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Puja Dey

Department of Plant Pathology,
 Biswanath College of
 Agriculture, Assam Agricultural
 University, Biswanath Chariali,
 Assam, India

Gawtham K Routhu

Department of Plant Pathology,
 College of Agriculture, Assam
 Agricultural University, Jorhat,
 Assam, India

Munmi Borah

Department of Plant Pathology,
 College of Agriculture, Assam
 Agricultural University, Jorhat,
 Assam, India

Manoj Kumar Kalita

Department of Plant Pathology,
 Biswanath College of
 Agriculture, Assam Agricultural
 University, Biswanath Chariali,
 Assam, India

Budha Bora

Department of Plant Pathology,
 Biswanath College of
 Agriculture, Assam Agricultural
 University, Biswanath Chariali,
 Assam, India

Palash Deb Nath

Department of Plant Pathology,
 College of Agriculture, Assam
 Agricultural University, Jorhat,
 Assam, India

Ranima Mishra

Department of Plant Pathology,
 Biswanath College of
 Agriculture, Assam Agricultural
 University, Biswanath Chariali,
 Assam, India

Corresponding Author:

Puja Dey

Department of Plant Pathology,
 Biswanath College of
 Agriculture, Assam Agricultural
 University, Biswanath Chariali,
 Assam, India

Incidence and detection of cucurbit mosaic disease in Assam

Puja Dey, Gawtham K Routhu, Munmi Borah, Manoj Kumar Kalita, Budha Bora, Palash Deb Nath and Ranima Mishra

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Abstract

The cucurbits belong to the family Cucurbitaceae (gourd family) under the order Cucurbitales which include many economically important vegetable and fruit species. Among various factors, viruses are one of the important factors reducing in quantity as well as quality of cucurbits. These crops are severely affected by the cucurbit mosaic disease worldwide, which is the major viral disease of the cucurbits. Considering the importance of the disease, the present investigation was taken up for incidence, detection, host range study and characterization of viruses associated with cucurbit mosaic disease in Assam. Survey was conducted in 5 districts of Assam (Sonitpur, Biswanath, Jorhat, Sivasagar, Dibrugarh) during 2021-22. Symptomatology revealed presence of various mosaic symptoms in the surveyed areas. Among the five surveyed locations, the symptoms observed in Sonitpur district were severe, whereas Biswanath district were moderate and mild symptoms were observed in Jorhat, Sivasagar, and Dibrugarh districts. Vector incidence study showed that aphid is the predominant vector. Molecular assay confirmed the presence of Cucumber Mosaic Virus in the collected cucurbit samples of surveyed areas; with severe incidence of mosaic disease in Sonitpur (90.91%), moderate in Biswanath (66.67%), mild in Jorhat (14.29%), Sivasagar (11%) and Dibrugarh (25%) districts. Molecular characterization of Cucumber mosaic virus cucurbit isolate from Jorhat district of Assam, was done through partial sequencing and similarity analysis showed 90.94 to 94.74 per cent sequence homology with different worldwide isolates of Cucumber mosaic virus.

Keywords: Assam, cucumber mosaic virus, reverse-transcription polymerase chain reaction

Introduction

Cucurbits are affected by various pests and diseases which cause a huge economic loss in the production. Amongst other factors, Cucurbit mosaic disease is one of the most serious problems faced by cucurbit growers worldwide. It has been reported to be caused by different viruses; viz., cucumber mosaic virus (CMV), watermelon mosaic virus (WMV), zucchini yellow mosaic virus (ZYMV), Pumpkin yellow vein mosaic virus (PYVMV) as well as papaya ringspot virus-watermelon strain (PRSV-W) (Biswas and Ghosh, 2018; Kumar, *et al.*, 2008) [6, 12]. Viruses such as Cucumber mosaic virus (CMV) cause losses as high as 100% (Khan, *et al.*, 2015) [11].

According to FAO, total area under cucurbits cultivation in India is 4.29 M ha with a total productivity of 10.52 t/ha (Anon, 2019) [1]. Cucurbit cultivation consists of about 5.6% of India's total vegetable production (Anon, 2019) [1]. China produces the largest quantity of cucumber (56.24 million tonnes) which is almost 75% of total global production (77.90 million tonnes) of cucumber (Anon, 2022) [2].

In India cucurbits are generally grown during summer (January to March) and rainy season (June to July). In hills it is sown in April-May. Among the North-Eastern states of India, Assam is one of the major cucurbit growing states (Anon, 2020) [3]. The mosaic disease in cucurbits has a worldwide occurrence and it has also been recorded from many cucurbit growing areas of Assam causing huge economic loss.

