 | HS |
| PAU BG-48 | Downward curling | 20 | 80 | 4 | S |
| PAU BG-53 | Curling and blistering | 22 | 100 | 5 | HS |
| PAU BG-57 | Curling | 22 | 50 | 3 | MS |
| PAU BG-61 | Downward curling, mosaic | 20 | 40 | 2 | MR |
| PAU BG-63 | Curling and mosaic | 23 | 100 | 5 | HS |
| PAU BG-70 | Severe curling, blistering and mosaic | 18 | 100 | 5 | HS |
| PAU BG-73 | Curling, blistering and slight mosaic | 20 | 50 | 3 | MS |
| PAU BG-77 | Curling, white dots on leaf | 18 | 100 | 5 | HS |
| PAU BG-86 | Curling and mosaic | 24 | 70 | 4 | S |
| PAU BG-87 | Curling | 25 | 50 | 3 | MS |
| PAU BG-88 | Curling and mosaic | 22 | 100 | 5 | HS |

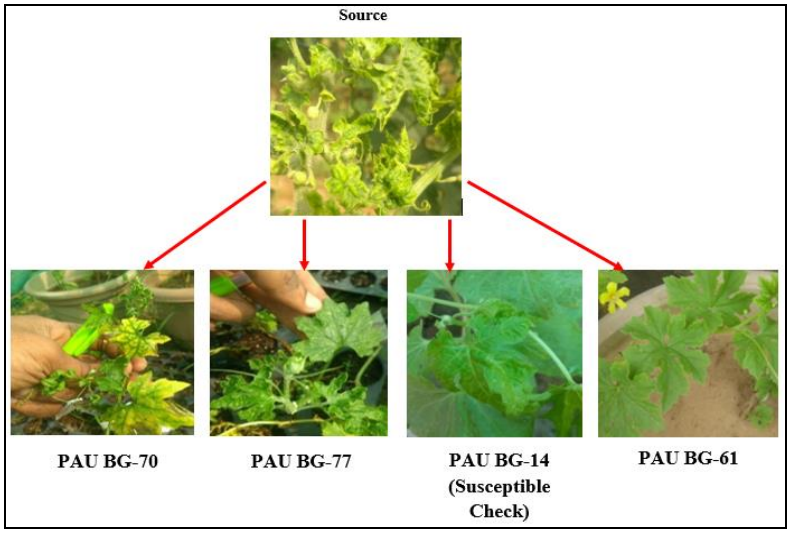


**Fig 1:** Reaction of bitter gourd germplasm in screening experiment

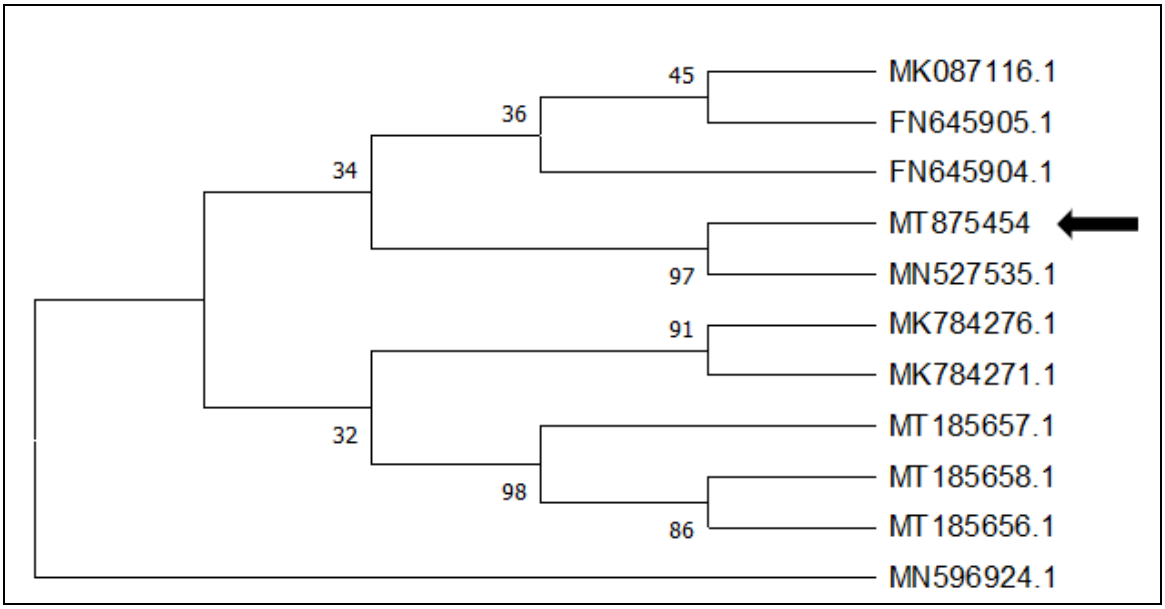


**Fig 2:** Molecular phylogenetic analysis of query sequence by maximum likelihood method

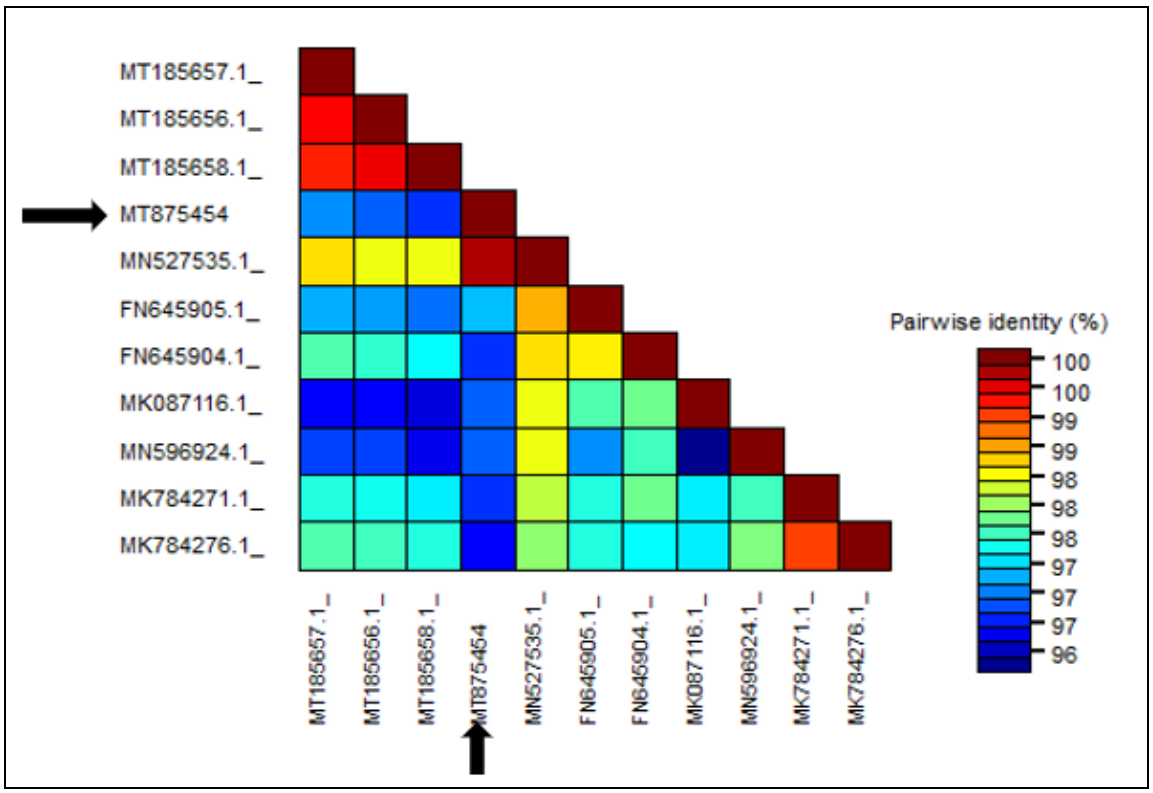


**Fig 3:** Pairwise distance analysis of query sequence along with closely related sequences of begomoviruses using SDT V1.2

**Acknowledgements**
The authors are grateful to the Dean, College of Agriculture, PAU, Ludhiana for providing the facilities to carry out this work.

**References**

1. Rajinimala N, Rabindran R, Ramaiah M. Management of Bitter gourd yellow mosaic virus (BGYMV) by using virus inhibiting chemical, biocontrol agents, antiviral principles (AVP) and insecticide. Archives of Phytopathology and Plant Protection. 2009; 42:738-750.
2. Giri BK, Mishra MD. A whitefly transmitted virus disease of bitter gourd. National seminar on whitefly-transmitted plant virus diseases. IARI , New Delhi. 1986, 22.
3. Mohanan A, Sharma A. Prevalence and incidence of bitter gourd yellow mosaic disease in Punjab. Plant Disease Research. 2020; 35(1):59-63.
4. Mohanan A, Sharma A. Transmission studies on viruses associated with yellow mosaic disease of bitter gourd. Indian Phytopathology. 2020; 73(2):329-337.
5. Lodhi MA, Ye GN, Weeden NF, Reisch BI. A simple and efficient method for DNA extractions from grapevine cultivars and Vitis species. Plant Molecular Biology Reporter. 1994; 12:6-13.
6. Rojas MR, Gilbertson RL, Maxwell DP. Use of degenerate primers in the polymerase chain reaction to detect whitefly transmitted *Geminiviridae*. Plant Disease. 1993; 77:340-347.
7. Zhou XP, Liu YL, Robinson DJ, Harrison BD. Four

DNA-A variants among Pakistai isolates of Cotton leaf curl virus and their affinities to DNA-A of geminivirus isolates from okra. Journal of General Virology. 1998; 79:915-923.
8. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research. 1994; 22:4673-4680.
9. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular biology and evolution. 2018; 35(6):1547-1549.
10. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution. 1993; 10:512-526.
11. Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. Evolution. 1985; 39:783-791.
12. Muhire B, Martin DP, Brown JK, Navas-Castillo J, Moriones E, Zerbini FM *et al*. A genome-wide pairwise-identity-based proposal for the classification of virus in the genus Mastrevirus (*Geminiviridae*). Archives of Virology. 2013; 9:1411-1424.
13. Arunachalam P. Breeding for resistance to Distortion mosaic virus in bitter gourd (*Momordica charantia* L.). Ph.D. Dissertation, Kerala Agricultural University, India. 2002.
14. Zaffalon V, Mukherjee SK, Reddy VS, Thompson JR, Tepfer M. A survey of geminiviruses and associated satellite DNAs in the cotton-growing areas of northwestern India. Archives of Virology. 2012; 157:483-495.
15. Khan JA, Siddiqui MK, Singh BP. The association of begomovirus with bitter melon in India. Plant Disease. 2002; 86:328.
16. Raj SK, Khan MS, Singh R, Kumari N, Prakash D. Occurrence of yellow mosaic geminiviral disease on bitter gourd (*Momordica charantia*) and its impact on phytochemical contents. International Journal of Food Science and Nutrition. 2005; 56:185-192.
17. Tiwari AK, Sharma PK, Khan MS, Snehi SK, Raj SK, Rao GP. Molecular detection and identification of Tomato leaf curl New Delhi virus isolate causing yellow mosaic disease in bitter gourd (*Momordica charantia*), a medicinally important plant in India. Medicinal Plants. 2010; 2:117-123.
18. Nagendran K, Kumar SM, Manoranjitham SK, Karthikeyan G. Molecular detection and characterization of Tomato Leaf Curl New Delhi Virus causing mosaic disease on bitter gourd in Tamil Nadu, India. Trends in Biosciences. 2014; 7:3925-3931.