In the year 1916, CMV was first reported as the disease causing agent of cucurbits by Doolittle in Michigan and Jagger in New York (Palukaitis and Arenal, 2008) [18]. In the year 1975, Raychaudhari and Verma reported this disease for the first time in India and identified their aphid vectors as *Myzus persicae* and *Aphis gossypii* (Raychaudhari and Varma, 1999; Biswas and Ghosh, 2018) [20, 6]. Cucumber mosaic virus (CMV) is under genus Cucumovirus and family Bromoviridae. CMV is a single-stranded positive-sense RNA virus (Hirsch and Moury, 2021) [9].

In the year 1965, from Rio Grande Valley of Texas, watermelon mosaic virus (WMV) was recognized as an economically important virus of many states such as California, Illinois, New Jersey (Rajbanshi and Ali, 2016) [19]. Watermelon mosaic virus (WMV) is under genus potyvirus and family potyviridae (Anon, 2022) [4]. Zucchini yellow vein mosaic virus (ZYMV) was first isolated on squash from northern Italy in the year 1973 and described in 1981. (Desbiez and Lecoq, 1997) [7]. It was reported for the first time from the Mediterranean region (Lecoq and Desbiez, 2012) [14]. Zucchini yellow vein mosaic virus (ZYMV) is under genus potyvirus and family potyviridae (Anon, 2021) [5]. During early 1940s, yellow vein disease in pumpkin plants were reported for the first time from northern part of India (Vasudeva and Lal, 1943; Munniyappa, *et al.*, 2003) [21, 17]. Pumpkin yellow vein mosaic virus (PYVMV) is under genus begomovirus and family geminiviridae (Munniyappa, *et al.*,

2003) [17]. The first epidemic of PYVMV was recorded from South India in 2004 (Maruthi, *et al.*, 2007) [16].

Several aphid species such as *Aphis gossypii* and *Myzus persicae* are responsible for non-persistent transmission of the cucurbit viruses. Cucumber beetles are also reported as minor contributor of cucurbit virus infections. Whitefly is responsible for persistent transmission of one of the DNA viruses of cucurbit; *viz.*, PYVMV (Jayashree, *et al.*, 1999) [10]. Some of these viruses can also be transmitted mechanically through hands or tools, grafting, through dodder or other plants.

Materials and Methods

The survey was conducted in five major cucurbit growing districts of Assam; *viz.*, Sonitpur, Biswanath, Jorhat, Sivasagar and Dibrugarh (Fig1) to determine the per cent incidence of cucurbit mosaic disease.

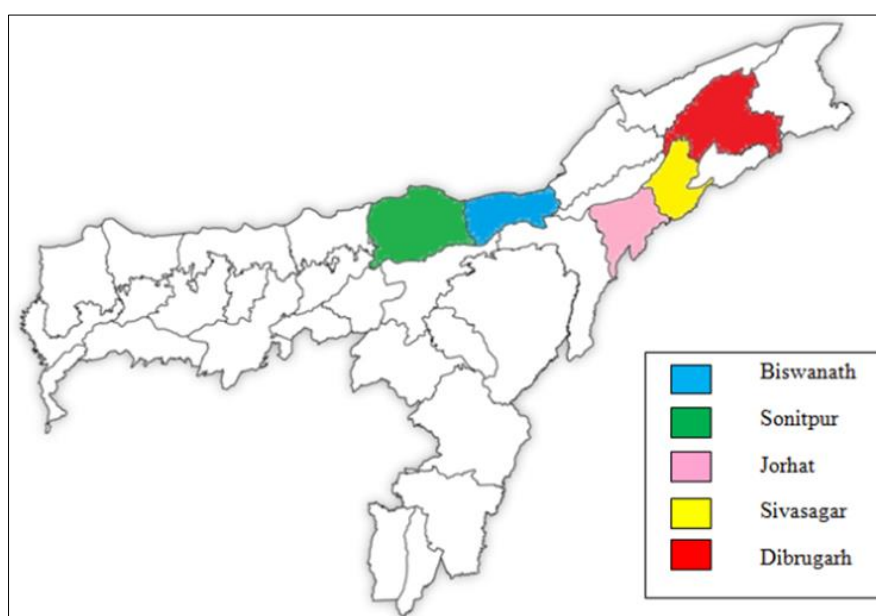


Fig 1: Map of Assam showing the surveyed districts

Leaf samples from both symptomatic and asymptomatic plants were collected following a roving survey from farmer's fields in the surveyed areas during November to December of 2021 and January to July of 2022. Samples were wrapped in Aluminium foil and stored at $-20\text{ }^{\circ}\text{C}$ in the laboratory for further molecular characterization.

Cucurbit plants were examined for mosaic disease symptoms and presence of vectors in the farmer's field. Based on, severity of the symptom expression in the surveyed areas, the symptoms were classified as mild (less than 50% plants infected), moderate (50-75% plants infected) and severe (more than 75% plants infected) for different locations (Fig 2, Table 2). The per cent vector incidence were calculated using the same formula and vector population were classified as low (less than 50% plants infected), medium (50-75% plants infected) and high (more than 75% plants infected) for different locations (Table 2).

Molecular analysis was done to detect the viruses associated with cucurbit mosaic disease. Total RNA was extracted following CTAB method described by Lodhi and Landolino. (Lodhi, *et al.*, 1994; Landolino, *et al.*, 2004) [22, 17] for detection of RNA viruses such as Cucumber mosaic virus, Watermelon mosaic virus and Zucchini yellow vein mosaic

virus and DNA isolation was done following CTAB method described by Doyle (Doyle, *et al.*, 1990) [10] for the DNA virus; *viz.*, Pumpkin yellow vein mosaic virus followed by one-step reverse transcription polymerase chain reaction (RT-PCR) and polymerase chain reaction (PCR), respectively; using specific primers (Table 3).

Results and Discussions

Different symptoms observed in surveyed districts of Assam were mosaic and increased leaf roughness (Fig 3. a), upward curling (Fig 3. b), yellowing of leaves (Fig 3. c), vein banding (Fig 3. d), vein clearing (Fig 3. e) as well as small, crinkled and deformed leaves (Fig 3. f). The highest disease incidence was recorded in Sonitpur district (96.25%), followed by Biswanath district was (74.23%). The disease incidences recorded in Jorhat, Sivasagar and Dibrugarh were 47.59%, 46.67%, and 49.66% respectively (Table 1). Symptoms were severe in Sonitpur, moderate at Biswanath and mild in Jorhat, Sivasagar, Dibrugarh (Table 2).

During survey, aphids were found to be the predominant vectors in all the investigated areas. Vector incidence was high in Sonitpur, medium in Biswanath and low in Jorhat, Sivasagar and Dibrugarh (Table 2).

Molecular assay confirmed the presence of CMV in the cucurbit samples of surveyed areas; while the assay showed negative results for the presence of other three viruses; viz., WMV, ZYMV, PYVMV. The thermal cycling process was standardized for the used primer pair; viz., MB 2CMV (F) and MB 2CMV (R) with an annealing temperature of 55.45 °C to obtain the desired 830 bp bands in Agarose gel electrophoresis (Fig 4). Further, molecular characterization of the coat protein gene was done by partial sequencing of the RT-PCR product. Similarity analysis showed 90.94 to 94.74 per cent sequence homology with different worldwide isolates

of CMV. The phylogenetic tree revealed that CMV isolate of Passion fruit from Imphal, Manipur was found to be residing in the immediate sister taxon with CMV Jorhat isolate of Pumpkin (Fig 5) and the isolate belonged to the larger cluster containing the majority of the CMV worldwide isolates. Per cent disease incidence was calculated in the surveyed areas through molecular assay which showed severe mosaic disease incidence in Sonitpur (90.91%), moderate in Biswanath (66.67%), mild in Jorhat (14.29%), Sivasagar (11%) and Dibrugarh (25%) districts (Table 4).

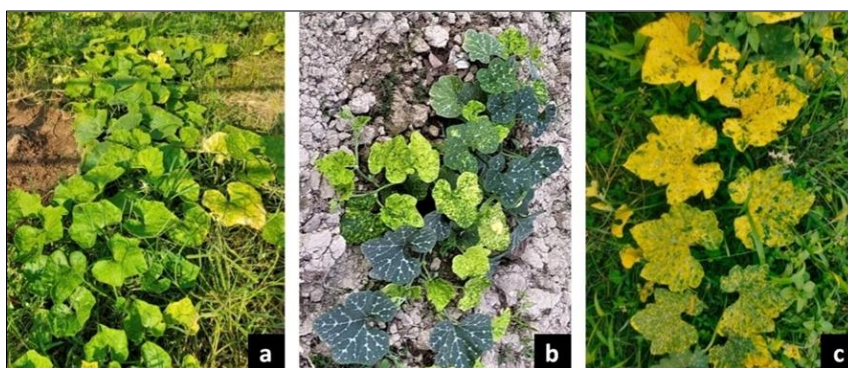


Fig 2: Mosaic affected cucurbit fields at surveyed locations. a= mild symptoms. b= moderate symptoms. c= severe symptoms

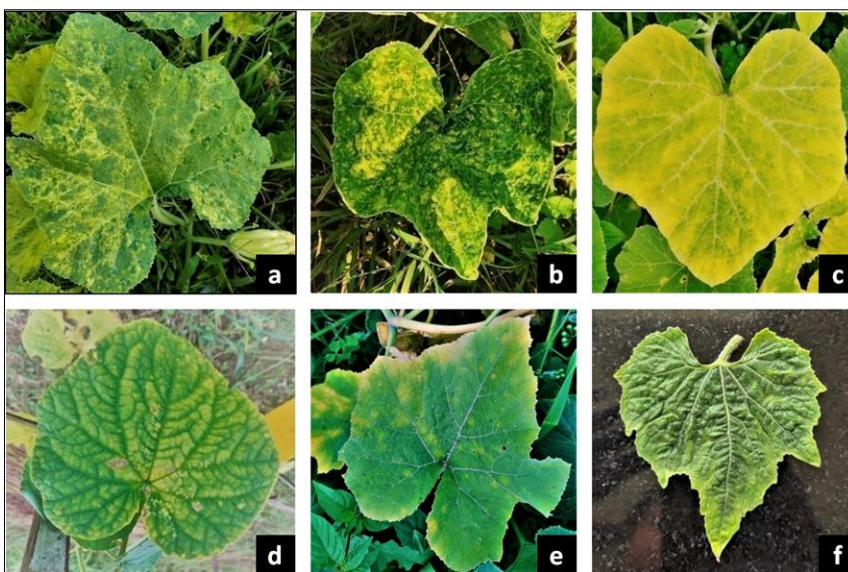


Fig 3: Different symptoms observed on cucurbit fields of surveyed area. a= mosaic and increased leaf roughness. b= upward curling. c= yellowing. d= vein banding. e= vein clearing. f= small, crinkled and deformed leaf

Table 1: Cucurbit mosaic disease incidence on different cucurbit crops from surveyed districts of Assam based on symptomatology

Locations	Name of the crop	No. Of sample observed	No. of positive sample observed	Infection percentage
Biswanath chariali	Pumpkin	120	113	74.23
	Cucumber	80	63	
	Ash gourd	40	24	
	Bottle gourd	25	16	
Sonitpur	Pumpkin	60	59	96.25
	Cucumber	80	77	
Jorhat	Pumpkin	55	22	47.59
	Cucumber	90	47	
Sivasagar	Pumpkin	20	11	46.67
	Cucumber	10	3	
Dibrugarh	Pumpkin	100	61	49.66
	Cucumber	35	7	
	Ash gourd	12	5	

Table 2: Severity of cucurbit mosaic symptoms and vector population count in surveyed area

Location	Symptoms observed in the field	Vectors
Biswanath chariali	++	**
Sonitpur	+++	***
Jorhat	+	*
Sivasagar	+	*
Dibrugarh	+	*

(+) = Mild, (++) = Moderate, (+++) = Severe
 (*) = Low, (**) = Medium, (***) = High

Table 3: Primers used for RT-PCR and PCR assay

Primer	Sequence	Target virus
MB 2 CMV F	ATGGACAAATCTGAATCAACC	Cucumber mosaic virus
MB 2 CMV R	TCAAAGTGGGAGCACCCCC	
WMV (F)	GGCTTCTGAGCAAAGATG	Watermelon mosaic virus
WMV (R)	CCCAYCAACTGTYGGAAG	
ZYMV (F)	GCTCCATACATAGCTGAGAC	Zucchini yellow vein mosaic virus
ZYMV (R)	AACGGAGTCTAATCTCGAGC	
PYVMV Deng B (F)	TAATATTACCKGWKGVCCSC	Pumpkin yellow vein mosaic Virus
PYVMV Deng B (R)	TGGACYTTRCAWGGBCCTCACA	

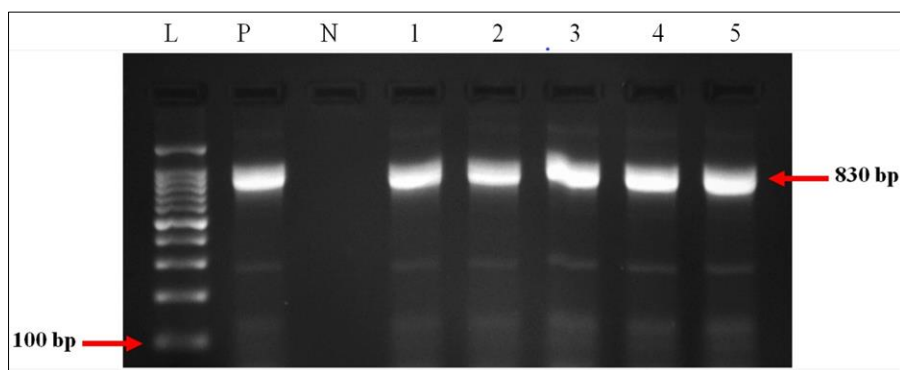


Fig 4: Agarose gel electrophoresis showing amplification of RT-PCR products. L= 100 bpdna ladder; n= negative control; p= positive control; 1-5= leaf samples

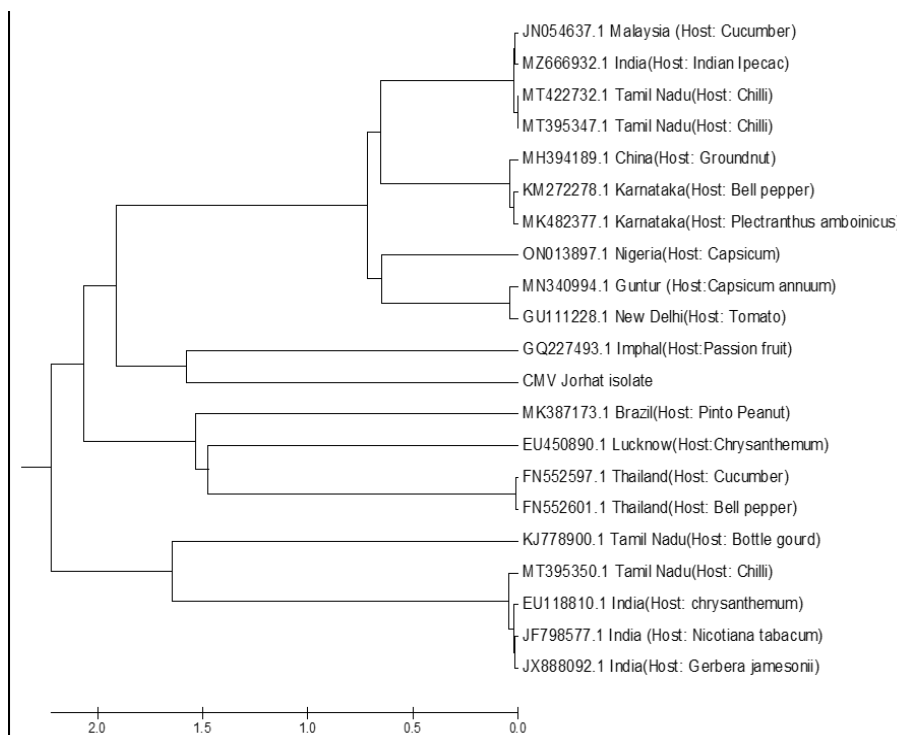


Fig 5: Phylogenetic relationship of CMV Jorhat isolate with worldwide isolates using UPGMA method. Scale bar indicates the evolutionary distances which were computed using the maximum composite likelihood method.

Table 4: Per cent disease incidence of cucurbit mosaic disease in surveyed area through molecular assay

Locations	No. of samples tested	No. of positive sample	Disease incidence (Per cent)	Severity
Sonitpur	11	10	90.91	***
Biswanath	12	8	66.67	**
Jorhat	7	1	14.29	*
Sivasagar	9	1	11.00	*
Dibrugarh	8	2	25.00	*

(* = Mild, (**) = Moderate, (***) = Severe

Conclusion

Mosaic disease is a serious problem of cucurbits in Assam. The viral infections are significant cause for low productivity of cucurbits globally. To reduce economic loss, integrated management strategies play an important role. Molecular characterization is of the virus isolates present, resistance breeding by identifying resistance genotype can pave way for formulating management strategies to maximise economic return for greater benefit of the farmers.

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